TEXTE
28/2022

Sind Stoffe persistenter als die Testsysteme glauben lassen? – Überprüfung der Testsysteme zur Persistenzbewertung am Beispiel der Hydrolyse

Influence of particles being present in aquatic environments on the hydrolytic degradation of organic substances

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Influence of particles being present in aquatic environments on the hydrolytic degradation of organic substances

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On behalf of the German Environment Agency

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Abstract

Hydrolysis is an abiotic process that is crucial for the fate of many water-soluble substances in an aquatic environment. The ability of chemicals to undergo hydrolysis is currently tested in purified lab water in accordance with OECD guideline 111. It has been suggested that the addition of particles such as sediment or microplastic fibers may result in a change in the rate of hydrolysis or in the formation of different transformation products. This study therefore tested the hydrolysis of three environmentally relevant compounds with and without addition of microplastic, sediment, or humic acids. The target compounds used were the fungicide trifloxystrobin (TFX), the anti-anxiety drug oxazepam, and (Methoxycarbonylmethyl)triphenylphosphonium (MCM-TPP) bromide, an intermediate product of industrial processes. None of the interfering materials had a significant effect on the hydrolysis of TFX. No significant influence was observed for oxazepam either, although the addition of sediment or humic acids resulted in a slight (almost significant) increase of the oxazepam hydrolysis half-live. However, for MCM-TPP the addition of humic acids and sediment resulted in a small, but significant decrease of the hydrolysis rate, while the addition of microplastic fibers had no effect on the hydrolysis.
It was thus shown that sediment particles and humic substances commonly occurring in aquatic environments can lead to a reduced hydrolysis rate of organic substances, probably especially for sorbing cationic compounds. However, this effect is small compared to the influences known to occur for changes of pH or temperature. The addition of interfering material (IMs) when testing the hydrolysis behavior of chemicals in OECD guideline 111 is thus not recommended. However, it is recommended to quantity in addition to the target compound also its main hydrolysis products to close the mass balances.

Kurzbeschreibung

Die Hydrolyse ist ein entscheidender Prozess für den Abbau vieler wasserlöslicher Substanzen in der Umwelt. Hydrolysestudien mit neuen Substanzen werden derzeit in Reinstwasser durchgeführt. Es besteht die Möglichkeit, dass in natürlichen Gewässern vorkommende (natürliche oder künstliche) Partikel einen Einfluss auf die Hydrolyse von Spurenstoffen haben.

Um dies zu testen, wurde in dieser Studie der hydrolytische Abbau von drei Substanzen in reinem Wasser sowie in Anwesenheit von Mikroplastikfasern, Sediment und Huminsäuren untersucht. Bei den ausgewählten Substanzen handelte es sich um das Fungizid Trifloxystrobin (TFX), das Benzodiazepin Oxazepam sowie um (Methoxycarbonylmethyl)triphenylphosphonium-(MCM-TPP-)bromid, ein Zwischenprodukt bei der Synthese von Alkenen.

Im Fall von TFX konnte kein Einfluss der Störstoffe auf die Hydrolyse nachgewiesen werden. Auch mit Oxazepam wurde kein signifikanter Einfluss beobachtet, obwohl die Anwesenheit von Sediment und Huminsäuren zu einem leichten, aber nicht signifikanten, Anstieg der Halbwertszeit führte. Im Fall von MCM-TPP führte die Zugabe von Sediment als auch von Huminsäuren zu einer geringen, aber signifikanten Verlangsamung des Abbaus, während Mikroplastikfasern keinen Einfluss auf die Hydrolysegeschwindigkeit hatten.

Die in natürlichen Gewässern vorkommende Sediment-Partikel und Huminstoffe können zu einer reduzierten Hydrolyserate bestimmter Spurenstoffe führen. Dies ist vermutlich für sorbierende kationische Verbindungen besonders wahrscheinlich. Dieser Effekt ist allerdings relativ klein im Vergleich zum Einfluss anderer Parameter wie der Änderung von pH-Wert und Temperatur.

Auf Grundlage dieser Ergebnisse ergibt sich nicht die Notwendigkeit, die Berücksichtigung verschiedener Störstoffe bei der Bewertung von Chemikalien zu fordern. Allerdings wäre es sinnvoll, zusätzlich zur Konzentration der Ausgangsverbindung auch immer die Konzentration der wichtigsten Hydrolyseprodukte zu bestimmen, um geschlossene Massenbilanzen zu erhalten.

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List of Abbreviations

ASE	Accelerated Solvent Extraction
a.u.	Arbitrary units
CAS	Chemical Abstracts Service
CB-TPP	Carboxymethyl(triphenylphosphonium) (hydrolysis product of MCM-TPP)
CNT	Carbon nanotubes
DDD	Defined daily dose
IM	Interfering material
MCM-TPP	(Methoxycarbonylmethyl)triphenylphosphonium
OECD	Organisation for Economic Co-operation and Development
PBT	persistent, bio-accumulating or toxic
PET	Polyethylene terephthalate
REACH	Registration, Evaluation, Authorisation and Restriction of Chemicals
TFX	Trifloxystrobin
TFXA	„Trifloxystrobin acid“, (E)-2-(methoxyimino)-2-(2-((((E)-(1-(3-(trifluoromethyl)phenyl)ethylidene)amino)oxy)methyl)phenyl)acetic acid
TOC	Total Organic Carbon
TP	Transformation product
TPP	Triphenylphosphonium *or* triphenylphosphine
WWTP	Wastewater treatment plant

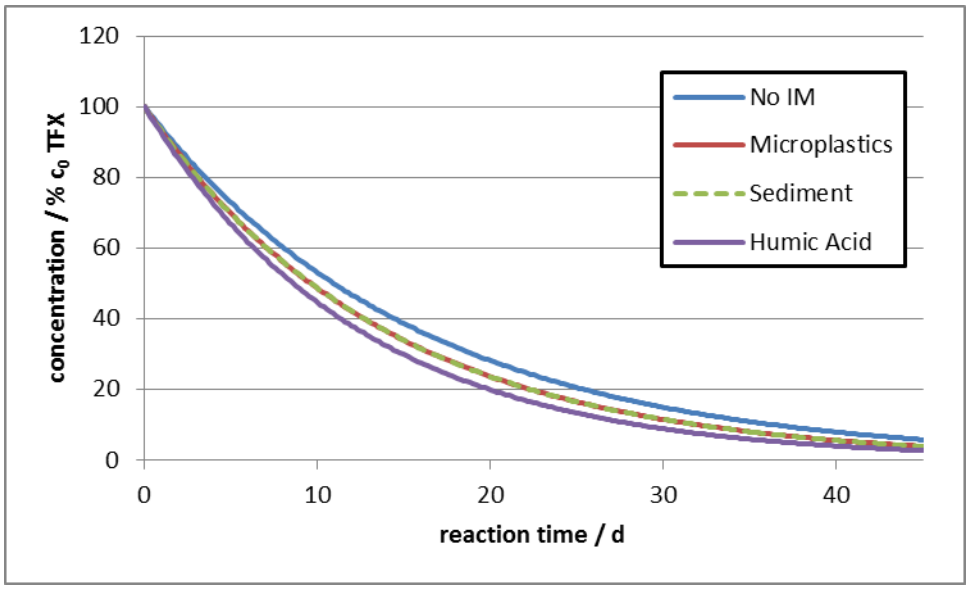
Summary

Hydrolysis is an abiotic process that is crucial for the fate of many water-soluble substances in the aquatic environment. Chemicals containing several functional groups such as amides, esters, or epoxides can be degraded by their reaction with water. The ability of new chemicals to undergo hydrolysis is currently tested in purified lab water according to OECD guideline 111.[1] It has been suggested that the addition of particles such as sediment, microplastic fibers, or humic acids may result in a change in the rate of hydrolysis or in the formation of different transformation products. An increase in the reaction rate could be caused by catalysis of the reaction by acid groups in the particles or by stabilization of the transition state, while a decrease could be caused if the sorbed portion of a chemical is no longer hydrolyzed.

This study therefore tested the hydrolysis of three environmentally relevant compounds with or without addition of microplastic, sediment, or humic acids. The target compounds used were the fungicide trifloxystrobin (TFX), the anti-anxiety drug oxazepam, and (Methoxycarbonylmethyl)triphenylphosphonium (MCM-TPP) bromide, an intermediate product in industrial processes. They were used in individual experiments with concentrations of 100 µg/L. The used microplastic consisted of polyethylenterephthalate (PET) fibers that were intended as filling material for pillows; the sediment was a fine sandy silt material gathered at the Ehrenbreitstein marina on the Rhine river; and the humic acids were acquired as a commercial standard. They all were applied in concentrations of 30 mg/L. The experiments were carried out in solution that had been sterilized by autoclaving. The pH was individually adjusted for each analyte to enable an appreciable hydrolysis which should not be too fast to determine the degradation kinetics. Individual samples were used for every sampling point, with the last sample being taken after 45 days. All samples were analyzed using an LC-Orbitrap mass spectrometer.

In the case of TFX, no significant effect of any of the three added materials was observed. In the experiments, carried out at pH 7.5, the half-life of TFX was found to be 10.9 ± 1.4 d when no interfering material (IM) was added. It was slightly, but not significantly lower when microplastic fibers, humic acids, or sediment were added. Figure S1 shows a direct comparison of the degradation of TFX in the different experiments.

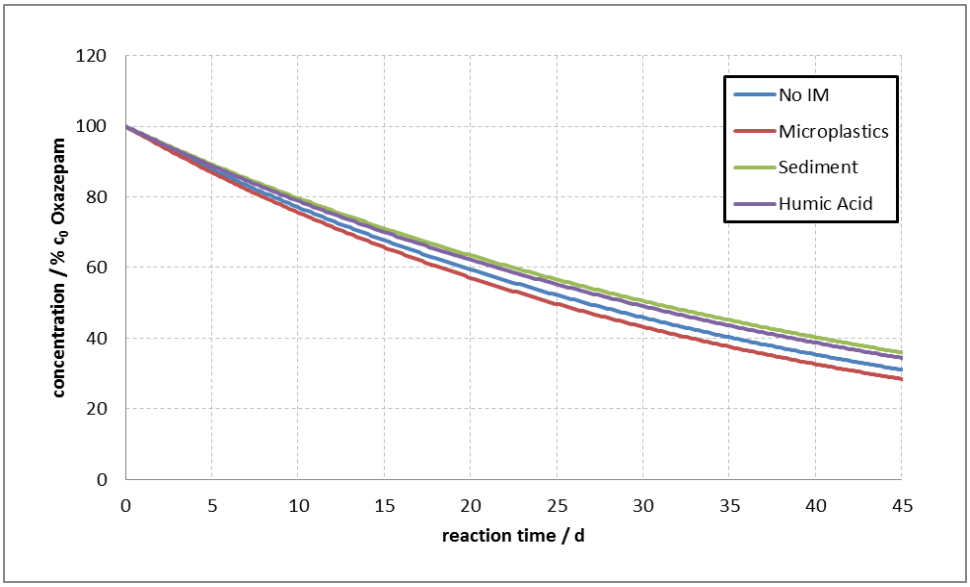
Figure S1 : Direct comparison of degradation of TFX under different conditions



Calculated curves based on the average of all experiments, based on non-linear regression of the untransformed data. Curves for the degradation in the presence of microplastic (red) and sediment (green) are not identical, but so similar that the green curve has to be represented by a dashed line.

No significant influence was observed for oxazepam either, although the addition of sediment or humic acids resulted in a slight (almost significant) increase of the oxazepam hydrolysis half-live. The half-life of oxazepam (measured at pH 9.0) was found to be 26.7 ± 2.1 d with no added IM, 24.8 ± 1.8 d with added microplastics, and slightly, but not significantly, higher at 30.5 ± 4.4 d with added sediment and 29.2 ± 2.5 d with added humic acids. A graphic comparison of the degradation of oxazepam under these different conditions is shown in Figure S2.

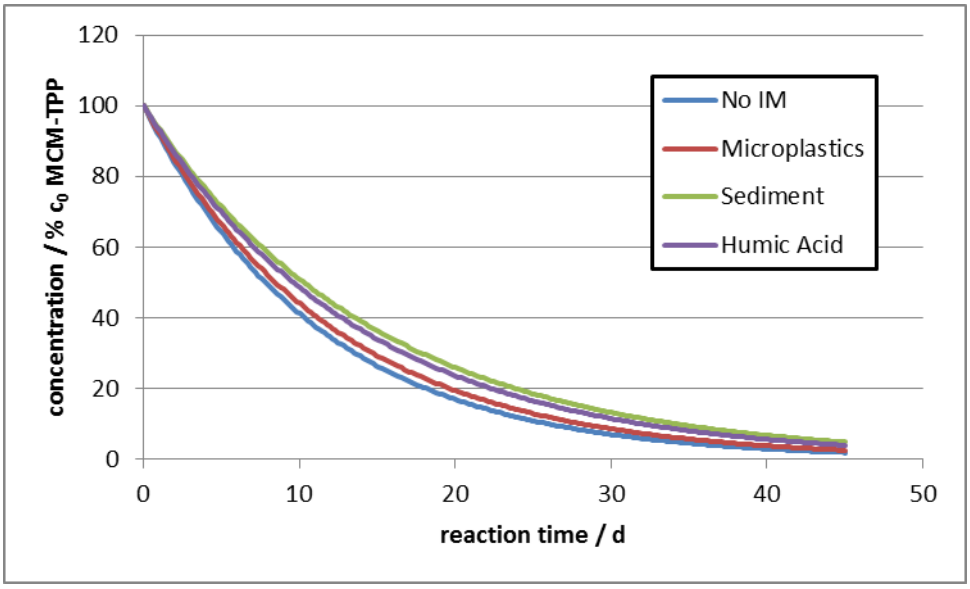
Figure S2 : Direct comparison of degradation of oxazepam under different conditions



Calculated curves based on the average of all experiments, based on non-linear regression of the untransformed data.

However, for MCM-TPP the addition of humic acids and sediment resulted in a small, but significant decrease of the hydrolysis rate, while the addition of microplastic fibers had no effect on the hydrolysis. The experiments in this case were carried out at pH 6.2, and the half-life was found to be 7.8 ± 0.4 d without any added material. With addition of microplastic fibers, the half-life was very similar at 8.5 ± 0.6 d. The addition of sediment resulted in an increased half-life of 10.3 ± 1.3 d, while the addition of humic acids resulted in a half-life of 9.7 ± 0.8 d. These are small, but significant increases.
A direct graphic comparison of the degradation of MCM-TPP under these different conditions is shown in Figure S3.

Figure S3 : Direct comparison of degradation of MCM-TPP under different conditions



Calculated curves are based on non-linear regression of the untransformed data of all experiments.

It was thus shown that sediment particles and humic substances commonly occurring in aquatic environments can lead to a reduced hydrolysis rate of organic substances, at least for sorbing cationic compounds such as MCM-TPP. However, this effect is small in comparison to the influences known to occur due to changes of pH or temperature. It should be noted that the concentration of IMs was 30 mg/L in all experiments. While this is a high concentration for microplastics in the environment, concentrations of humic acids and suspended matter can in some cases reach higher concentrations. Under the experimental conditions we used, sorption to the IMs was almost negligible compared to the dissolved fractions. As consequence, experiments with the addition of interfering material (IMs) when testing the hydrolysis in OECD 111 is not recommended. If IMs are to be included, it would probably be sufficient to include them in sorption tests (e.g. according to OECD guideline 106). If no significant sorption of a chemical occurs to any IM at environmentally relevant concentrations, it seems unlikely that the IMs will affect the hydrolysis of that chemical.

However, it is recommended to quantify in addition to the target compound also its main hydrolysis products to close the mass balances enabling to differentiate between sorption and hydrolysis leading to the dissipation of the target compound concentrations. The toxicological potential of the hydrolysis products could then be modelled or assessed experimentally. Overall, a revision of OECD guideline 111 to include the effect of interfering particles seems to be unnecessary based on our results and a literature review.

Zusammenfassung

Die Hydrolyse ist ein entscheidender Prozess für den Abbau vieler wasserlöslicher Substanzen in der Umwelt. Chemikalien mit häufig vorkommenden funktionellen Gruppen wie Ester, Amide oder Epoxide können durch eine Reaktion mit Wasser abgebaut werden.

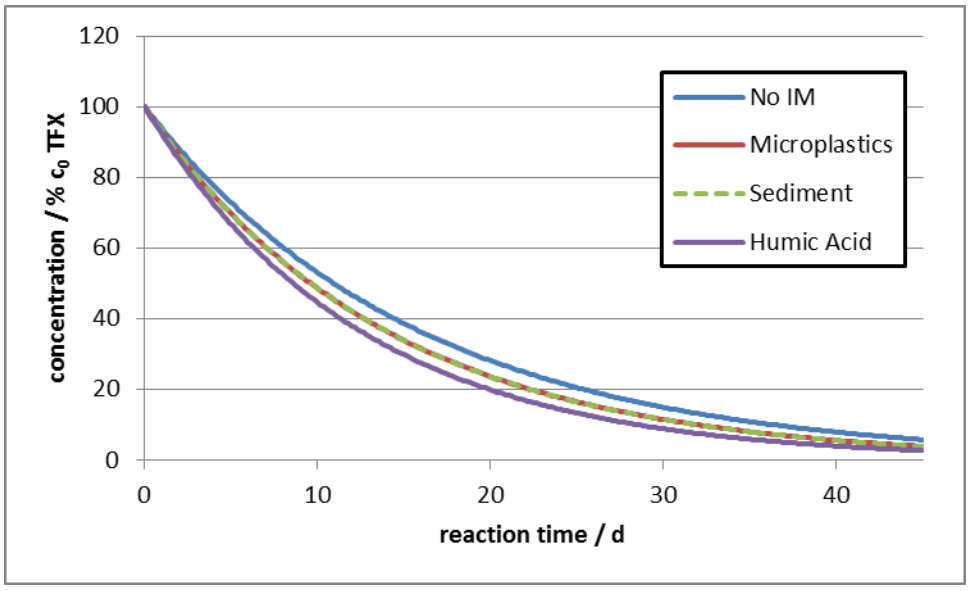
Hydrolysestudien mit neuen Substanzen werden derzeit nach OECD-Richtlinie 111 in Reinstwasser durchgeführt.[1] Es besteht die Möglichkeit, dass in natürlichen Gewässern vorkommende (natürliche oder künstliche) Partikel einen Einfluss auf die Hydrolyse von Spurenstoffen haben. Dabei ist sowohl eine Erhöhung der Abbaugeschwindigkeit möglich, wenn zum Beispiel Säuregruppen in einem Störstoff wie Huminsäure einen Abbau katalysieren, als auch eine Verringerung der Abbaugeschwindigkeit, wenn sorbierte Chemikalien nicht für einen hydrolytischen Abbau zur Verfügung stehen.

Um dies zu testen, wurde in dieser Studie der hydrolytische Abbau von drei Substanzen in reinem Wasser sowie in Anwesenheit von Mikroplastikfasern, Sediment und Huminsäuren untersucht. Bei den ausgewählten Substanzen handelte es sich um das Fungizid Trifloxystrobin (TFX), das Benzodiazepin Oxazepam sowie um (Methoxycarbonylmethyl)triphenylphosphonium-(MCM-TPP-)bromid, ein Zwischenprodukt industrieller Prozesse. Diese Substanzen wurden in Konzentrationen von 100 µg/L eingesetzt.

Die Mikroplastikfasern wurden von PET-Kügelchen entfernt, die als Füllmaterial für Kissen oder Stofftiere gedacht waren; bei dem eingesetzten Sediment handelte es sich um einen sandigen Schluff aus dem Koblenzer Rheinhafen Ehrenbreitstein; die Huminsäure stand als kommerzieller Standard zur Verfügung. Alle Störstoffe wurden in Konzentrationen von 30 mg/L eingesetzt.

Die Versuche wurden in autoklavierten Lösungen bei Raumtemperatur durchgeführt und liefen über 45 Tage. Der pH-Wert wurde dabei für jeden Analyten so gewählt, dass ein signifikanter, aber nicht zu schneller Abbau erwartet werden konnte. In regelmäßigen Abständen wurden aus individuellen Reaktionsgefäßen Proben entnommen und mittels Orbitrap-MS analysiert.
Im Fall von TFX konnte kein Einfluss der Störstoffe auf die Hydrolyse nachgewiesen werden. Die Versuche wurden hierbei bei einem pH-Wert von 7.5 durchgeführt. Die errechnete Halbwertszeit lag in dem Versuch ohne zugesetzten Störstoff bei 10.9 ± 1.4 d. In den Versuchen mit Störstoff war sie insignifikant kleiner. Abbildung S1 zeigt einen direkten Vergleich des Abbaus von TFX unter den verschiedenen Bedingungen.

Abbildung S1: Direkter Vergleich des TFX-Abbaus unter verschiedenen Bedingungen

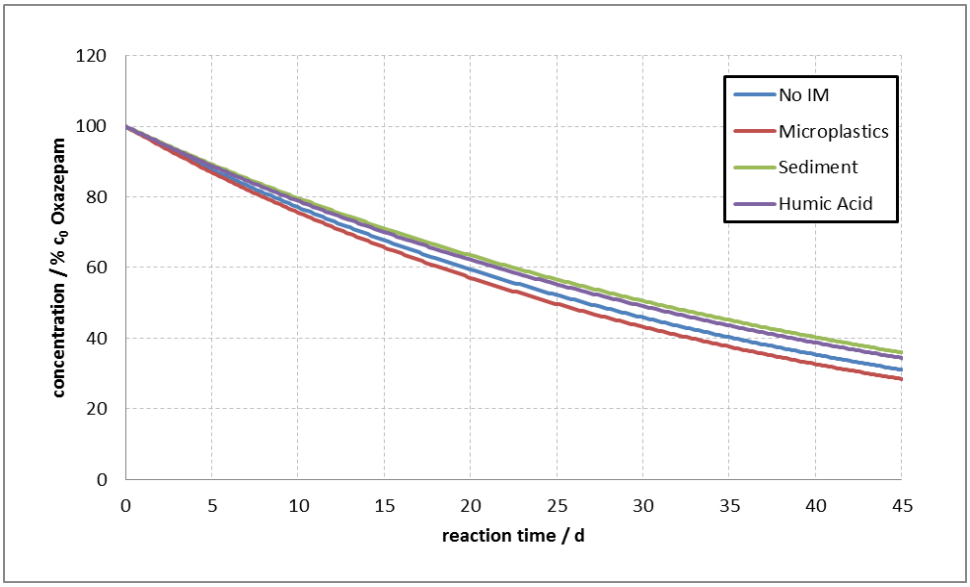


Berechnete Abbaukurven, aus den Ergebnissen aller Experimente mittels direkter nichtlinearer Regression der Daten errechnet. Die Abbaukurven für die Versuche mit Mikroplastik (rot) und mit Sediment (grün) sind nicht identisch, aber so nahe bei einander, dass die Sediment-Abbaukurve gestrichelt dargestellt werden muss.

Auch mit Oxazepam wurde kein signifikanter Einfluss beobachtet, obwohl die Anwesenheit von Sediment und Huminsäuren zu einem leichten, aber nicht signifikanten, Anstieg der Halbwertszeit führte. Tatsächlich konnte der Unterschied im Fall von Huminsäure als klein, aber signifikant angesehen werden, wenn die Daten mittels Auftragung des Logarithmus der Konzentration gegen die Zeit ausgewertet wurden, was eine immer noch häufig verwendete Vorgehensweise darstellt. Wurden die kinetischen Parameter stattdessen mittels nichtlinearer Regression der nicht-transformierten Daten ermittelt, so ergab sich eine Halbwertszeit (bei pH 9.0) von 26.7 ± 2.1 d ohne Störstoff und von 24.8 ± 1.8 d bei Zusatz von Mikroplastikfasern. Die Werte aus den Versuchen mit Sediment (30.5 ± 4.4 d) und Huminsäure (29.2 ± 2.5 d) waren leicht, aber nicht signifikant erhöht.

Eine graphische Zusammenfassung der Abbaukurven von Oxazepam unter allen verschiedenen Reaktionsbedingungen findet sich in Abbildung S2.

Abbildung S2: Direkter Vergleich des Oxazepam-Abbaus unter verschiedenen Bedingungen

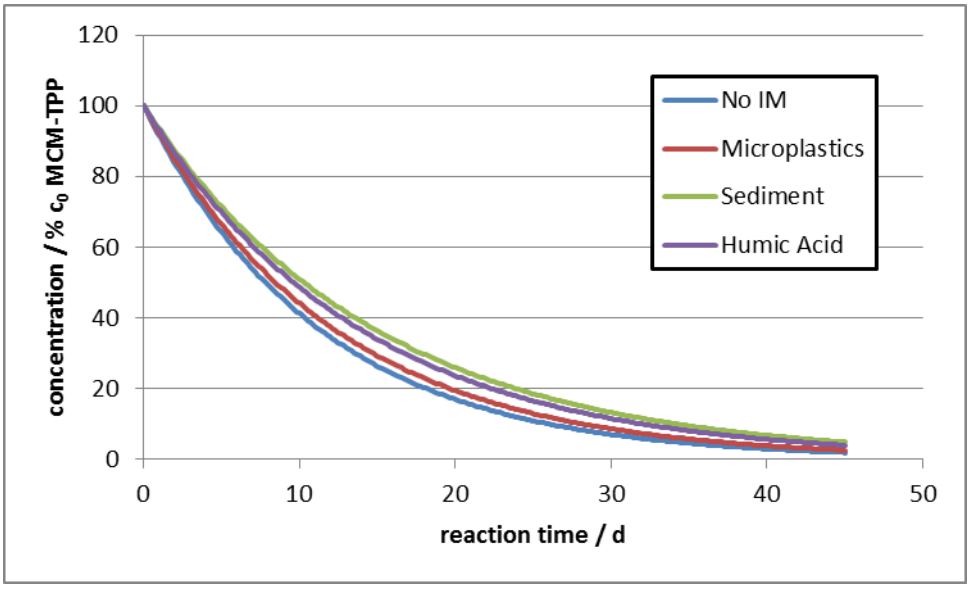


Berechnete Abbaukurven, aus den Ergebnissen aller Experimente mittels direkter nichtlinearer Regression der Daten errechnet.

Im Fall von MCM-TPP führte sowohl die Zugabe von Sediment als auch von Huminsäuren zu einer geringen, aber signifikanten Verlangsamung des Abbaus, während Mikroplastikfasern keinen Einfluss auf die Hydrolysegeschwindigkeit hatten. In den Versuchen, die bei einem pH-Wert von 6.2 durchgeführt wurden, wurde für MCM-TPP eine Halbwertszeit von 7.8 ± 0.4 d ohne Zusatz von Störstoffen und von 8.5 ± 0.6 d bei Zusatz von Mikroplastik ermittelt. Die Zugabe von Sediment führte dagegen zu einer Erhöhung der Halbwertszeit auf 10.3 ± 1.3 d, die Zugabe von Huminsäure zu einer Halbwertszeit von 9.7 ± 0.8 d. Dies ist in beiden Fällen eine geringe, aber signifikante Zunahme.

Ein direkter graphischer Vergleich des Abbaus unter diesen verschiedenen Bedingungen findet sich in Abbildung S3.

Abbildung S3: Direkter Vergleich des Abbaus von MCM-TPP unter verschiedenen Bedingungen



Berechnete Abbaukurven, aus den Ergebnissen aller Experimente mittels direkter nichtlinearer Regression der Daten errechnet.

Die in natürlichen Gewässern vorkommende Sediment-Partikel und Huminstoffe können also zu einer reduzierten Hydrolyserate bestimmter Spurenstoffe führen. Dies ist vermutlich für sorbierende kationische Verbindungen besonders wahrscheinlich. Dieser Effekt ist allerdings relativ klein im Vergleich zum Einfluss anderer Parameter wie der Änderung von pH-Wert und Temperatur.
Die eingesetzten Störstoffkonzentrationen von 30 mg/L lagen für Mikroplastik weit oberhalb der normalerweise zu erwartenden Umweltkonzentration, während für Huminsäure und insbesondere für Sediment auch wesentlich höhere Werte vorkommen. Unter den von uns gewählten Versuchsbedingungen spielte die Sorption an die Oberfläche der Störstoffe nur eine geringe Rolle.
Auf Grundlage dieser Ergebnisse ergibt sich augenscheinlich nicht die Notwendigkeit, allgemein die Berücksichtigung verschiedener Störstoffe bei der Untersuchung von Chemikalien nach OECD-Richtlinie 111 zu fordern. Um den Einfluss von Störstoffen zu berücksichtigen, könnte ein Sorptionstests (z.B. nach OECD-Richtlinie 106) vorgeschaltet werden. Sollte bei umweltrelevanten Störstoffkonzentrationen keine signifikante Sorption der Substanz auftreten, erscheint es unwahrscheinlich, dass der Störstoff die Hydrolyse der Chemikalie beeinflusst.

Andere Änderungen der Vorgehensweise scheinen sinnvoller, beispielsweise eine stärkere Berücksichtigung der Frage, ob beim hydrolytischen Abbau tatsächliche eine Verringerung des Gefahrenpotentials erfolgt, das von einer neuen Chemikalie ausgeht. D.h. der Bildung der stabilen Hydrolyseprodukte sollte mehr Beachtung geschenkt werden. Zum einen kann hierdurch die Massenbilanz geschlossen werden, also nachweislich Abbau und Sorption unterschieden werden, und zum anderen kann das toxikologische Potenzial der Hydrolyseprodukte modelliert oder sogar experimentell ermittelt werden.

Eine grundlegende Überarbeitung von OECD-Richtlinie 111 scheint auf Grundlage unserer Ergebnisse und der verfügbaren Literatur nicht notwendig zu sein.

1 Introduction

Anthropogenic contaminants are constantly being emitted into the aquatic environment via different pathways such as effluents of wastewater treatment plants (WWTPs) or discharges by several diffuse pollution sites such as run-offs from agricultural lands or urban areas.[2-8]

The fate of emerging contaminants in WWTPs as well as in the aquatic environment is of high relevance for the toxicity of emerging contaminants on aquatic organisms. In addition to a microbial degradation under aerobic and anaerobic conditions,[5, 9-11] abiotic processes also have to be considered. Abiotic processes are direct and indirect photolysis and redox reactions as well as hydrolysis.[12-14] In all cases the abiotic reactions lead to the degradation of the emerging pollutant; any possible toxicity of the pollutant may thus disappear or be reduced. Hydrolysis therefore has to be classified an important process for the assessment of the persistence of water-soluble substances in the aquatic environment,[15] and the ability of substances to undergo hydrolysis is tested during the registration of new chemicals in REACH.[16]

Of special concern are substances that are persistent, bioaccumulative and toxic (PBT substances). PBT substances are especially problematic due to their potential long term adverse effects on human health or ecosystems. Furthermore, long-term effects are difficult to predict, and any substance that is hydrolyzed reduces the likelihood of long-term effects which are caused in the environment by the respective substance. The ability of chemicals to undergo hydrolysis is currently tested according to OECD guideline 111 („Hydrolysis as a function of pH“) in purified lab water without the addition of particles.[1]

It can be hypothesized that testing under these idealized lab-conditions (purified water without particles) might underestimate the environmental stability of certain emerging contaminants. If a substance is partially sorbed onto suspended matter/sediments or microplastic, hydrolysis might be slowed down and cease to be an effective degradation pathway.[17] On the other hand, the hydrolysis of emerging contaminants might be even catalyzed by the presence of particles.[18, 19]

1.1 Abiotic hydrolysis of micropollutants in the aquatic environment

Hydrolysis is an abiotic degradation process involving the attack of water on a molecule. Several functional groups in a molecule can potentially undergo hydrolysis. Among these functional groups are:

- halogenated aliphatic hydrocarbons
- epoxides
- anhydrides
- organophosphates
- amides
- carbamates
- esters

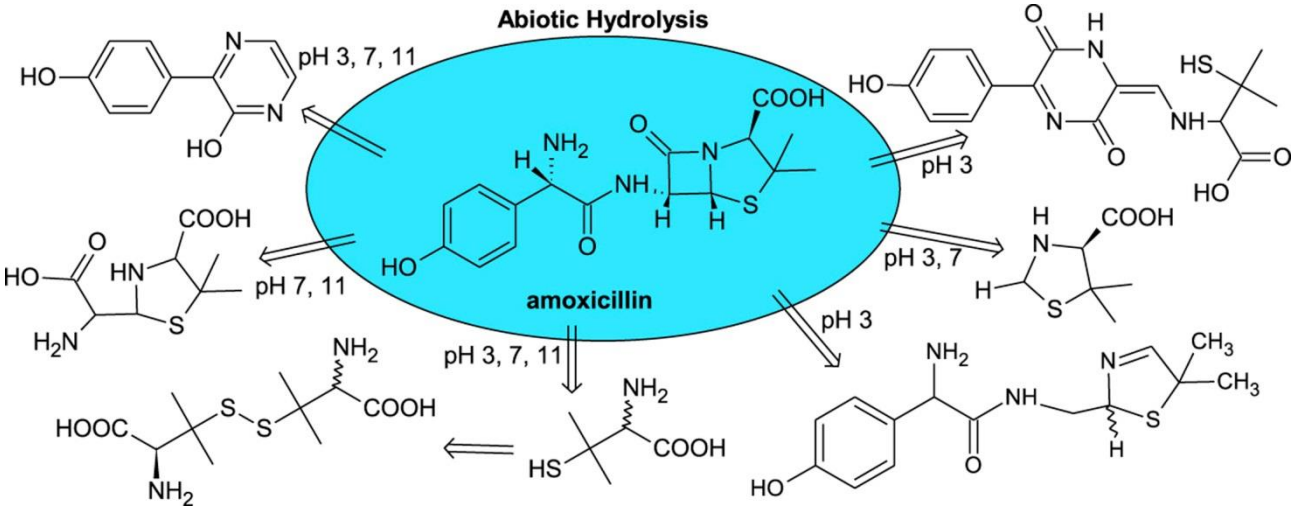
These functional groups are present in a multitude of anthropogenic contaminants such as biocides, pharmaceuticals, personal care products, cleaning agents or industrial chemicals. Detailed studies for the fate of such substances and their hydrolytic degradation in the aquatic environment exist only for few compounds. Furthermore, the majority of compounds for which such studies exist are pesticides or herbicides. Examples include sulfosulfuron.[20, 21] clomazon,[22] terbufos[23], and isoxaflutol.[24-26] Chamberlain *et al.* (2011)[27] tested the hydrolytic stability of a total of 62 pesticides at three different pH values (pH 2, 7, and 12). They found that only 7 of the 62 compounds (11%) were degraded by at least

half their initial concentration after 7 days at neutral pH. At acidic pH, 10 pesticides were degraded by at least that amount, while almost half of all tested pesticides (28 of 62, 45%) were degraded by at least 50% in the same time at pH 12. In contrast to this, Bialk-Bielinska *et al.* (2012)[28] analyzed the hydrolysis of sulfonamides and found that 10 out of 12 tested compounds were hydrolyzed (by at least 10% within 7 days) at pH 4, while only 3 of them were hydrolyzed at neutral pH, and none at all were hydrolyzed significantly at a pH of 9.

There have been several reports on the hydrolysis of different emerging pollutants recently. Below, some of these studies are discussed to emphasize the range of hydrolysis reactions and the relevance of the formed hydrolysis products.

Hirte *et al.* (2016)[29] described the hydrolysis of amoxicillin (AMX), a beta-lactam antibiotic. The authors confirmed that not only the hydrolysis rate, but also the pattern of transformation products (TPs) was strongly pH-depended. The first three TPs (AMX penicilloic acid, AMX 2´,5´-diketopiperazine, AMX penilloic acid) were formed by cleavage of the beta lactam ring. By a subsequent decarboxylation, they were transformed even further into 23 TPs. (See Figure 1.) Thus, a multitude of subsequent reactions follow the first attack on AMX by a water molecule. The instability of beta-lactam rings are the main reason for the hydrolysis reactions. A cleavage of the lactam ring by hydrolysis has also been reported for Penicillin G leading to penicilloic acid, penilloic acid and iso-penillic acid[30] as well as for four cephalosporin antibiotics.[31]

Figure 1: Hydrolysis of amoxicillin



Source: Hirte *et al.*[29] The TPs formed depended strongly on the pH at which the experiment was conducted.

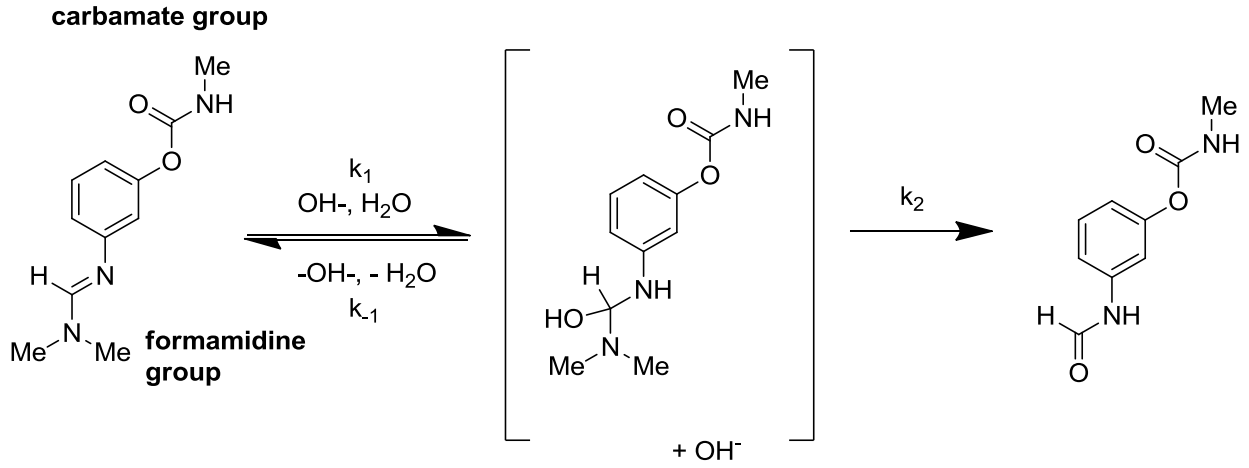
The hydrolysis of the two amphenicols chloramphenicol and florfenicol and the two macrolide antibiotics spiramycin and tylosin[32] was reported to be most relevant at pH<5 and pH>8 as well as at elevated temperatures (25-60°C). In those compounds, alkyl fluorides, amides and lactone (cyclic ester) moieties are hydrolyzed. However, the authors noted that under ambient environmental conditions (pH 7, low temperature) the investigated antibiotics are rather recalcitrant regarding hydrolysis. At pH 8 however, spiramycin and chloramphenicol were already significantly hydrolyzed.

Su *et al.* (2016)[33] reported the pH dependence of the hydrolysis of 16 organophophate tri-esters used as flame retardants and plasticizers. While the tri-alkylphosphonate triesters and (chloro)trialkylphosphonate triesters were not hydrolyzed at pH 7, 9 and 11, the tri-bromoarylphosphonate tri-esters were hydrolyzed at all pH values (7, 9, 11, 13) tested. As major hydrolyze products the dibromoarylphosphonate diesters were identified because the released negative charge of the phosphate OH moiety obviously inhibited a further cleavage of another brominated aryl moiety.

Ramezani *et al.* (2008)[34] reported that three imidazolinone herbicides are slowly hydrolyzed at pH 9, while they are completely stable at pH 3 and The half-life reported at pH 9 was 6.5 months for imazaquin, 9.2 months for imazethapyr, and 9.6 months for imazapyr.

Divito *et al.* (2007)[35] elucidated the hydrolysis of the insecticide formetanate, which contains two functional groups: a formamidine group and a carbamate. By analyzing the TPs, the researchers were able to show that the compound is hydrolyzed at the foramidine group, while the carbamate moiety is mostly stable. This is an example for a hydrolysis reaction in which it is important to identify the TPs to understand the reaction. (See Figure 2.)

Figure 2: Hydrolysis of formetanate



According to Divito *et al.*[35] In the bifunctional pesticide formetanate, the formamidine group is hydrolyzed under basic conditions, while the carbamate group remains stable.

1.2 Hydrolysis

Hydrolysis is an abiotic process which might lead to a degradation of emerging contaminants in the aquatic environment. A compound undergoing hydrolysis reacts with water according to the following reaction scheme:

$$R_1\text{-}R_2 + H_2O \xrightarrow{H^+ \text{ or } OH^-} R_1\text{-}OH + R_2\text{-}H$$

Thus Hydrolysis causes the cleavage of a certain moiety. It can be catalyzed by both H^+ and OH^- ions and therefore occurs at reduced rates at neutral pH, while more rapid hydrolysis is found under basic and acidic conditions. Since hydrolysis is a reaction between two molecules (one of them being water), it follows a second-order rate law. However, since water is present in a large surplus, its concentration over the course of the reaction can be seen as constant. Thus the reaction can be modelled by *pseudo-first* order kinetic:

$$A \xrightarrow{k_1} B$$

k_1 is the rate constant, which describes the speed at which the reaction occurs. It is typically strongly dependent on the pH, and also changes with temperature.

The concentration of the substance undergoing hydrolysis changes according to the following equation:

$$v = -\frac{d[A]}{dt} = k \cdot [A]$$

Integrating this equation yields:

$$[A]_t = [A]_0 \cdot e^{-k \cdot t}$$

$[A]_t$: concentration of A at time t, $[A]_0$: starting concentration of A, k: rate constant

It is common to use the logarithmic form of this equation, as shown below:

$$\ln[A]_t = (-k \cdot t) + \ln[A]_0$$

This is particularly useful because it means that plotting $\ln([A]_t/[A]_0)$ against the time results in a straight line with the reaction constant k as its (negative) slope. This offers an easy way to visually judge whether a reaction follows first-order kinetics, and allows determination of kinetic parameters using linear rather than non-linear regression analysis. However, this logarithmic transformation also means that in fitting the kinetic parameters, it is the logarithm of the errors that will be minimized rather than the errors themselves. This is equivalent to weighing the data $(1/(\text{fitted value}))^2$ and can thus result in distorted values for the fitted parameters.[36] When tools for non-linear regression analysis are available, it is preferable to determine all kinetic parameters from the untransformed data.

Under the assumptions that there is only one reaction product B and that B is stable once formed, we can also take advantage of the fact that the sum of A and B remains constant over the course of the reaction:

$$[A]_t + [B]_t = [A]_0$$

The concentration of B at any given time thus only depends on the starting concentration of A and the reaction constant k, according to the following equation:

$$[B]_t = [A]_0 \cdot (1 - e^{-k \cdot t})$$

The rate constant k of this reaction is also temperature-dependent. This is expressed by the Arrhenius equation, which links the rate of the reaction with its activation energy.

$$k = Ae^{-E_a/RT}$$

A: pre-exponential factor, E_a: activation energy of the reaction, R: universal gas constant, T: temperature

The pre-exponential factor A in that equation is a constant for each chemical reaction that describes how often the reactant molecules collide in the correct orientation. Together with the activation energy, this factor determines the temperature dependence of the reaction. These factors are typically determined by measuring the rate of a reaction at different temperatures and plotting it on a logarithmic scale according to the logarithmic form of the Arrhenius equation:

$$\ln(k) = \ln(A) + \frac{1}{T}\frac{-E_a}{R}$$

When ln(k) is plotted against 1/T in this manner, the result is a straight line with ln(A) as its y-intercept and a slope of $-E_a/R$, which allows the calculation of both the pre-exponential Arrhenius factor A and the activation energy E_a.

1.3 Hydrolysis testing according to OECD guidelines

Chemicals are currently tested to assess their hydrolytic transformation according to OECD guideline 111, "Hydrolysis as a function of pH".[1] The principle of the test is that sterile buffer solution of different pH values (pH 4, 7, and 9) are treated with the test substance and incubated in the dark at a constant temperature. At regular intervals, buffer solutions are analyzed for the remaining amount of the test substance and for hydrolysis products. The most important instructions from these guidelines are described in the following paragraphs.

According to the guideline, the study should be performed in glass containers under dark and sterile conditions. Alternative materials (such as Teflon) may be used if it is known or likely that the test substance adheres to glass.

The reaction containers must be kept at constant temperature, which requires temperature-controlled water baths or thermostatically-controlled incubators. All glassware and solutions should be sterilized to avoid biological degradation of the test substance.

The test substance is typically applied as an aqueous solution; or as a solution in a small amount of water miscible solvent (such as acetonitrile) if this is necessary for adequate dissolution. If organic solvents are used, their concentration in the reaction mixture should not exceed 1 % v/v, unless it can be shown that this has no effect on the hydrolysis of the test substance.

To avoid precipitation issues, the employed concentration of the test substance should not exceed 0.01 M or half the saturation concentration.

The test should be performed at pH values of 4, 7, and 9. Suitable buffers for these pH values, namely biphthalate, citrate, borate, and phosphate buffers, are suggested in the annex of the guidelines.

The most typical temperature for hydrolysis testing is 25°C, although the guidelines suggest 10-70°C as a suitable range; the temperature only needs to be kept constant during the experiment. If the hydrolytic behavior of the test substance is unknown, a preliminary test at a temperature of 50°C should be conducted – this increased temperature allows for a faster determination of whether the test substance is hydrolytically stable. A compound is thus generally regarded as stable at the tested pH if it is degraded by less than 10% during 5 days at 50°C, which implies a half-life of more than a year at room temperature.

The test is performed in the dark to avoid photolytic degradation of any test substance. Since dissolved oxygen in the water could also lead to increased degradation of some test substances, measures should be taken to avoid oxygen, e.g. by bubbling an inert gas through the solution before the start of the experiment.

It is further advised to use individual reaction vessels for each sampling point, since the use of a single bulk vessel from which samples are drawn at each sample point can lead to contamination of the test solution and also does not allow for an analysis of data variability. Samples should be prepared at least in duplicate for each sample point.

According to the guidelines, any hydrolysis products that represent at least 10% of the starting concentration of the analyte should be identified.

Regarding data handling and reporting, the guideline demands that log-transformed data of the test substance concentrations should be presented graphically, but also states that more accurate kinetic models should be used to determine the kinetic parameters such as half-lives.

1.4 Studies employing OECD guideline 111

Guideline 111 was adopted by the OECD in 2004.[1] Since then, there have been a number of studies that explicitly used the guideline in the determination of the hydrolysis of emerging contaminants.

Maszkowska *et al.* (2014)[37] investigated the hydrolytic stability of beta-blockers to evaluate the environmental risk assessment (ERA) proposed by Kuester *et al.* (2009)[38]. However, they concluded that the beta-blockers propranolol and metoprolol are not hydrolyzed at all at pH 7 and only to a very small extent at pH 4 or pH 9. Only nadolol exhibited a small tendency to hydrolysis which increased from pH 4 to pH 9, but is reported to have a half-life of more than 1 year at pH 7. Bialk-Bielinska *et al.* (2012)[28] conducted a hydrolysis study of 12 sulfonamides used as antibiotics mainly in veterinary medicine, with some of them also being used in human medicine. The authors confirmed that the hydrolysis reaction rates increased for pH 9 < pH7 < pH4 due to the protonation of the sulfonamide moiety, which is negatively charged at elevated pH values. Nevertheless, under normal environmental conditions the sulfonamides are rather stable with regard to hydrolysis. A similar conclusion was reported by Rayne and Forest who modelled that the sulfonamide group of perfluorinated compounds containing such a group should not be hydrolyzed.[39, 40]

Michel *et al.* (2014)[41] reported the hydrolysis of polyether trisiloxanes using OECD 111. At pH 7 with MilliQ water, the hydrolysis rate was extremely low or even negligible, while a rapid hydrolysis was observed at pH 9. Furthermore, a clear increase of the hydrolysis rate constants was found with increasing temperature. They even observed a small increase of about 10% of the rate constants if the river water used was filtrated. This could be an indication that the particulate matter reduces the hydrolysis rate constants, although to a very minor extent.

1.5 Influence of IMs

Hydrolysis testing is typically performed in purified water. In natural waters, the presence of particles might influence the rate of hydrolysis in various ways. For most substances, it is believed that they hydrolyze only in the aqueous phase, but remain stable while sorbed onto sediment or other particles.[42] This would imply that the hydrolysis of trace substances is slowed down by the presence of particles onto which the substance can be sorbed. This effect has been observed in a number of studies.[17, 43]

On the other hand, the presence of particles has been some cases also been found to increase the apparent rate of hydrolysis of trace substances. This was for instance observed for the herbicide atrazine.[44] To explain this effect, acid hydrolysis and stabilization of the transition state have been suggested.[17, 45] Surface-catalyzed hydrolysis has also been shown to occur in the presence of metal oxides or activated carbon.[18, 46, 47]

Macalady and Wolfe in 1985 studied the hydrolysis of the insecticide chlorpyrifos.[43] They suggested that hydrolysis in this case can in fact also occur in the sorbed state, at least under neutral conditions. For alkaline conditions, they did not observe hydrolysis in the sediment-sorbed phase comparable to that in the aqueous phase.

Perdue *et al.* studied the influence of the presence of humic substances on the hydrolysis kinetics of (2,4-dichlorophenoxy)acetic acid in 1982. They found that the apparent rate of hydrolysis was re-

duced in the presence of humic substances, which they explained with reversible adsorption of the analyte to the surface of the humic substances.[48]

Walse *et al.* in 2002 reported the influence of suspended solids on the oxidation and hydrolysis of the insecticide endosulfan and its degradation products. They observed a catalytic effect for some solids (sea sand, TiO_2, Fe_2O_3, FeOOH, Laponite, and SiO_2), while suspended creek sediment inhibited the hydrolysis.[46]

Salvestrini (2013)[19] studied the abiotic degradation of phenylurea herbicides in soil-water systems catalyzed by low molecular weight humic acids like compounds. They concluded that the presence of benzoic acid derivatives catalysis the hydrolysis of diuron herbicides. The influence of dissolved humic acids on the hydrolysis of γ-hexachlorocyclohexane and 1,1,2,2-tetrachloroethane was investigated by Georgi *et al.* (2008).[17] They found that 1,1,2,2-tetrachloroethan was not affected by the addition of 2 g humic acids at pH 10, while the hydrolysis of γ-hexachlorocyclohexane was reduced, since the sorbed molecules are protected from the attack of OH^- by the negative charge of the humic acids.

In a previous study conducted at the German Federal Environmental Agency (Umweltbundesamt), a reduced hydrolysis of Metilox (methyl 3-(3,5-di-tert-butyl-4-hydroxyphenyl)propanoate) was observed when ion-exchange resin, carbon nanotubes, or activated sludge were added as interfering material (IM).[49]

1.6 Aim of this study

This project tested whether an appreciable amount of particles can influence the hydrolysis rate or lead to the formation of different transformation products. For this, three emerging contaminants, which were known to be hydrolyzed from literature studies, were tested under a variety of conditions in purified water that contained particles such as sediment or microplastic.

The overall aim of the project was to study the hydrolysis of emerging contaminants in order to find out whether current test systems underestimate their persistence.

2 Preliminary considerations

2.1 Selection criteria for analytes

At the start of the project, analytes had to be selected which can be used in the hydrolysis tests to be performed. Crucial criteria for suitable analytes were:

1. Half-lives of hydrolysis are < 120 d and > 2 d
2. Standards are commercially available
3. Analytical methods are known or easy to develop for environmental concentrations
4. Hydrolysis products are (mostly) known and their standards are ideally commercially available
5. Target compounds should belong to different classes of chemicals (e.g. pesticides, pharmaceuticals)
6. Environmental relevance (detected in surface water, potential ecotoxicity)
7. Known or likely interactions with particles

Potential analytes considered for this project are listed in Table 1.

Table 1: Potential analytes that were considered for the project

Name	CAS Number	Description
Trifloxystrobin	141517-21-7	Fungicide
Dithianon	3347-22-6	Fungicide
Captan	133-06-2	Fungicide
2,3,4,5-Tetrahydro-7,8-dinitro-3-(trifluoroacetyl)-1,5-methano-1H-3-benzazepine	230615-59-5	Intermediate industrial compound
2-Propylheptyl-octanoate	868839-23-0	Oil found in cosmetics
(Methoxycarbonylmethyl)triphenylphosphonium bromide	1779-58-4	Industrial by-product
1-Butoxy-2,3-difluorobenzene	136239-66-2	Intermediate industrial compound
2-Morpholinothio-benzothiazole	102-77-2	Additive in rubber
Diazolidinyl urea	78491-02-8	Preservative found in cosmetics
Mefenpyrdiethyl	135590-91-9	Herbicide
Isotianil	224049-04-1	Fungicide
Amoxicillin	26787-78-0	Antibiotic drug
Oxazepam	604-75-1	Anti-anxiety drug

Of these compounds, several had to be discarded after preliminary tests. Dithianon, 2-Propylheptyl-octanoate, captan, 2,3,4,5-Tetrahydro-7,8-dinitro-3-(trifluoroacetyl)-1,5-methano-1H-3-benzazepine, 1-Butoxy-2,3-difluorobenzene, and Isotianil all proved to be difficult to detect at concentrations of 100 µg/L. 2-Morpholinothio-benzothiazole was found to hydrolyze too fast, with complete degradation after less than 24 h at 25°C. The half-life of the potential analytes needs to be short enough to guarantee that a significant degradation occurs over the course of the experiment, but long enough so that several samples can be taken before the analyte is completely degraded.

We also preferred using analytes that are representatives of different classes of compounds, e.g. a pharmaceutical, a pesticide, an intermediate and a final industrial compound, or a food additive. Regulations for the evaluation and registration of these different classes of compounds differ widely. The potential analytes that best met the criteria were trifloxystrobin, MCM-TPP, and oxazepam, although it should be noted that not all criteria could be met for all of these analytes. Trifloxystrobin is known to undergo hydrolysis with a half-life of approximately 36 d. The compound and its main hydrolysis product are available as commercial standards. Analytical methods for its detection and quantification are known, and as a commonly used fungicide, its fate and degradation in the environment is highly relevant. With a log Kow of 4.5, it can also be expected that the compound interacts with IM surfaces.

MCM-TPP is positively charged, so an interaction with the typically negatively charged surfaces of sediments[50, 51] as well as with negatively charged functional groups of humic acids are likely. Hydrolysis of the easily accessible ester group also seems likely. An analytical method (described in section 3.3) could easily be established. Since the compound is just an industrial by-product that is not sold at large quantities, isotope-labelled standards were not available, although we were able to also obtain the main hydrolysis product. Its environmental relevance is unknown, but it can be seen as a representative of cationic compounds in general.

Oxazepam as a pharmaceutical belongs to a completely different class of chemicals than the other two analytes. It can easily be commercially obtained together with an isotope-labelled standard, and analytical methods are already established. Oxazepam is known to undergo hydrolysis,[52] and it also known to interact with sediment particles.[53]

The selected analytes are discussed in more detail in section 3.1.1.

2.2 Selection criteria for interfering materials

There is a wide variety of potentially interfering compounds that could be found in the aquatic environment, of which only a small number could be employed in this study. The criteria for selecting IMs were as follows:

- Environmental relevance
- (Commercial) Availability
- Interactions with analytes is known or likely
- Defined and reproducible properties
- Ease of experimental handling
- Particles are stable for the duration of the experiment (45 d)

Potential IMs that were considered for this project are listed in Table 2.

Table 2: Potential interfering materials considered for this project

IM	Description/comment
(Sterilized) activated sludge	Structural change during sterilization likely
Sediment	Many different sediments available; structural change during sterilization possible
Humic acids	Partly dissolved in water[54, 55]
Microplastic fibers	Different materials possible, e.g. polystyrol, fleece, etc.
Carbon (nanoparticles, activated carbon)	Nanotubes containing heavy metals are unsuitable because of their catalytic effects[56, 57]
Natural particles (such as dried leaves, wood fibers)	Highly diverse material[58]
Plankton	Highly diverse material, difficult to reproduce; structural changes during sterilization likely[59-61]
Lignin	Might de-polymerize at high pH[62-64]

Activated sludge was excluded because its main expected effect on organic compounds in the environment would be expected to be the biological degradation caused by the microorganisms it contains. Sediments occur in all natural waters and are thus highly relevant, although there are many different types of sediments. We chose a sediment that was easily available and has been used in several studies already.[65, 66] Humic acids also occur ubiquitously in the aquatic environment. Their abundance of functional groups makes interactions with organic compounds very likely. Commercial standards of humic acids are available.

Some other natural compounds (such as plankton) are too variable to make good standard materials.

Microplastics are released into the environment by human activities. Their fate and behavior in the environment is a topic of high scientific and ecological relevance. Many different kinds of plastics exist, so we chose to work with one that is easily commercially available and has a high likelihood of occurring in the aquatic environment.
The analytes that were ultimately chosen (microplastic fibers, sediment, and humic acids) are discussed in more detail in section 3.1.2.

3 Materials and methods

3.1 Materials

3.1.1 Analytes

The hydrolysis of three environmentally relevant compounds was investigated in this project: a) the fungicide trifloxystrobin; b) an intermediate for the synthesis of olefins, (Methoxycarbonylmethyl)triphenylphosphonium bromide; and c) the anti-anxiety drug oxazepam. The three compounds are introduced in more detail in the following sections.

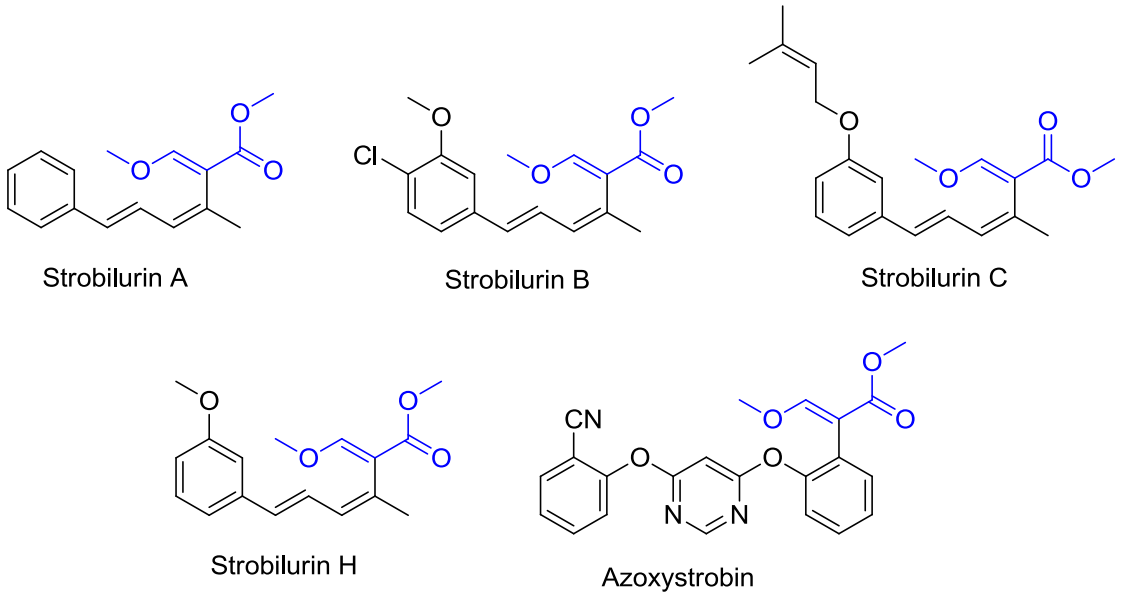
3.1.1.1 Trifloxystrobin

Trifloxystrobin (TFX) is a fungicide from the strobilurin class used in agriculture.

Strobilurins are a class of synthetic fungicides which act similar to the naturally occurring strobilurin A, which is produced by fungi.[67] It was first extracted from the small mushroom *Strobilurus tenacellus*, which was known for its ability to defend itself against other fungi.[68, 69]

The toxophore (the chemical group which causes the toxic effect) in all strobilurins is the β-methoxyacrylate moiety, which is shown in blue in Figure 3. This group binds tightly to the mitochondrial coenzyme Q_0, thus interrupting the respiratory chain of the fungus and inhibiting its growth.[70, 71] Strobilurins therefore belong to the larger group of quinone outside inhibitors, or QoI fungicides.[72] Strobilurin-producing fungi are immune to their own toxins.

Figure 3: Chemical structure of some other strobilurins



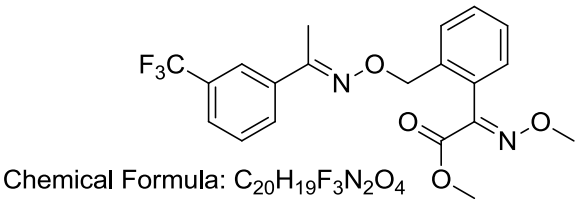
The β-methoxyacrylate group, which produces the toxic effect in strobilurins, is highlighted in blue.

Because of its broad spectrum of effects for fungal diseases, TFX has been widely used in various crops, for instance in cereals, citrus fruit and rice cultivation.[73-75] In the European Union, the use of trifloxystrobin was first approved in 2003,[76] and the approval period was most recently extended by the Commission Implementing Regulation 2016/950 until July 31th, 2017.[77]

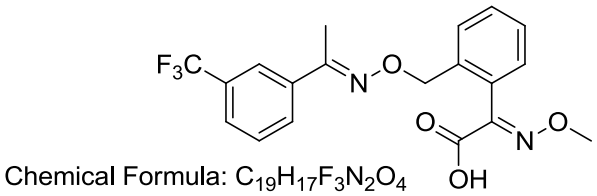
Since the common toxophore of the strobilurins contains an ester group, they all are susceptible to hydrolysis. The degradation of trifloxystrobin by hydrolysis and photolysis was described by Liu *et al.* in 2014.[78]

The chemical structure of TFX is presented in Figure 4. TFX has a molar mass of 408.4 g/mol, an octanol-water partition coefficient (log K_{OW}) of 4.5 and a water solubility of 0.61 mg/L at 25 °C.[79] It does not possess any groups that could readily be protonated or deprotonated and can be expected to be present predominantly as an uncharged molecule at any pH found in surface waters.

Figure 4: Chemical structure of TFX (left) and its main hydrolysis product



TFX



TFXA
CAS 252913-85-2

Table 3: Physicochemical values of TFX

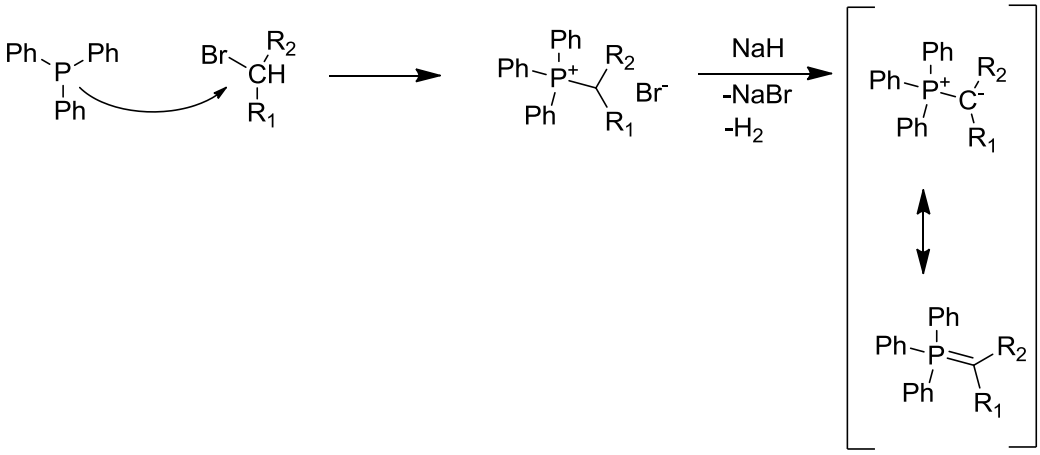
Property	Value
Solubility in water	0.61 mg/L
log Kow	4.5
log Koc (MCI method)	6.48*
log Koc (Kow method)	3.35*

Values marked with * are estimated in EPI suite.

3.1.1.2 MCM-TPP

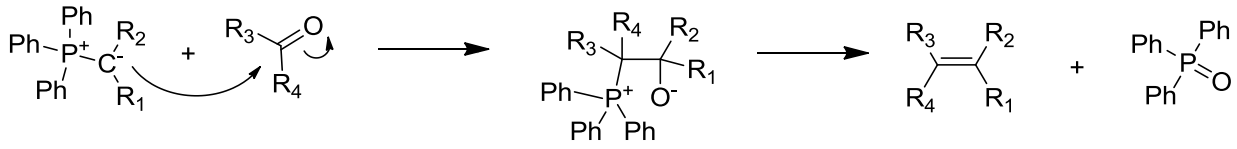
(Methoxycarbonylmethyl)triphenylphosphonium (MCM-TPP), which was used as a bromide salt, is an intermediate product of the Wittig reaction. This reaction, discovered in 1954 by Georg Wittig, is nowadays used at an industrial scale to form alkenes from ketones or aldehydes using phosphonium ylides.[80, 81] The general reaction mechanism is shown in Figure 5 and Figure 6.[82]

Figure 5: Reaction mechanism of the Wittig reaction, part I: formation of the ylide



To generate the ylides, triphenylphosphine is first quarternized by reaction with an alkyl halide. This results in an alkylphosphonium salt, for which MCM-TPP bromide is an example. In the Wittig reaction, the alkylphosphonium salt is then deprotonated with a strong base to form an ylide/ylene, which reacts with the ketons or aldehydes by forming olefin moieties.

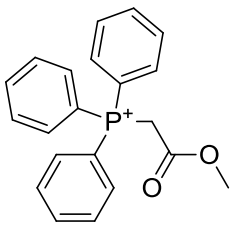
Figure 6: Reaction mechanism of the Wittig reaction, part II: formation of the alkene



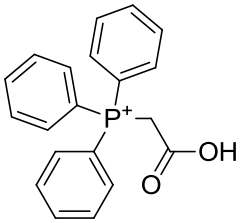
When the hydrolysis of phosphonium salts is discussed, this usually means a reaction at the central phosphor which results in the formation of triphenylphosphine oxide.[83-85] Byrne *et al.* only proved in 2015 that this mechanism involves the intermediate formation of P-hydroxytetraorganophosphorane.[86]

With MCM-TPP however, the hydrolysis of the ester group in the side chain is the prevailing process.

Figure 7: Chemical structure of MCM-TPP (left) and its main hydrolysis product



Chemical Formula: $C_{21}H_{20}O_2P^+$
Exact Mass: 335.1195
MCM-TPP



Chemical Formula: $C_{20}H_{18}O_2P^+$
Exact Mass: 321.1039
CB-TPP
CAS 1530-45-6

MCM-TPP has a molar mass of 415.3 g/mol as a bromide salt. It can be expected to be present as a cation at any pH found in natural waters. Although no experimental values values for Kow and Koc were

available and prediction software (such as EPI suite)[87] seems unreliable when handling permanently charged molecules, it can be expected that phosphonium as a cationic compound will sorb quite strongly to soil and sediments. EPI suite predicts a log Koc of 5.44 or 2.86, depending on the method used.

Table 4: Physicochemical values of MCM-TPP

Property	Value
Solubility in water	6.20 mg/L*
log Kow	3.61*
log Koc (MCI method)	5.44*
log Koc (Kow method)	2.86*

Values marked with * are estimated in EPI suite.

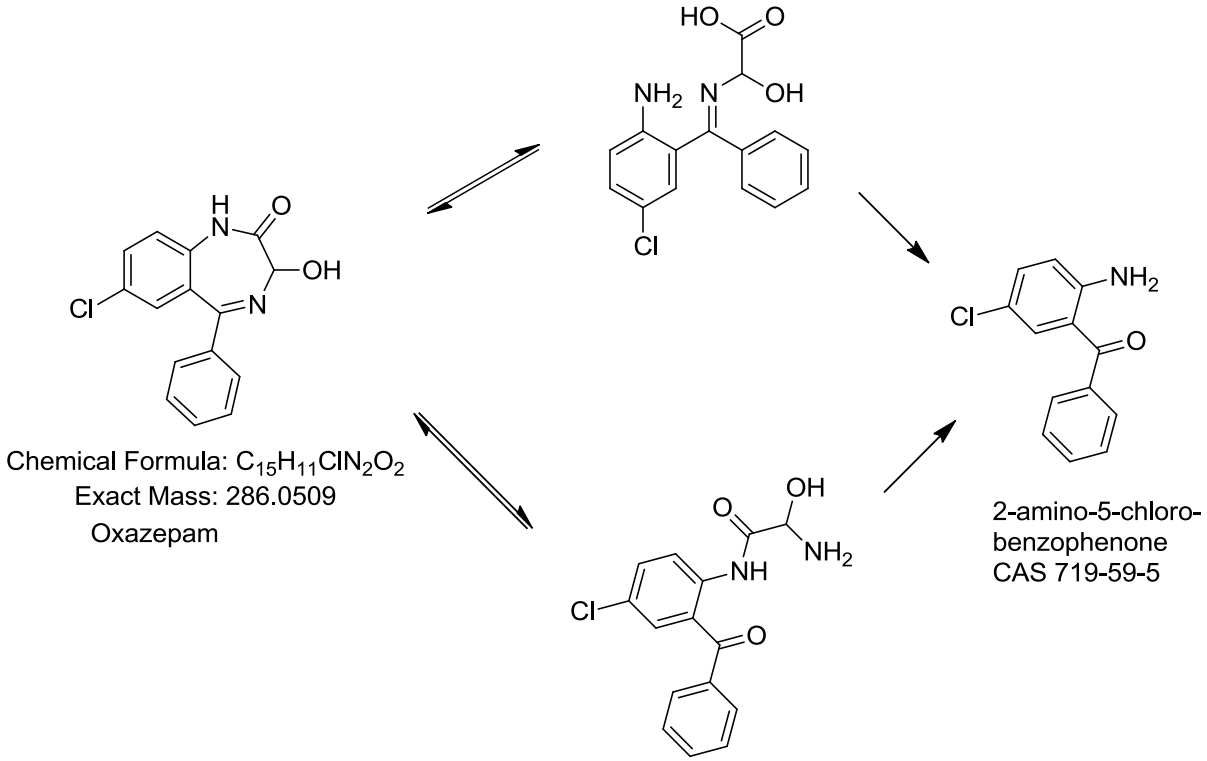
3.1.1.3 Oxazepam

Oxazepam is a psychoactive drug commonly used for the treatment of anxiety and insomnia. It belongs to the benzodiazepine class of drugs and is also a human metabolite of several other benzodiazepines such as diazepam and nordiazepam.

Benzodiazepines are used extensively worldwide and are known to occur in WWTP effluent and many surface waters.[6, 88-91] There is some concern that oxazepam at environmental concentrations may influence the behavior of fish populations.[92] This was demonstrated for the European perch (*Perca fluviatilis*) by Brodin *et al*. in 2013.[93]

The mechanisms and kinetics of benzodiazepine hydrolysis were described by Han *et al.* in a series of articles in 1976 and 1977.[52, 94, 95] The proposed degradation pathway is presented in Figure 8.

Figure 8: Chemical structure and hydrolysis pathway of oxazepam



Oxazepam is known to be moderately persistent in the environment, with a tendency to sorb onto sediment.[53] It has been shown to be relatively stable toward photodegradation, with a half-life of roughly 4 sunny summer days.[96]

It has a molar mass of 286.7 g/mol, a log K_{OW} of 2.24 and a water solubility of 20 mg/L at 22 °C.[97, 98] Oxazepam has a pKa of 11.3 and its corresponding acid has a pKa of 1.7 – Oxazepam can therefore be expected to be present predominantly as an uncharged molecule at any pH commonly found in surface waters.

Table 5: Physicochemical values of oxazepam

Property	Value
Solubility in water	20 mg/L
log Kow	2.24
log Koc (MCI method)	2.76*
log Koc (Kow method)	1.73*

Values marked with * are estimated in EPI suite.

3.1.2 Interfering materials

Humic acids, microplastic fibers and sediments were used as interfering materials (IMs) in our experiments. For better comparability, all IMs were typically used in the same concentration of 30 mg/L. This concentration is comparable to the level at which humic acids occur in aquatic environments,

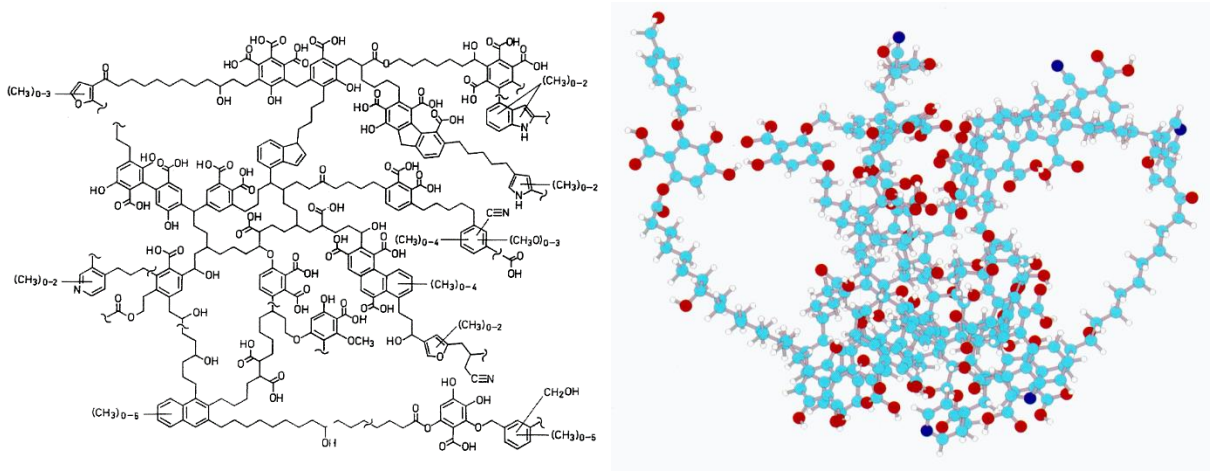
while microplastic fibers occur in much lower concentrations. For sediment, using a much larger amount could also be justified. OECD guideline 308 for instance, which describes the procedure for testing the transformation of chemicals in aquatic sediment systems, suggests using a water:sediment volume ratio between 3:1 and 4:1.[99] However, particulate matter can be present in rivers and streams in the range of 30 mg/L.[100]

3.1.2.1 Humic acids

Humic acids are a diverse mixture of high-molecular weight compounds containing many carboxyl and phenolic moieties.[54] They are formed by the partial biological degradation of plants and represent a significant part of the organic component present in soil and the aquatic environments. Humic acids are found everywhere in the aquatic environment: in soils[101], lakes[102], rivers[103], and in the sea.[104, 105]

The 2D and 3D structure of a molecule of humic acid, as proposed by Schulten et al., is shown as an example in Figure 9.[106]

Figure 9: Structure of a humic acid molecule

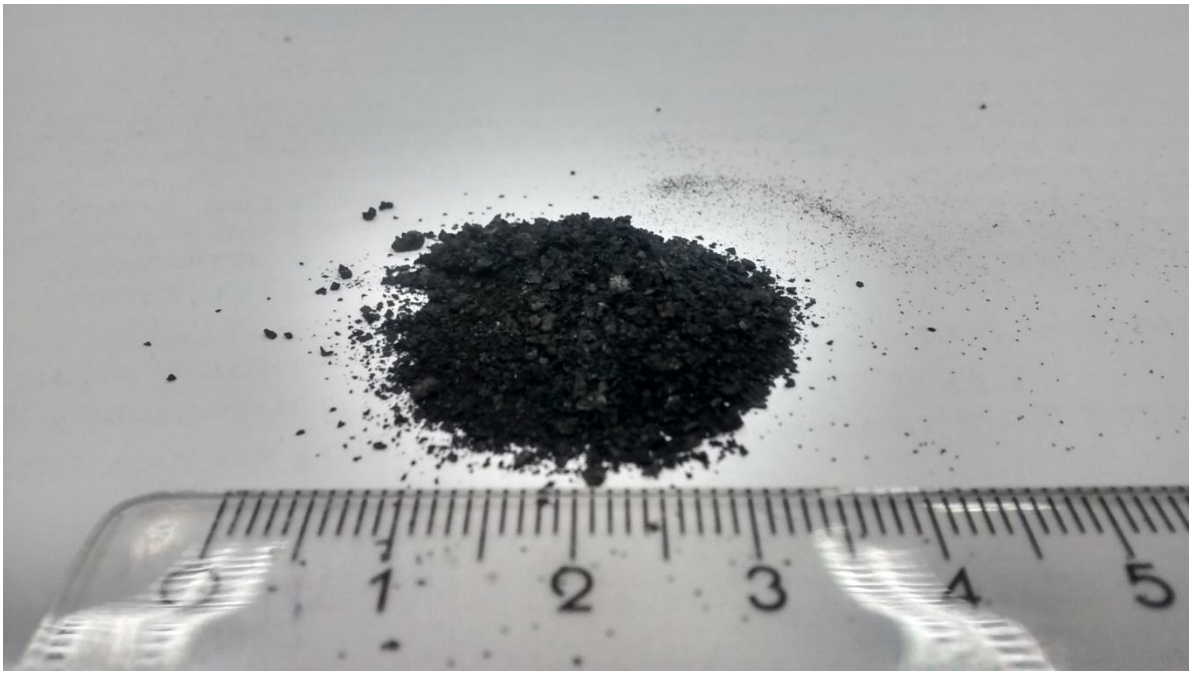


Element colors in the 3D model: carbon (cyan); hydrogen (white); oxygen (red); and nitrogen (blue). Source: Schulten et al. 1997.[106]

In this study, we used a commercially available humic acid standard provided by Sigma Aldrich (article number 53680, CAS 1415-93-6). The carbon content of this standard was determined as 39.4 %. The use of commercial humic acids has been criticized since they may be significantly different from naturally occurring aquatic NOM even if their elemental composition is similar.[107] However, they still allow improved ease of handling and greater comparability of results with those of other researchers.

It is well known that humic acids can increase the apparent solubility of otherwise insoluble substances.[108] This is because the substances can be sorbed onto the humic acid, which itself is partly dissolved in water.

Figure 10: Picture of humic acid used as an IM



Scale in cm.

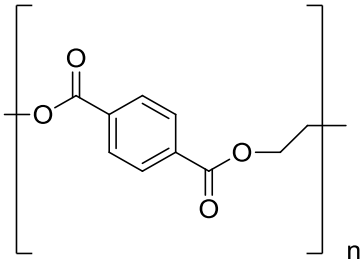
3.1.2.2 Microplastic fibers

Plastic waste can be found everywhere in the aquatic environment. The first reports of plastic litter in the ocean date back to the 1970s.[109, 110] This includes large plastic items as well as microscopic fragments and fibers.[111] These so-called microplastics and their behavior in the environment have been studied extensively in recent years.[112-121] They have been found in oceans[122], rivers[118, 123], and sediments[123, 124] as well as in living organisms.[116, 124]

Microplastics are both formed from larger plastic objects in the environment and released into the environment by a number of different sources.[120, 125, 126] Domestic sources of microplastic fibers include the washing of polyester fleece textiles[127] as well as some personal care products.[120, 128]

Microplastics in aquatic environments have the potential to absorb persistent organic pollutants and transfer them to marine organisms after being ingested.[129] There is some concern that the microplastics used in laboratory experiments are not consistent with those found in the field.[130] The most commonly used types of plastic are polyethylene (PE), polypropylene (PP), polyvinylchloride (PVC), polystyrene (PS), and polyethylene terephthalate (PET) (see Figure 11), which together account for approximately 90% of the global plastic production.[131]

Figure 11: Structure of PET



The **microplastic fibers** employed in this study were purchased as small PET balls (approximately 1 cm diameter) that were sold as filling material for pillows or stuffed toys. Similar fibers are used in

mixed-fiber textiles. The fibers should therefore be comparable to fibers that are released into the environment during the domestic washing of clothes. Individual fibers (length up to several cm, diameter <0.1 mm) were manually detached from the PET balls prior to use.

Figure 12: Picture of microplastic fibers used

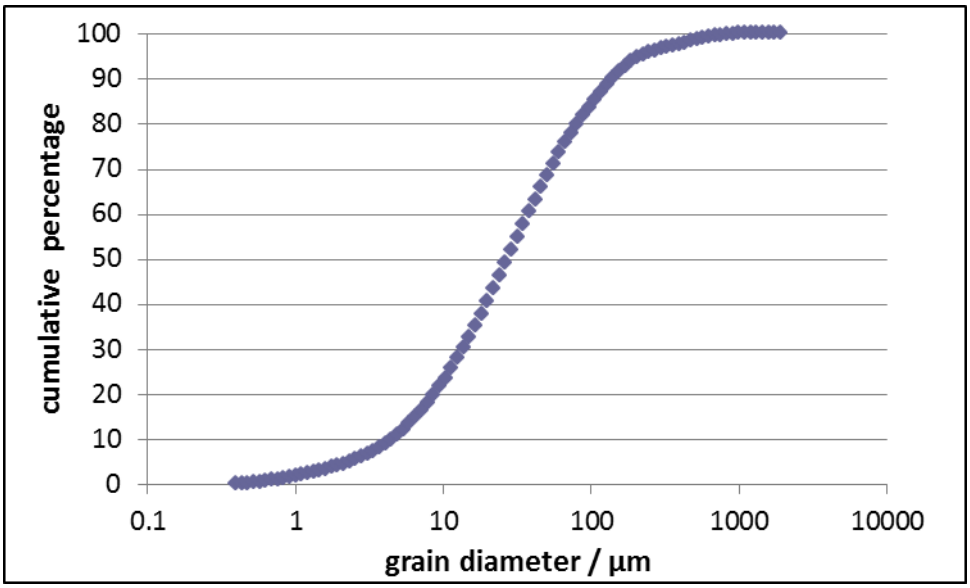
Scale in cm.

3.1.2.3 Sediment

Sediments are naturally occurring materials that are broken down by erosion and weathering and are subsequently transported by the action of water or other natural means.[132] Sediments are classified by grain size, reaching from clay (<3.9 µm) to coarse sand (0.5-2 mm). Even larger particles such as gravel, cobble, and even boulders can be considered sediment, as long as they are transported, e.g. by glaciers.[133]

The used **Rhine sediment** is a sandy silt sediment sampled at the Ehrenbreitstein marina at Koblenz. It was filtered through a 2-mm sieve prior to its application in the lab-scale experiments. The grain size distribution of the sediment can be seen in Figure 13.

Figure 13: Grain size distribution of the Ehrenbreitstein sediment



The Ehrenbreitstein sediment thus consists of approximately 4.4% clay, 70.2% silt, and 25.4% sand. It has a dry matter content of 32.8% with an organic portion of 4.3%.

3.2 Experimental methods

3.2.1 Buffer systems

Buffer systems used in this project were acetic acid/acetate buffer for experiments at low pH (up to pH 6), phosphate buffer for near-neutral experiments (up to pH 8), and glycine-NaOH buffer for basic conditions. Buffers were used in concentrations of 0.1 mol/L.

Acetic acid/acetate buffer was prepared by dissolving 6 mL glacial acetic acid and 16.4 g sodium acetate each in 1 L purified water separately, then slowly adding acetic acid solution to the sodium acetate solution while stirring vigorously until the desired pH is reached.

Phosphate buffer was prepared by dissolving 10.206 g KH_2PO_4 in 1.5 L purified water, then slowly adding sodium hydroxide solution (c = 0.1 mol/L) until the desired pH is reached.

Glycine-NaOH buffer was prepared by dissolving 15.0 g glycine and 11.7 sodium chloride in 2 L purified water and adjusting to the desired pH by slowly adding sodium hydroxide solution (c = 0.1 mol/L) until the desired pH is reached.

3.2.2 Lab-scale experiments

For a series of the experiment, a buffer solution (approximately eight liters) at the desired pH was prepared and distributed to individual reaction vessels (100 mL brown glass bottles), containing a magnetic stirring bar and 3 mg of IM (if any). The whole solutions were autoclaved at 121 °C with a sterilization time of 15 min using a Systec VE-75 (Systec, Linden). 100 µL of analyte stock solution (100 mg/L in MeOH) was added to the buffer solution for a resulting concentration of 100 µg/L. The reaction vessels were then placed in a climate cabinet at 25 °C in the dark. At each sampling point, a complete vessel was sacrificed for analysis. The first sample of each experiment (d 0) was drawn 30 min after placing the samples in the climate cabinet.

The reaction mixture was filtered through a syringe filter. The filter was washed with 3x10 mL of the buffer solution. Finally, an internal standard was added to the filtrate, which was then stirred for 5 min. An aliquot of 1 mL was analyzed immediately at the end of the experiment, and another 4 mL were retained as a backup sample. Both samples were stored at -20 °C.

The reaction vessels and the filters were also extracted using 3x10 mL methanol to cover the sorbed quantities. In some experiments, this was done separately for the vessel and the filter; in later experiments, the methanol was first poured into the vessel and shaken vigorously, then the same methanol was used to wash the filter. Internal standards were also added to the extracts, and they were stored until analysis at -20 °C.

3.2.2.1 Single-vessel experiments

A simplified experimental set-up was used for the lab-scale experiments by using larger vessels containing 1 L of the reaction mixture, which was sampled repeatedly at defined intervals. However, this layout was not in accordance with OECD method 111 and has severe drawbacks in that microbial contamination of the reaction vessel during sampling was difficult to avoid. Due to inconsistent data the experiments were stopped (data not shown). Thus, all results shown in this report were achieved by using individual vessels for each sampling point.

3.2.2.2 Differences from the standard method

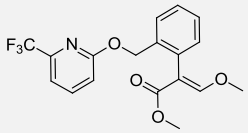
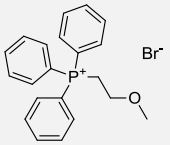
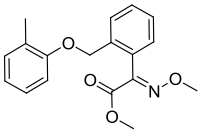
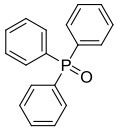
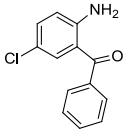
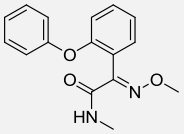
Except for the single-vessel experiments described in the previous section, all experiments followed the standard experimental layout described in OECD method 111 closely, with one important difference: preliminary tests were carried out at three (or more) different pH values, but the main experiments (with added IMs) were only conducted at a pH at which the substances were found to have a half-life of about one to two weeks. At a much longer half-life, degradation would be difficult to observe over the course of an experiment that only lasts about one month. Much shorter half-lives on the other hand would be experimentally more challenging (they require faster sampling intervals so that not all of the analyte is consumed after just one or two sampling points), and they are also less relevant: if a substance is degraded very quickly, a slightly reduction of the rate of degradation does not matter as much as it does in a substance that is already relatively stable.

3.2.3 Available standards

In addition to an external calibration, internal standards were used for quantification of the target substances as well as the hydrolysis products. The surrogate standards were spiked to a sample at the beginning of sample preparation. The concentrations of the analytes were calculated as the ratio of the signal of the analyte and that of the internal standard, which allows to correct losses during sample preparation and storage, as well as to compensate variations in the sensitivity of the measurements.

For each of the selected compound the surrogate standards used for quantification in this study are shown in Table 6.

Table 6: Surrogate standards used for quantification

TFX standards	MCM-TPP standards	Oxazepam standards
 Picoxystrobin Exact Mass: 367.1031 CAS 117428-22-5	 (2-methoxyethyl)tpp bromide Exact Mass: 321.1403 (excluding bromide) CAS 55894-16-1	 Oxazepam-d5 Exact Mass: 291.0823 CAS 65854-78-6
 Kresoxim-methyl Exact Mass: 313.1314 CAS 143390-89-0	 Exact Mass: 278.0861 Triphenylphosphine oxide CAS 791-28-6	 (2-amino-5-chlorophenyl)(phenyl)methanone Exact Mass: 231.0451 CAS 719-59-5
 Metominostrobin Exact Mass: 284.1161 CAS 133408-50-1		

For TFX, an isotope-labelled standard were unfortunately not commercially available. Several suppliers were conducted to purchase TFX-d6, but without success. To amend this, three compounds with a similar chemical structure as TFX were purchased at Sigma-Aldrich and tested for their suitability as surrogate standard of TFX. All of them are also strobilurin class fungicides: picoxystrobin, kresoxim-methyl, and metominostrobin. Picoxystrobin, which has the greatest similarity to TFX, proved to be the most suitable compound for quantification, and ended up being used as the only internal standard. It has physicochemical properties that are reasonably similar to those of TFX, such as a log Kow of 3.6 (TFX: 4.5) and a water solubility of 3.1 mg/L (TFX: 0.6 mg/L).[134, 135] It shares with TFX not only the β-methoxyacrylate moiety that all strobilurins have in common, but also the trifluoromethyl group. TFXA, the main hydrolysis product of TFX was available as an un-labelled standard (Sigma-Aldrich).

Since no isotope-labelled standard was available for MCM-TPP, another TPP salt was used: (2-methoxyethyl)triphenylphosphonium bromide. Values for the physicochemical properties of these phosphonium salts are not readily available, since they are formed as undesired intermediate products and not sold at larger quantities that would require a complete registration. It can however be assumed that the sorption behavior of all TPP salts is dominated by the permanent positive charge. The main hydrolysis product CB-TPP was available as a standard. Triphenylphosphine oxide, another possible result of the hydrolysis of phosphonium salts, was also purchased (both Sigma-Aldrich).

Isotope-labelled oxazepam (oxazepam-d5) was purchased at Sigma-Aldrich. As for the hydrolysis product, (2-amino-5-chlorophenyl)(phenyl)methanone had been acquired, but turned out to be a product of minor importance under the conditions used in this project.

3.2.4 ASE method

In the ASE method used, 1 g of the IMs was filled in extraction cells (7.5 cm, 2 cm diameter) together with inert purified sea sand (Th. Geyer, Renningen). They were heated within 6 min to 120 °C, followed by 15 min of extraction at a pressure of 100 bar via MeOH/H_2O (80/20; v/v) containing 1% of formic acid. This extraction cycle was repeated three times. The volume of the ASE extracts was then reduced in a gentle nitrogen stream to a defined volume and analytes were measured using the methods described below.

3.3 Analytical methods

All samples were analyzed via LC-MS on an LTQ Orbitrap Elite (Thermo Scientific, Bremen, Germany). Separation was achieved using a Hydro-RP column (50x2 mm, 4 µm, Phenomenex, Aschaffenburg) at 30 °C. The chromatographic settings are listed in Table 1-3. The flow rate was 400 µL/min. All analyses were run in positive ionization mode at a resolution of 60,000 for the full scan and 15,000 for subsequent fragmentation experiments to identify hydrolysis products. The injection volume was set at 100 µl. For MCM-TPP, the m/z range was set to 180-1000. For oxazepam and trifloxystrobin, it was set to 180-400 or 180-600, respectively.

With these settings, all of the analytes could be reliably quantified at concentrations of 1 µg/L, corresponding to 1% of the starting concentration used in degradation experiments. With every set of experimental samples, an calibration curve was measured, consisting of samples at known concentrations of 0 µg/L, 1 µg/L, 2 µg/L, 5 µg/L, 10 µg/L, 20 µg/L, 50 µg/L, 100 µg/L, and 150 µg/L.

Table 7: Chromatographic settings for MCM-TPP experiments

time / min	water / %	acetonitrile / %
0	95	5
1.5	95	5
2	65	35
5	65	35
8	40	60
9	40	60
9.2	95	5
12	95	5

Table 8: Chromatographic settings for trifloxystrobin experiments

time / min	water / %	acetonitrile / %
0	60	40
1	60	40
2	30	70
5	10	90
10	10	90
10.5	60	40
15	60	40

Table 9: Chromatographic settings for oxazepam experiments

time / min	water / %	acetonitrile / %
0	80	20
1	80	20
2	40	60
5	20	80
10	20	80
10.5	80	20
15	80	20

3.4 Determination of sediment properties

3.4.1 Particle size distribution

The particle size distribution of the Ehrenbreitstein sediment was conducted by dynamic light scattering using a Beckman Coulter LS 200.

3.4.2 Dry matter

The dry matter was determined by drying samples to constant mass at 105°C in a drying oven. The difference in mass before and after drying can be used to calculate water content and dry matter.

3.4.3 TOC

The TOC content of sediment was determined by burning samples in a purified atmosphere in a Mitsubishi HF-210 and measuring the resulting amount of carbon dioxide (Compact IC pro 881, Metrohm, Herisau, Switzerland).

3.5 Statistical evaluation

Excel 2010 (Microsoft, Redmond, USA) was used for data management and simple calculations. Calculation of kinetic parameters in Excel is possible,[136, 137] but using specialized statistics software is much more comfortable. Therefore, SigmaPlot for Windows Version 13.0 (Systat Software, San Jose, USA) was used as well.

Hydrolysis typically is a first-order reaction, which can be described by the following equation:

$$Y_t = Y_0 \cdot e^{-k \cdot t}$$

$$Y_t: calculated\ concentration\ at\ time\ t, Y_0: calculated\ concentration\ of\ t = 0, k: rate\ constant$$

The parameters Y_0 and k were optimized via non-linear regression by minimizing the root of the sum of the squared differences between experimental data and theoretical curves. For every measured concentration c and the associated reaction time t, the error between c and the corresponding theoret-

ical concentration Y_t is calculated, the errors are squared (to obtain only positive values), and the sum of the squared errors is minimized by iteratively varying Y_0 and k using statistical analysis software.

Half-lives were calculated as:

$$DT_{50} = \frac{\ln(2)}{k}$$

Confidence intervals of k were calculated in SigmaPlot, and confidence intervals of the half-lives were calculated from these using simple rules for the propagation of uncertainty:

$$f(DT_{50}) = \frac{\ln(2)}{(k)^2} f(k)$$

4 Results and discussion

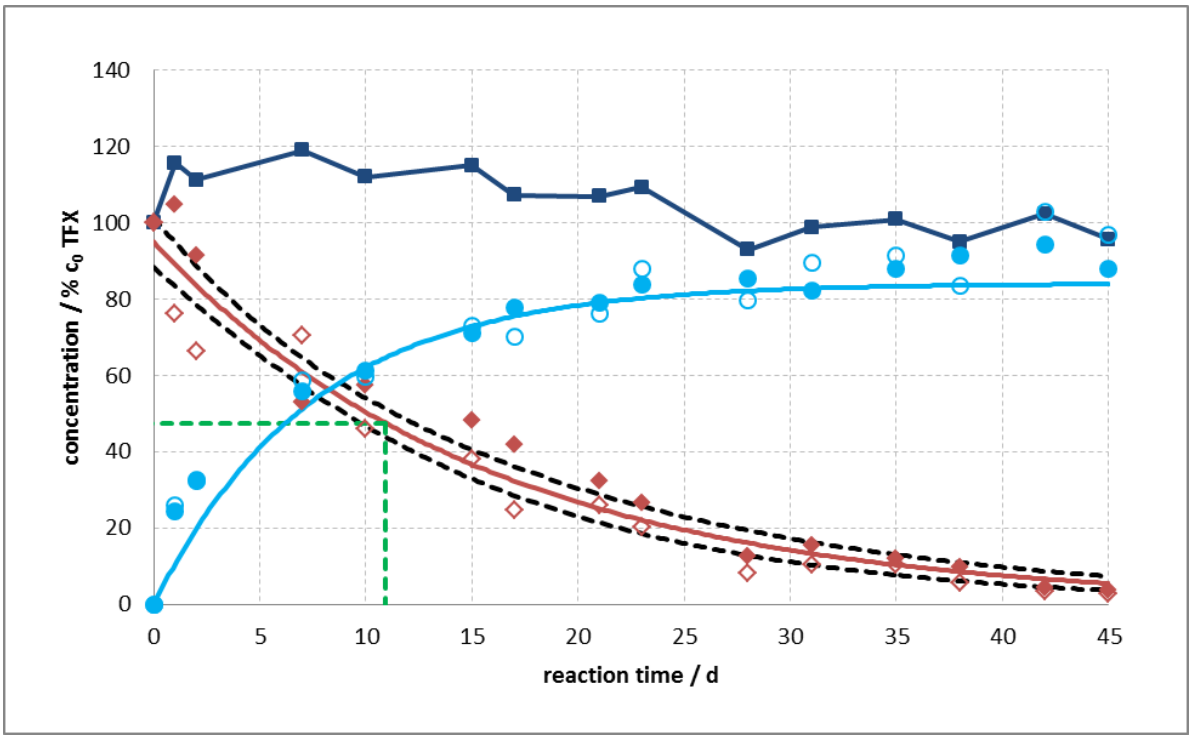
4.1 Main experiments

4.1.1 Trifloxystrobin

It was already known[78] that TFX is stable at pH 4 and still has a half-life of almost 40 days at neutral pH, but is completely degraded within just a few hours at pH 9. We therefore first conducted preliminary experiments at room temperature and pH 7.5, pH 8.0 and pH 8.5. Based on the results of these preliminary experiments, we decided to run the main degradation experiments at pH 7.5 in phosphate buffer. This would allow for a degradation that is fast enough for several half-lives to pass over the course of the experiment, but not so fast that all the TFX is consumed within just a few days. The concentration of the TFX hydrolysis product TFXA in the main experiments was also measured and a mass balance was calculated.

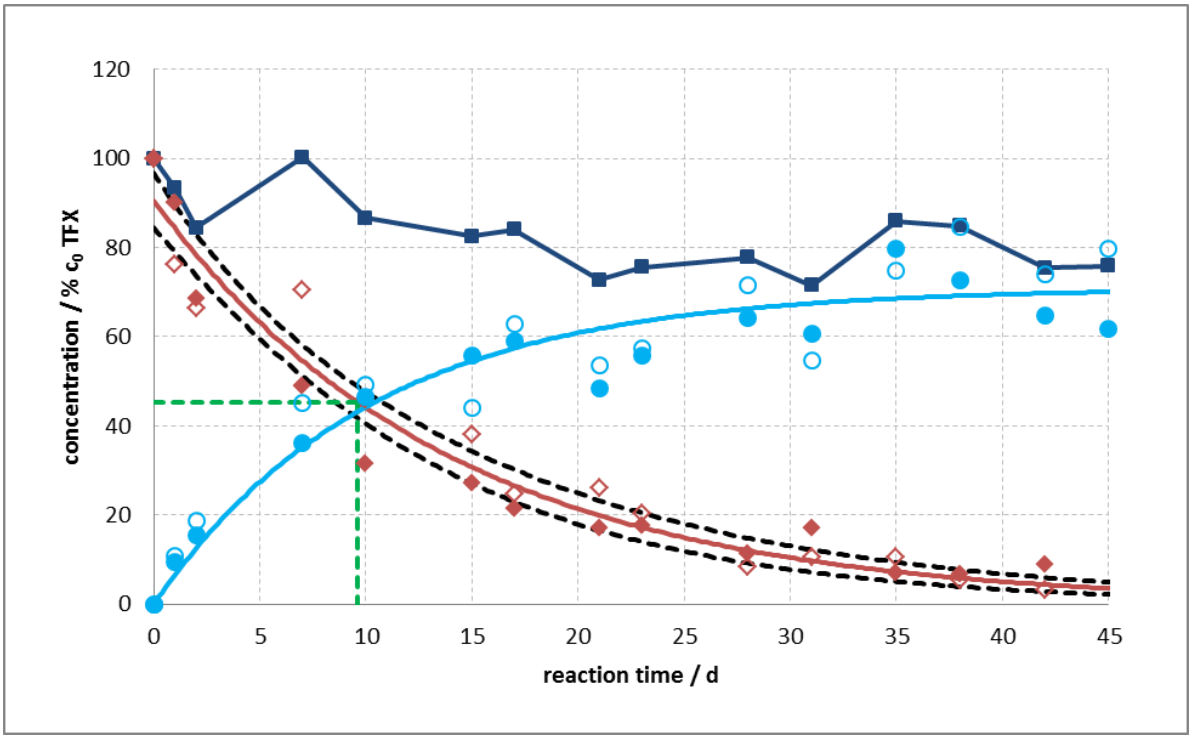
In the experiments without IM (Figure 14) and with sediment (Figure 16), the mass balances were always close to 100%. In the experiment with microplastic (Figure 15), the mass balance dropped down to about 80% early on, but remained at that level for the duration of the experiment, which might be caused by an under-determination of the starting concentration. In the experiments with humic acid on the other hand (Figure 17), the mass balance fluctuated somewhat stronger, with apparent values of up to 133%. The most likely explanation for this is that the concentration of the hydrolysis product (for which an internal standard was not available) was over-estimated. Overall, it is obvious from the mass balance that TFXA is the dominant hydrolysis product of TFX, and that TFXA is not further hydrolyzed.

Figure 14: Degradation of TFX with no IM



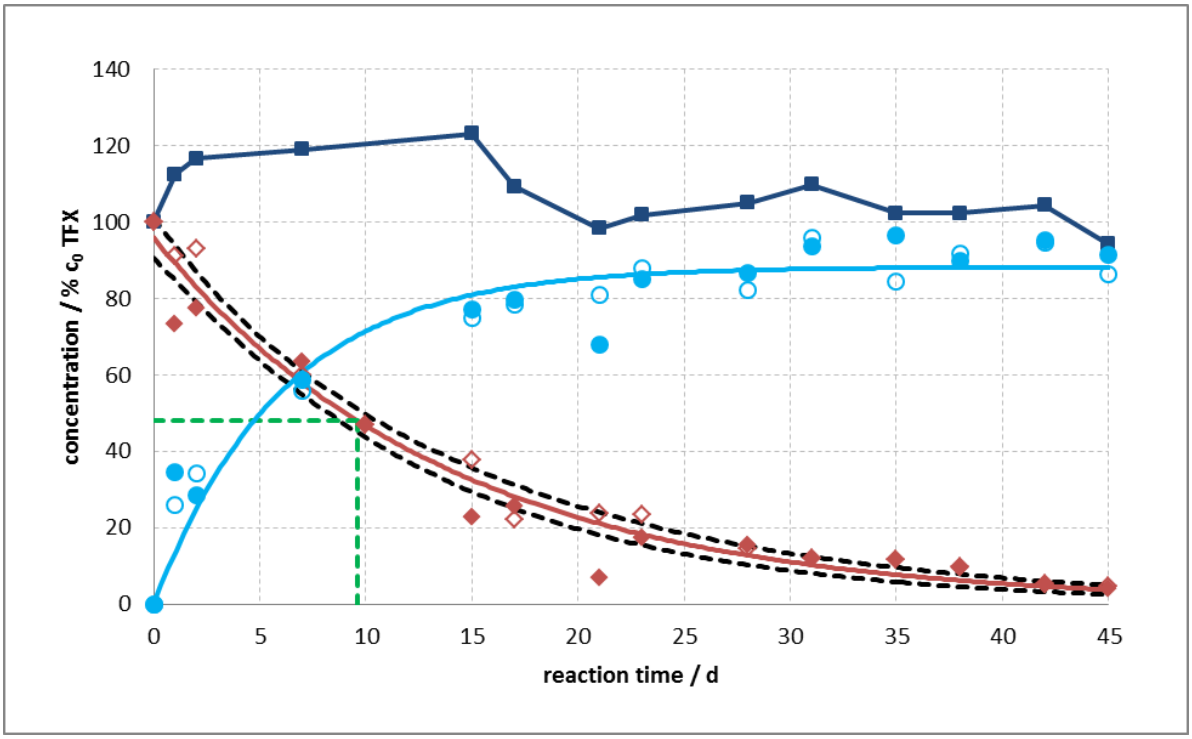
Red diamonds: TFX, light blue circles: TFXA, normalized on the molar mass of TFX (solid symbols: first run, hollow symbols: second run), dark blue squares: sum of TFX and TFXA (averaged for both runs). The black dashed lines represent the 95% CI, the green dashed line shows DT_{50}, pH 7.5.

Figure 15: Degradation of TFX in the presence of microplastics



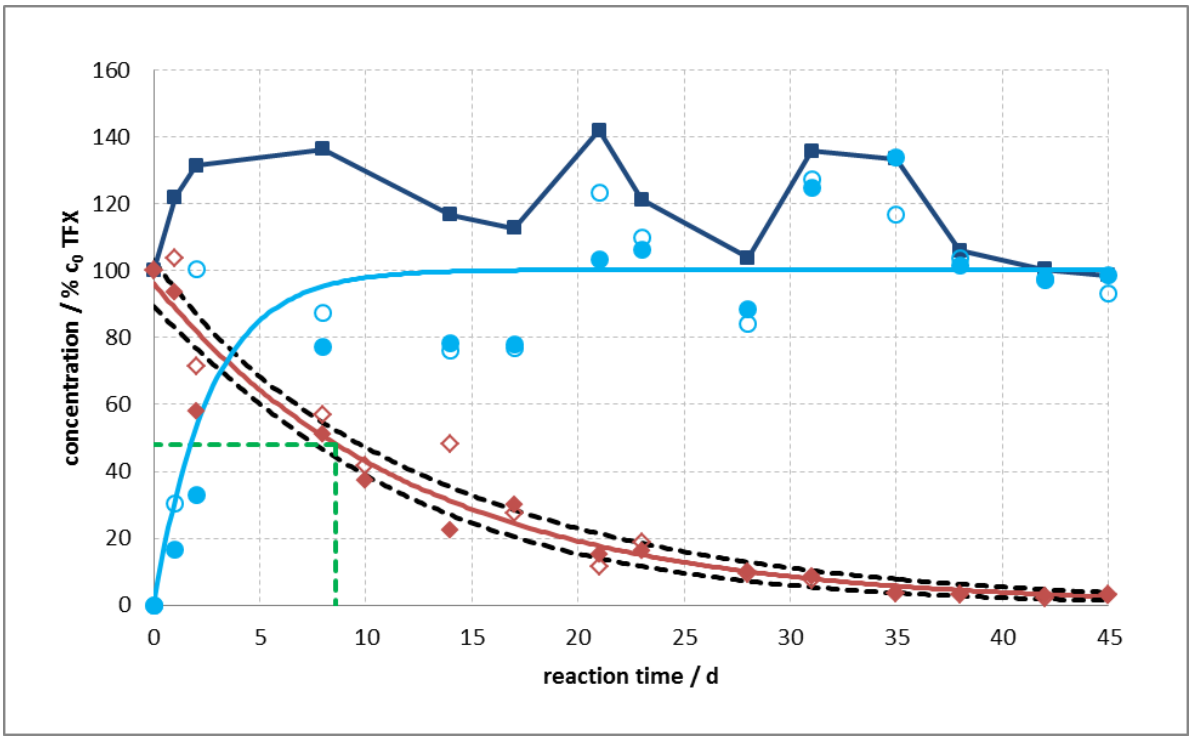
Red diamonds: TFX, light blue circles: TFXA, normalized on the molar mass of TFX (solid symbols: first run, hollow symbols: second run), dark blue squares: sum of TFX and TFXA (averaged for both runs). The black dashed lines represent the 95% CI, the green dashed line shows DT_{50}, pH 7.5.

Figure 16: Degradation of TFX in the presence of sediment particles



Red diamonds: TFX, light blue circles: TFXA, normalized on the molar mass of TFX (solid symbols: first run, hollow symbols: second run), dark blue squares: sum of TFX and TFXA (averaged for both runs). The black dashed lines represent the 95% CI, the green dashed line shows DT_{50}, pH 7.5.

Figure 17: Degradation of TFX in the presence of humic acids



Red diamonds: TFX, light blue circles: TFXA, normalized on the molar mass of TFX (solid symbols: first run, hollow symbols: second run), dark blue squares: sum of TFX and TFXA (averaged for both runs). The black dashed lines represent the 95% CI, the green dashed line shows DT_{50}, pH 7.5.

An overview of the rate constants and half-lives measured for TFX experiments is listed in Table 10. The half-life measured without addition of any IM was slightly higher (10.9 d) than for any of the experiments with addition of IM (ranging from 8.6 d to 9.6 d).

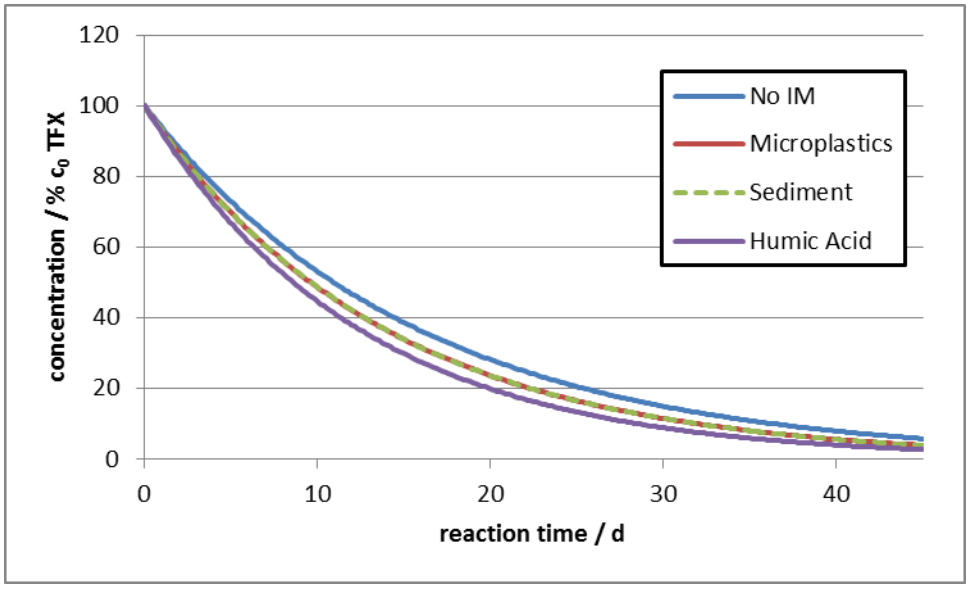
Table 10: Rate constants and half-lives for TFX

	No IM	Microplastics	Sediment	Humic acids
k	**0.063 ± 0.008**	**0.072 ± 0.009**	**0.072 ± 0.080**	**0.081 ± 0.011**
DT_{50}	**10.9 ± 1.4**	**9.6 ± 1.3**	**9.6 ± 1.0**	**8.6 ± 1.2**
k_{log}	**0.073 ± 0.007**	**0.065 ± 0.007**	**0.065 ± 0.007**	**0.085 ± 0.006**
$DT_{50,log}$	**9.5 ± 0.8**	**10.6 ± 1.1**	**10.7 ± 1.1**	**8.2 ± 0.6**

k and DT_{50} determined by non-linear regression of the untransformed data. k_{log} and $DT_{50,log}$ determined from the slope of a logarithmic plot of the concentration over time, pH 7.5 .

Liu *et al.* (2014)[78] report the half-life of trifloxystrobin at 25°C as 866.4 h (or 36.1 d) at pH 7 and 36.3 h (or 1.5 d) at pH 8; this was for experiments which, of course, did not include any IM. This is in good agreement with our finding of a half-life of about 10 d (or 240 h) at pH 7.5.

Figure 18: Direct comparison of degradation of TFX under different conditions.



Calculated curves based on the average of all experiments, based on non-linear regression of the untransformed data. Curves for the degradation in the presence of microplastic (red) and sediment (green) are not identical, but so similar that the green curve has to be represented by a dashed line.

Figure 18 shows a direct comparison of the degradation curves for all TFX experiments.

The observed rate of hydrolysis was slightly lower without the addition of IM, but the confidence intervals still overlap for all experiments. Although there is a tendency that the rate constants were lower with added IM, the difference was statistically not significant. The hypothesis that the IMs significantly reduce the rate of hydrolysis of TFX could therefore not be confirmed. Due to the closed mass balances, it can also be excluded that other transformations products are formed when IM are added.

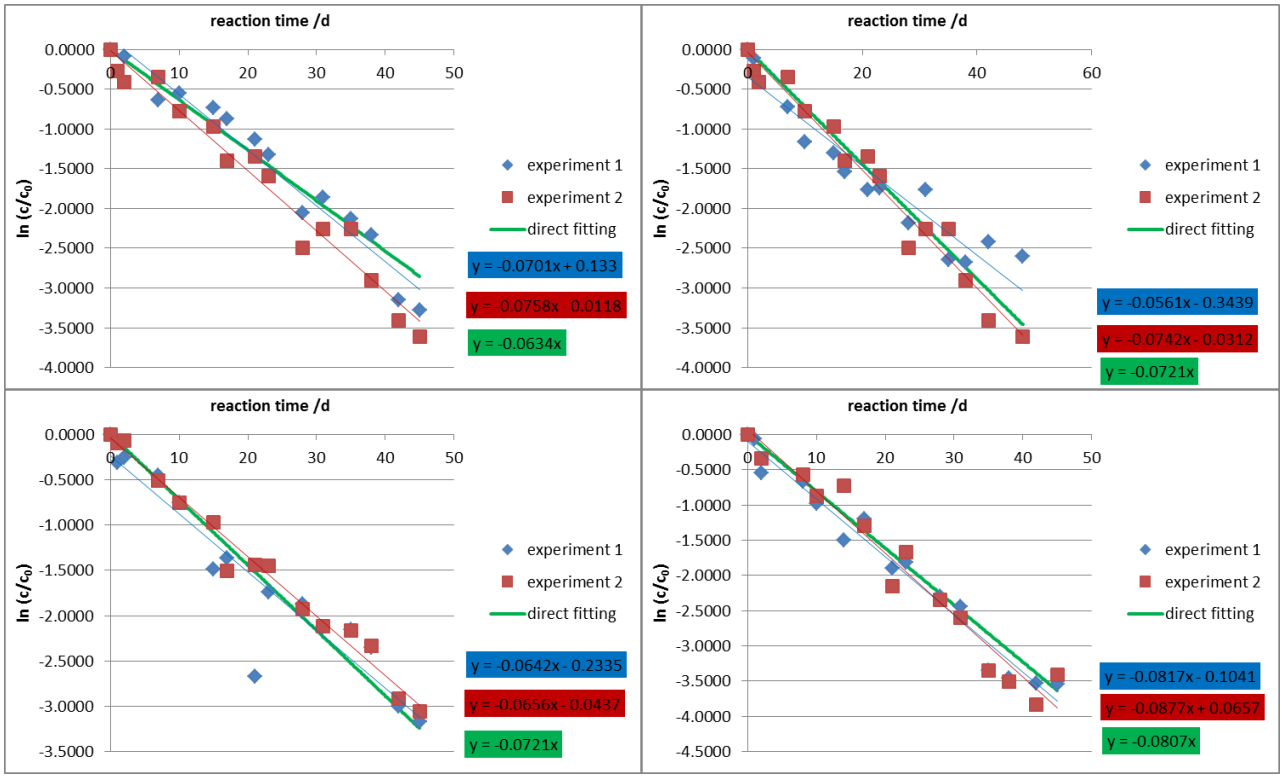
In absolute values, the measured starting concentration for all TFX experiments were very close to each other and to the theoretical concentration, with values of 99.3 µg/L in the experiment without added IM, 95.9 µg/L in the sediment experiment, 100.1 in the experiment with microplastic fibers, and 96.3 µg/L in the experiment with humic acid.

As mentioned in section 1.2, kinetic constants are frequently determined by plotting $\ln([A]_t/[A]_0)$ against the time, which results in a straight line with -k as its slope. For comparison, we also determined k (and the associated DT_{50}) that way. The plots can be seen in Figure 19, the kinetic data are also listed in Table 10.

The kinetic parameters determined this way differ from those determined by non-linear regression by about 10%. It is interesting to note that the order of experiments (if ranked by ascending reaction rate) has changed: while the rate constant for the experiment without added IM is lowest if determined by non-linear regression, k_{log} is lower for the experiments with added microplastic or sediment. This is caused by the fact that the last few data points are slightly below the modelled degradation curve for the experiment in pure water (see Figure 14), but slightly above that line in the microplastics and sediment experiments (see Figure 15 and Figure 16). The influence of these data points is increased in the logarithmic plot, which overvalues samples with low concentrations.

The confidence intervals for the experiment in pure water overlap with those of all other experiments in the logarithmic plot as well. This confirms the conclusion that the employed IMs have no significant effect on the hydrolysis of TFX.

Figure 19: Logarithmic plot of data from TFX experiments



Top row: no IM (left), microplastics (right). Bottom row: sediment (left), humic acid (right).

4.1.2 MCM-TPP

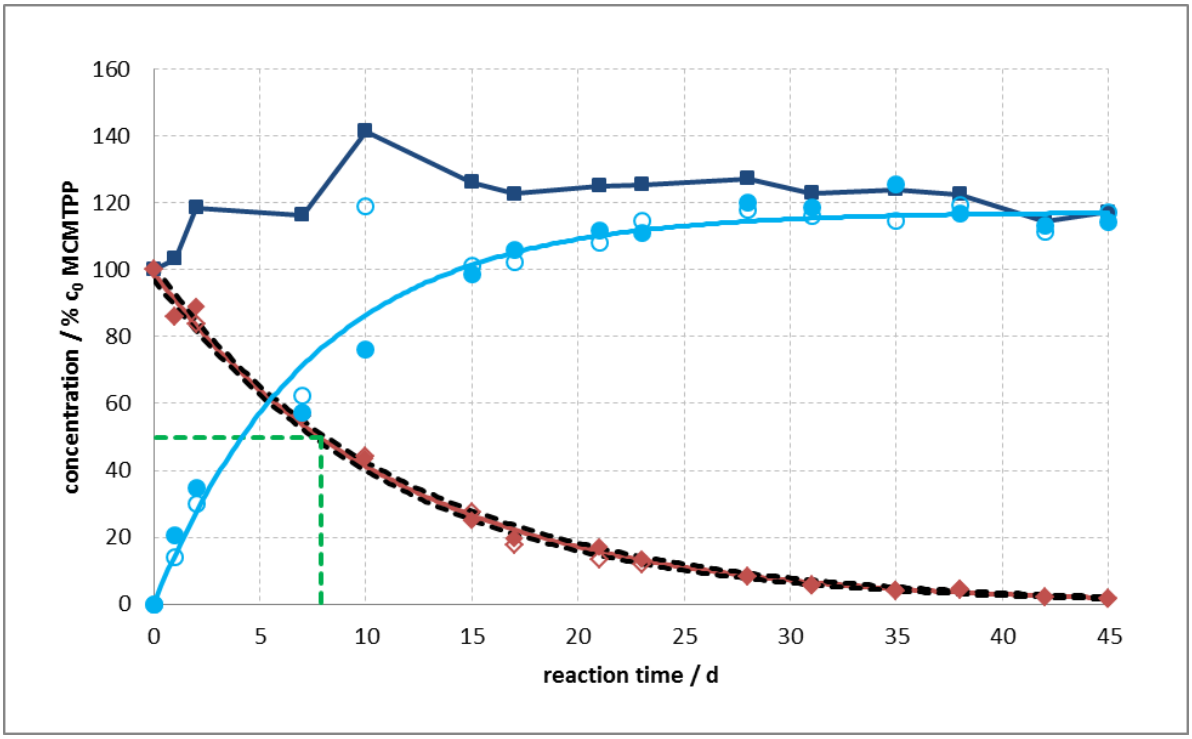
Since MCM-TPP is a positively charged compound, it is clear that its hydrolysis will be catalyzed by hydroxide ions and will thus be faster at elevated pH values. In preliminary experiments, we found that MCM-TPP is almost completely consumed within 3 days at room temperature and neutral pH. To slow down this fast degradation, additional preliminary tests were run at pH 4, pH 5, and pH 6. Degradation in all of these experiments was very slow, with less than 20% hydrolysis observed after two weeks at pH 6. After a final round of preliminary pH tests at pH 6.2, pH 6.5, and pH 6.8, we therefore decided to run the main MCM-TPP experiments in phosphate buffer at pH 6.2. For these main experiments, both MCM-TPP and its expected hydrolysis product carboxymethyl(triphenylphosphonium) (CB-TPP) were available as standards for an external calibration.

In addition, (2-methoxyethyl)triphenylphosphonium was added to the samples during sample preparation and was used as a surrogate standard for MCM-TPP quantification. In the experiments with sediment, samples from d 21 on showed vastly increased peak areas for the IS. Since the reason for this was not found, these samples were quantified using the average peak area of the IS in the samples taken on d 0-17.

In all experiments, it was found that CB-TPP is the dominant hydrolysis product, as can be seen from Figure 20-Figure 23. In the experiment without an added IM, the formation of CB-TPP initially seemed to be much faster than the degradation of MCM-TPP, which caused the mass balance to jump to 120%. This is most likely caused by an over-determination of the CB-TPP concentration due to the lack of an appropriate surrogate standard for CB-TPP yielding to a mass balance of 120 % even at the end of the experiment after 45 days.

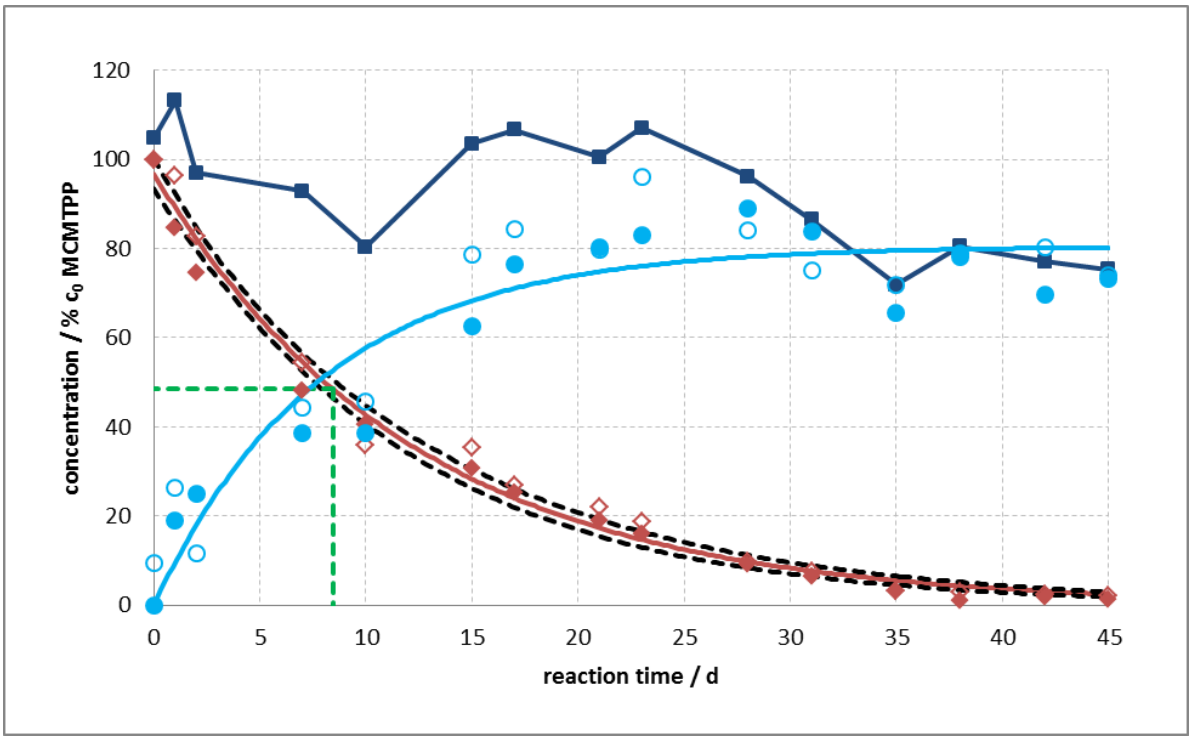
In the experiments with added sediment or humic acids, the sum of MCM-TPP and CB-TPP remained relatively constant over the course of the reaction, with only some inflated values of about 120% of the starting concentration. These are likely also the result of an over-determination of CB-TPP. In the microplastics experiments, the mass balance dropped to about 80% toward the end of the reaction, which was caused by an apparent decrease of the CB-TPP concentrations. No such decrease was observed in the other experiments. It is possible that some of the CB-TPP formed was slowly sorbed to the microplastics fibers. However, this effect was small and may also be caused by random fluctuations in the measurements of CB-TPP.

Figure 20: Degradation of MCM-TPP with no IM



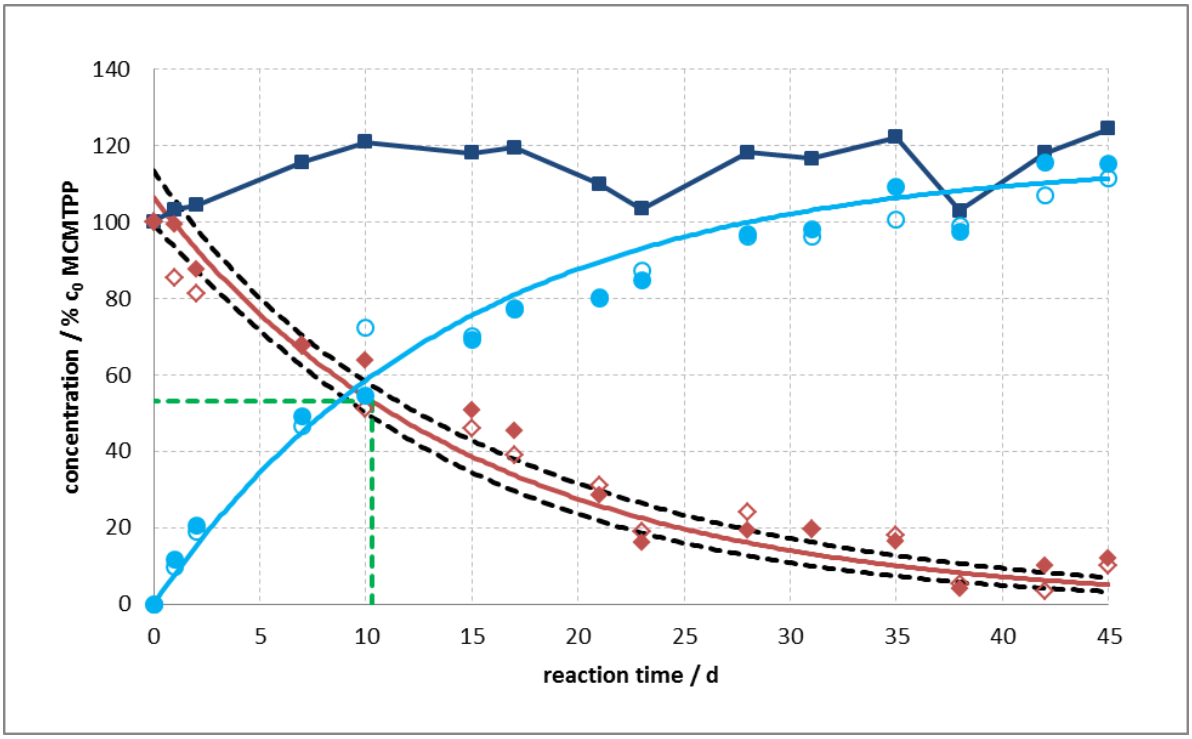
Red diamonds: MCM-TPP, light blue circles: CB-TPP, normalized on the molar mass of MCM-TPP (solid symbols: first run, hollow symbols: second run), dark blue squares: sum of MCM-TPP and CB-TPP (averaged for both runs). The black dashed lines represent the 95% CI, the green dashed line shows DT_{50}.

Figure 21: Degradation of MCM-TPP in the presence of microplastics



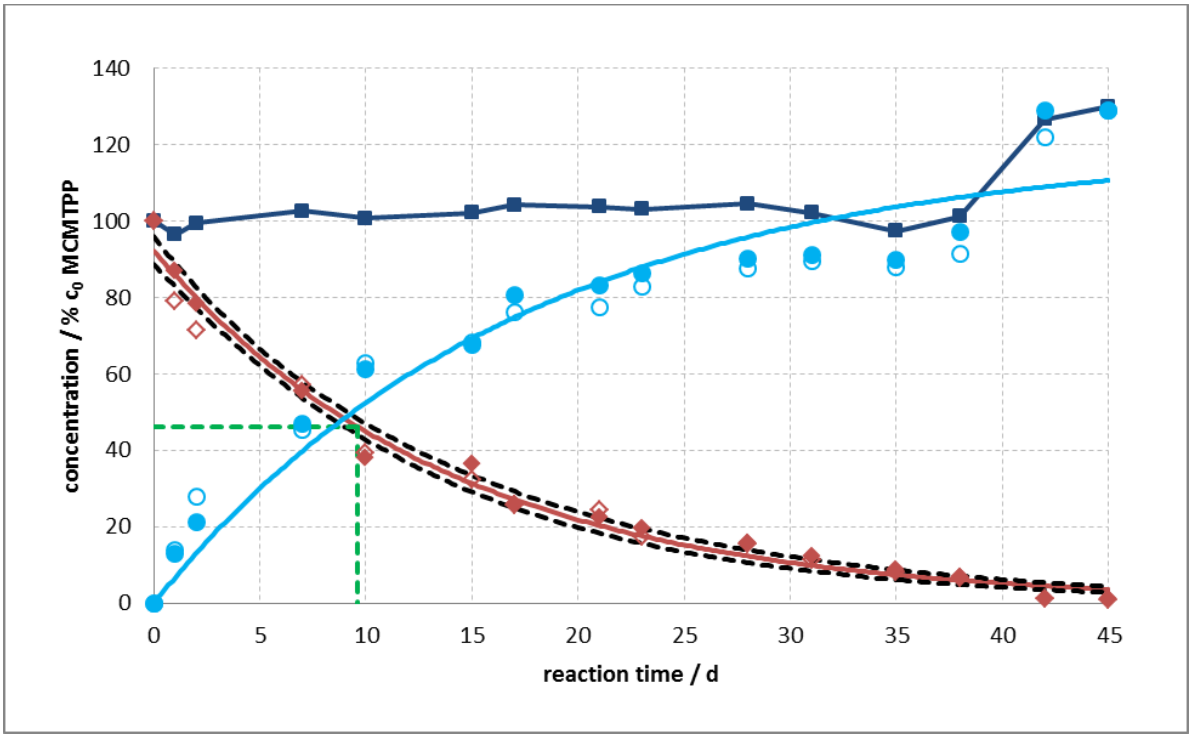
Red diamonds: MCM-TPP, light blue circles: CB-TPP, normalized on the molar mass of MCM-TPP (solid symbols: first run, hollow symbols: second run), dark blue squares: sum of MCM-TPP and CB-TPP (averaged for both runs). The black dashed lines represent the 95% CI, the green dashed line shows DT_{50}.

Figure 22: Degradation of MCM-TPP in the presence of sediment



Red diamonds: MCM-TPP, light blue circles: CB-TPP, normalized on the molar mass of MCM-TPP (solid symbols: first run, hollow symbols: second run), dark blue squares: sum of MCM-TPP and CB-TPP (averaged for both runs). The black dashed lines represent the 95% CI, the green dashed line shows DT_{50}. MCM-TPP concentrations from d21 on calculated without the use of an internal standard.

Figure 23: Degradation of MCM-TPP with in the presence of humic acids



Red diamonds: MCM-TPP, light blue circles: CB-TPP, normalized on the molar mass of MCM-TPP (solid symbols: first run, hollow symbols: second run), dark blue squares: sum of MCM-TPP and CB-TPP (averaged for both runs). The black dashed lines represent the 95% CI, the green dashed line shows DT_{50}.

In the experiment with added humic acids, the last two samples in both experimental runs differed clearly from the previous samples: they contained slightly lower amounts of MCM-TPP than expected, and clearly increased amounts of CB-TPP, resulting in an apparent mass balance of about 120%. It is possible that these observations were caused by an accelerated hydrolysis actually occurring in these samples, e.g. due to a slightly increased temperature in the climate cabinet after d 38. These points might be excluded as outliers, but this proved to have only a negligible effect on the calculated value of k. (0.071 ± 0.006 rather than 0.072 ± 0.006)

In addition to CB-TPP, no other quantitatively significant hydrolysis product can be expected, and in fact, the only further compound detected in any of the samples was triphenylphosphine oxide, which is discussed in the following section.

An overview of the kinetic data from MCM-TPP experiments are listed in Table 11. The half-life was slightly lower (7.8 d) in the experiment without added IM than in any of the other experiments (8.5 d to 10.3 d).

Table 11: Rate constants and half-lives for MCM-TPP

	No IM	Microplastics	Sediment	Humic acids
k	**0.089 ± 0.004**	**0.082 ± 0.006**	**0.068 ± 0.009**	**0.072 ± 0.006** **0.071 ± 0.006***
DT_{50}	**7.8 ± 0.4**	**8.5 ± 0.6**	**10.3 ± 1.3**	**9.6 ± 0.7** **9.7 ± 0.8***
k_{log}	**0.095 ± 0.010**	**0.095 ± 0.008**	**0.061 ± 0.009**	**0.088 ± 0.010** **0.064 ± 0.009***
$DT_{50,log}$	**7.3 ± 0.3**	**7.3 ± 0.6**	**11.4 ± 1.7**	**10.9 ± 1.6***

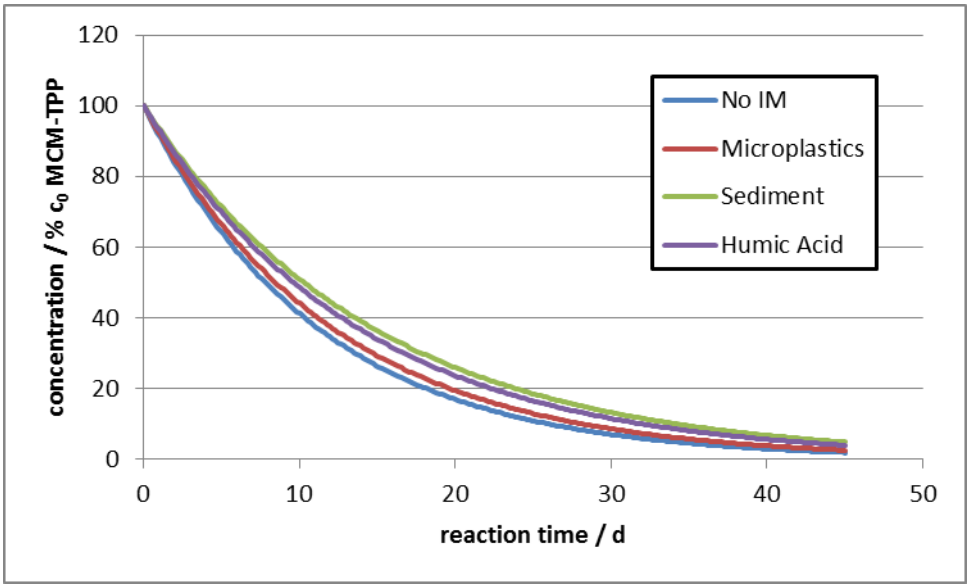
k and DT_{50} determined by non-linear regression of the untransformed data. k_{log} and $DT_{50,log}$ determined from the slope of a logarithmic plot of the concentration over time, (*) without outliers.

For the experiments without added IM on the one hand and the experiments with added sediment or humic acid on the other hand, there was no overlap of the 95% CI. This suggests that at least sediments and humic acids might have a decreasing effect on the hydrolysis rate of MCM-TPP. However, the increase of the rate constants for these two IMs was very similar and overlapped with the (insignificant) increase in the experiments with microplastics additions. This suggests that the inhibiting effect on the hydrolysis of MCM-TPP was quite small. A difference in the formed TPs was not observed.

As discussed in section 4.1.1, the experiments were also evaluated by plotting the logarithmic concentrations against the reaction time (see

Figure 25). The kinetic parameters derived from these plots were once again very similar to the parameters obtained by non-linear regression of the untransformed data, with the exception of the experiment with add humic acids. In this case, the last two data points from both runs of the experiment are somewhat lower than expected. This is obvious in the logarithmic plot, but difficult to see in the direct plot (see Figure 23). It might be best to exclude these points as outliers. If, however, they are included in the calculations, they strongly influence the kinetic constant derived from the logarithmic plot of the data (because they are data points at low concentrations), but barely matter for the constant obtained from non-linear regression.

Figure 24: Direct comparison of degradation of MCM-TPP under different conditions.



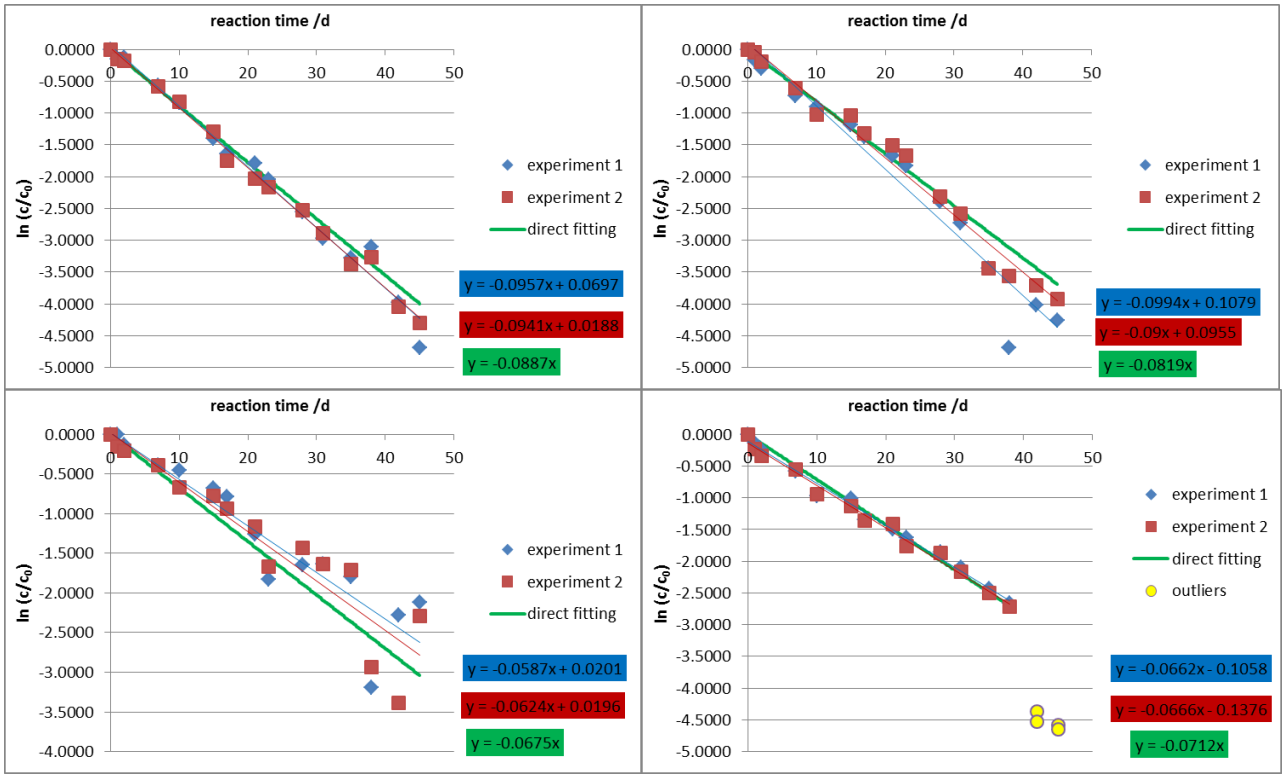
Calculated curves based on the average of all experiments, based on non-linear regression of the untransformed data.

This highlights advantages and disadvantages of the logarithmic plot beyond its ease of use: it makes it easier to judge whether a degradation actually follows first order kinetics, and it allows for an easier identification of potential outliers; but it also means that the calculated kinetic parameters are strongly influenced even by small variations in experimental data measured at low concentrations.

If the outliers are excluded, both the sediment and the humic acids experiment yield significantly decreased rate constants. This confirms the conclusion that sediment and humic acids may have an effect on the hydrolysis of MCM-TPP, but it also illustrates that this effect is relatively small.

A direct comparison of the degradation curves of all experiments with MCM-TPP can be seen in Figure 24. In absolute values, the measured starting concentration for three of the MCM-TPP experiments were close to each other and to the theoretical concentration, with values of 106.0 µg/L in the experiment without added IM, 108.5 µg/L in the sediment experiment, and 96.0 µg/L in the experiment with humic acid. In the experiment with added microplastic fibers, the measured starting concentration was somewhat lower at 88.6 µg/L. Since this fit very well with the other sampling points in this experiment as well as with the measured values for the hydrolysis product (which were also lower in absolute values in this experiment than in any of the others), it seems unlikely that this was the result of sorption to the microplastic. It is more convincing to assume that a slightly smaller amount of analyte was actually used in the preparation of the samples for this experiment, or that the quantification of the analyte was too low, for instance due to a bad calibration curve or sensitivity problems of the instrument.

Figure 25: Logarithmic plot of data from MCM-TPP experiments

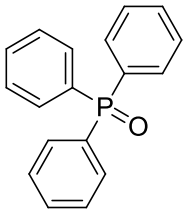


Top row: no IM (left), microplastics (right). Bottom row: sediment (left), humic acid (right).

4.1.2.1 TPP oxide

In addition to the expected hydrolysis product, only one other compound was found in samples from the MCM-TPP experiments: triphenylphosphine oxide (TPP oxide, structure shown in Figure 26). This compound was available as an external standard, so concentrations could also be determined.

Figure 26: Chemical structure of TPP oxide



Chemical Formula: $C_{18}H_{15}OP$
Exact Mass: 278.0861
TPP oxide

In the experiments without IM, around 20 µg/L of TPP oxide were determined toward the end of the reaction period. However, it was found that TPP oxide was already present as a contamination in the MCM-TPP standard, and most of the TPP oxide (around 70%) was already detected at the beginning of the experiment.

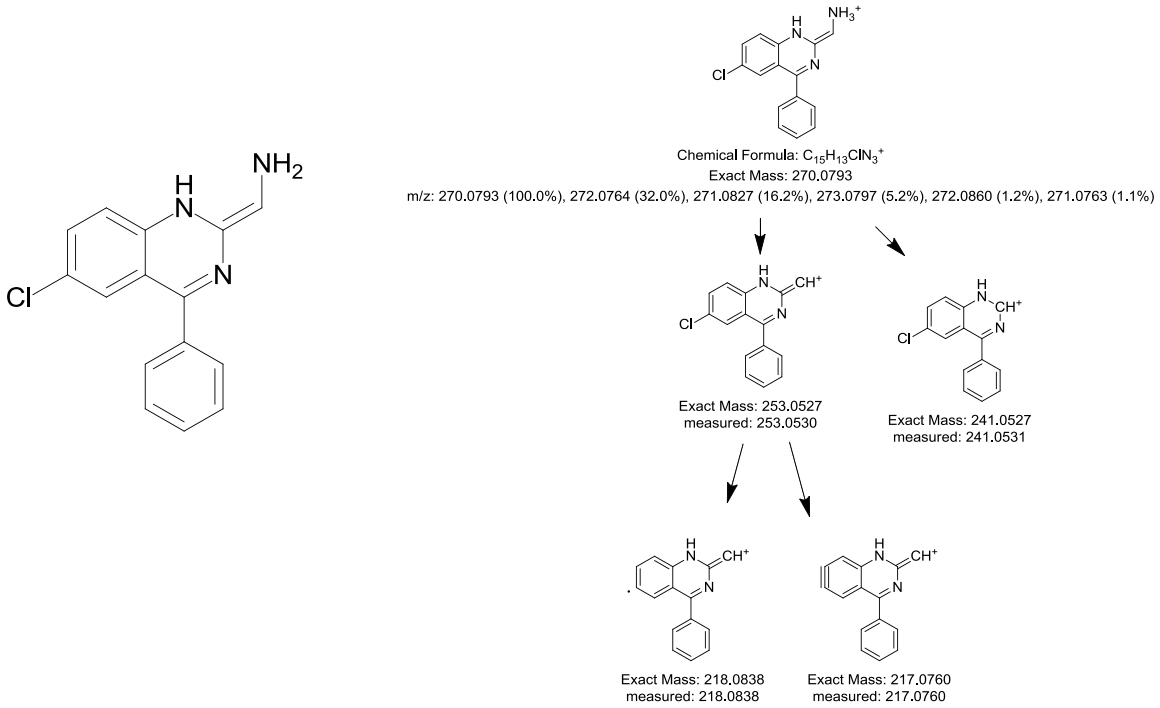
It is well known that hydrolysis of phosphonium compounds can result in the formation of phosphine oxide,[85, 138] and it seems plausible that this also occurred in our experiments to a minor extent. Because of the presence of TPP oxide in the MCM-TPP standard however, no further attempt to quantify TPP oxide in other experiments was undertaken.

4.1.3 Oxazepam

Preliminary tests at pH 4, pH 7, and pH 9, conducted at a temperature of 50 °C, showed that hydrolysis was quite slow at neutral pH (with only about 50% degradation within 4 days), and faster at pH 4 and pH 9, with about 90% degradation at pH 4 and about 95% degradation at pH 9. Since that degradation is overall relatively slow, we decided to conduct the main experiment series at pH 9, where the degradation was fastest.

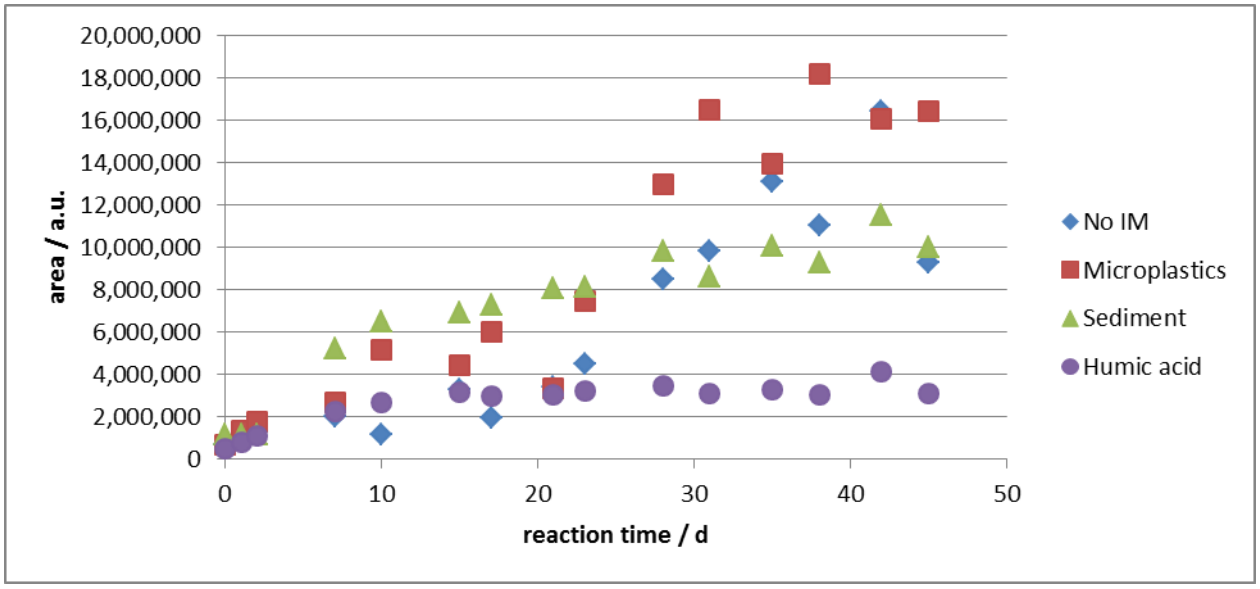
The hydrolysis of oxazepam is more complex than that of the other analyzed compounds, because both the amide and the imine function can be hydrolyzed. When this happens, the final product is (2-amino-5-chlorophenyl)(phenyl)methanone. However, it turned out that the buffer used in our experiment interfered with the formation of that transformation product in an unexpected manner. A glycine-NaOH buffer was used, which seemed to be a good choice since the side chain cleaved from oxazepam also is a glycine derivative. However, the glycine from the buffer reacted with one of the intermediate hydrolysis products to form the quinazoline derivative shown in Figure 27. The fragmentation pattern of the product that was used to suggest the structure is also shown in that figure.

Figure 27: Suggested hydrolysis product (in the presence of glycine) and its fragmentation pattern



Since no standard of this compound was available, an accurate quantification of this transformation product was impossible. A comparison between the different experiments showed that a similar formation was observed in experiments without IM and with the addition of sediment and microplastic fibers. However, in experiments with humic acids, after 10 days no further formation of the quinazoline derivative occurred although hydrolysis of oxazepam still took place; while such a plateau was not found in any of the other experiments. If another transformation product was formed or if sorption effects are causing this result cannot be concluded without further experiments, although no other products were detected in any of the samples.

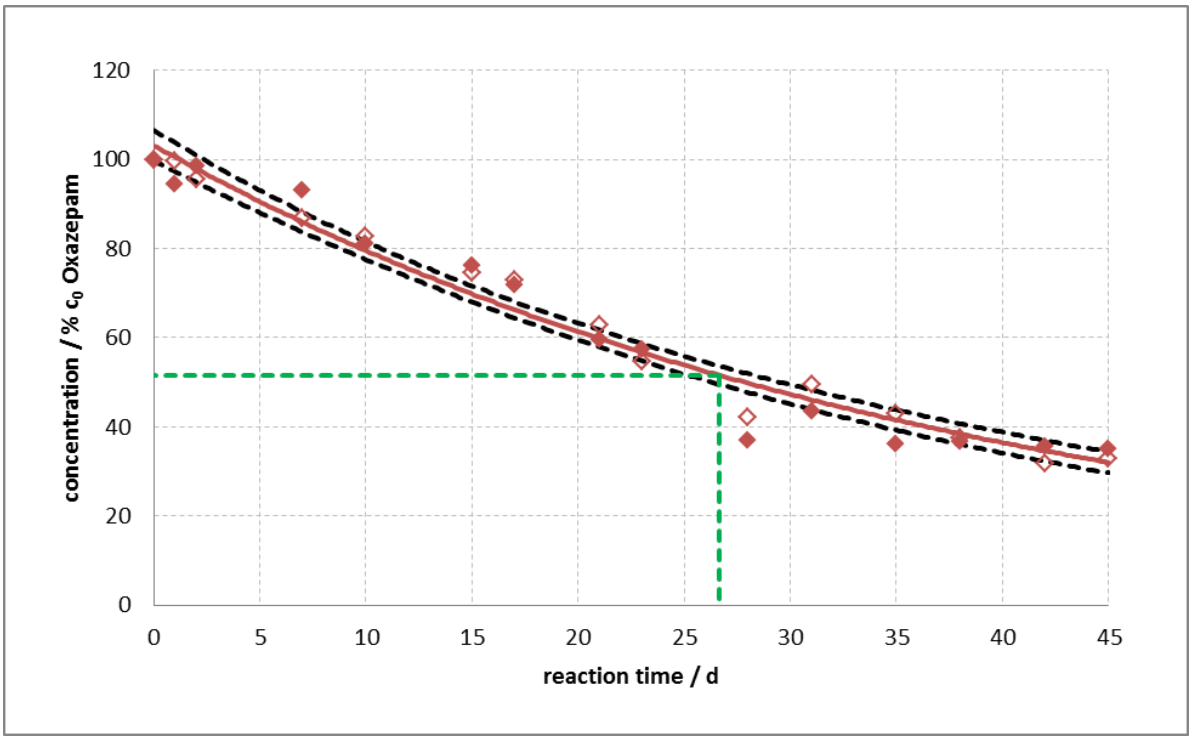
Figure 28: Formation of the oxazepam hydrolysis product



Peak areas plotted against the reaction time.

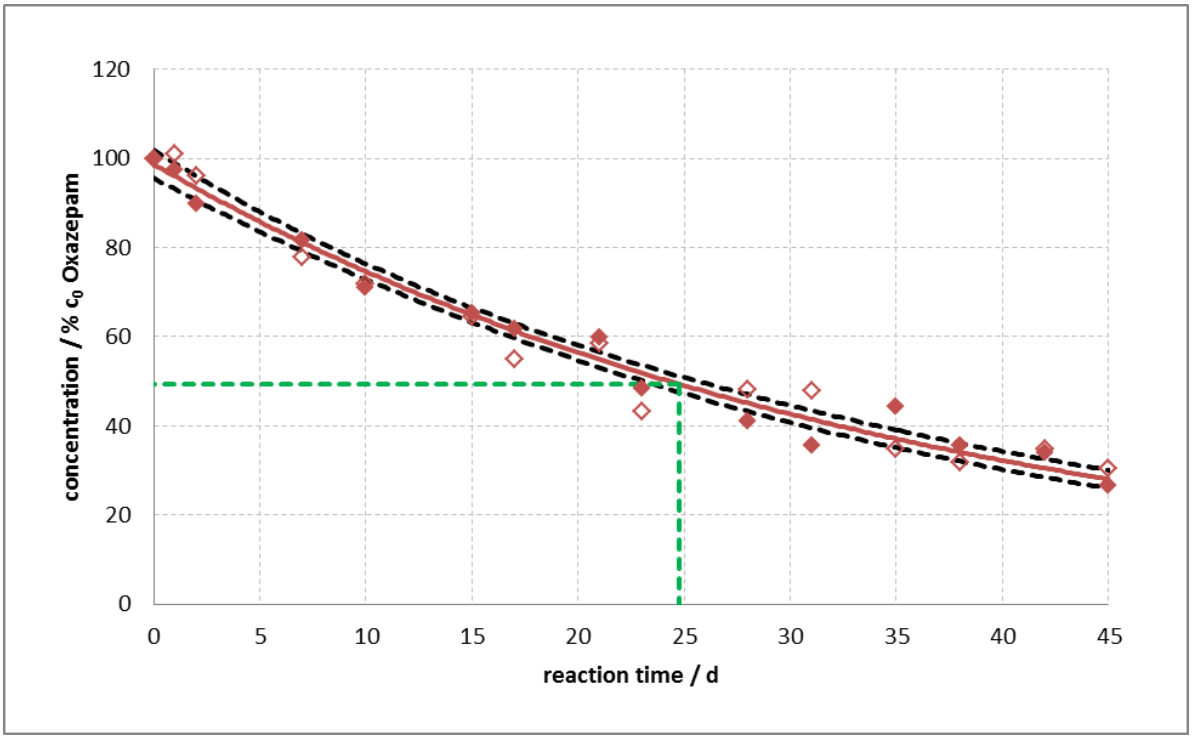
Graphs for the hydrolysis of oxazepam itself can be found in Figure 29-32.

Figure 29: Degradation of oxazepam with no IM



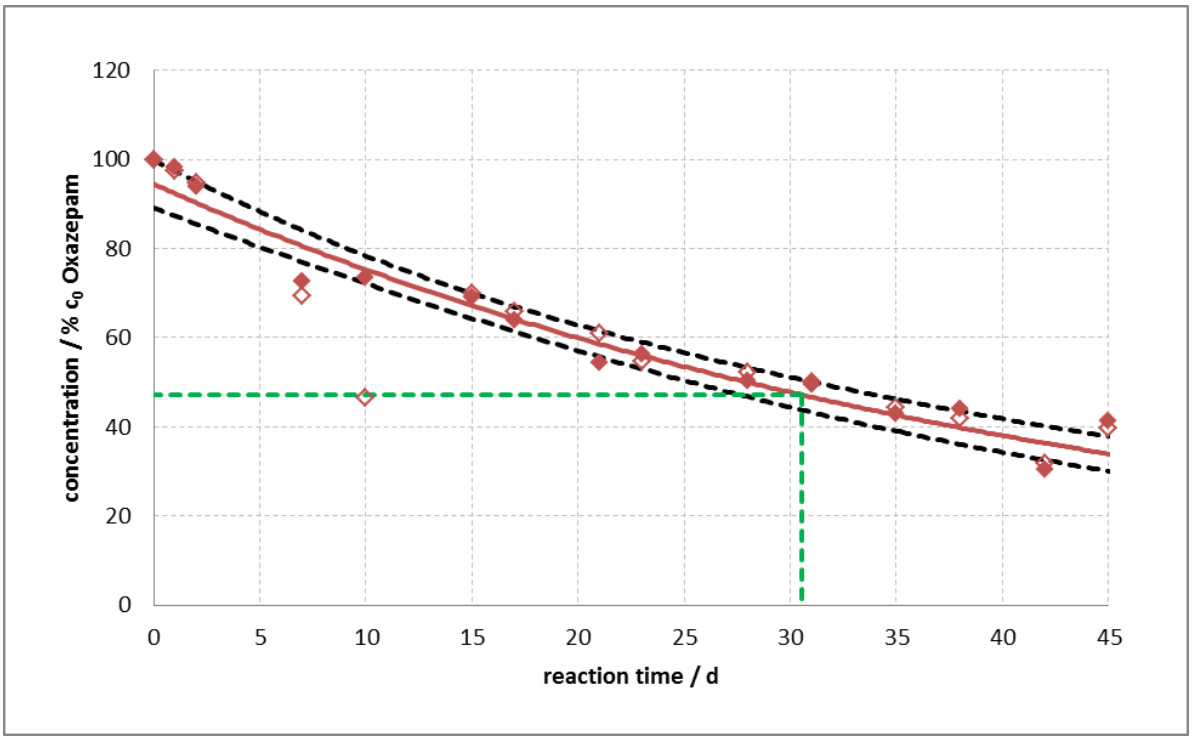
Red diamonds: oxazepam. (solid symbols: first run, hollow symbols: second run). The black dashed lines represent the 95% CI, the green dashed line shows DT_{50}.

Figure 30: Degradation of oxazepam in the presence of microplastics



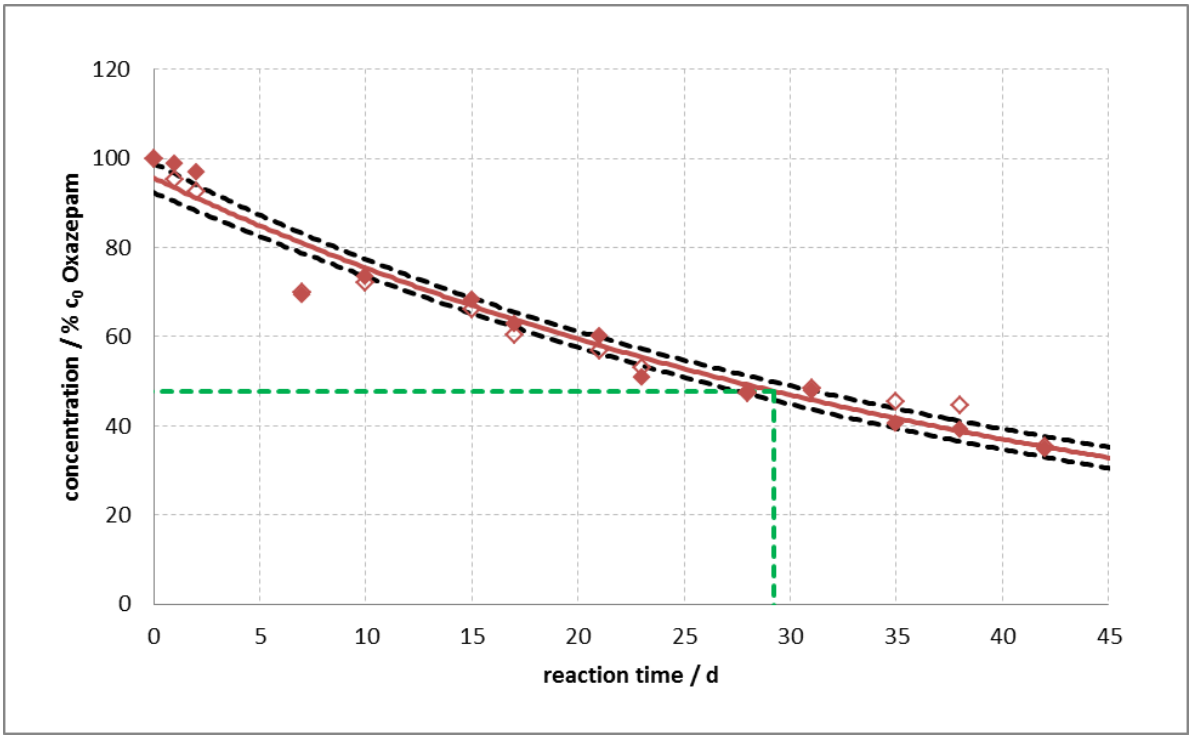
Red diamonds: oxazepam. (solid symbols: first run, hollow symbols: second run). The black dashed lines represent the 95% CI, the green dashed line shows DT_{50}.

Figure 31: Degradation of oxazepam in the presence of sediment



Red diamonds: oxazepam. (solid symbols: first run, hollow symbols: second run). The black dashed lines represent the 95% CI, the green dashed line shows DT_{50}.

Figure 32: Degradation of oxazepam in the presence of humic acids



Red diamonds: oxazepam. (solid symbols: first run, hollow symbols: second run). The black dashed lines represent the 95% CI, the green dashed line shows DT_{50}.

An overview of the kinetic data found in oxazepam experiments can be found in Table 12. The half-life was somewhat higher (26.7 d) in the experiment without added IM than in the experiment with added microplastic fibers (24.8 d), but lower than in any of the other experiments (29.2 d to 30.5 d). The differences were statistically not significant to confirm our hypothesis that the IMs significantly reduce on the rate of hydrolysis of oxazepam. Due to the missing mass balances, it cannot be excluded that other transformations products are formed when IM are added. However, no such products were detected in any experiment, so it seems unlikely that any further product was formed in appreciable amounts.

Table 12: Rate constants and half-lives for oxazepam

	No IM	Microplastics	Sediment	Humic acids
k	**0.026 ± 0.002**	**0.028 ± 0.002**	**0.023 ± 0.003**	**0.024 ± 0.002**
DT_{50}	**26.7 ± 2.1**	**24.8 ± 1.8**	**30.5 ± 4.4**	**29.2 ± 2.5**
k_{log}	**0.027 ± 0.002**	**0.027 ± 0.003**	**0.021 ± 0.003**	**0.022 ± 0.002**
$DT_{50,log}$	**26.0 ± 2.1**	**25.7 ± 2.0**	**32.4 ± 4.7**	**31.4 ± 2.4**

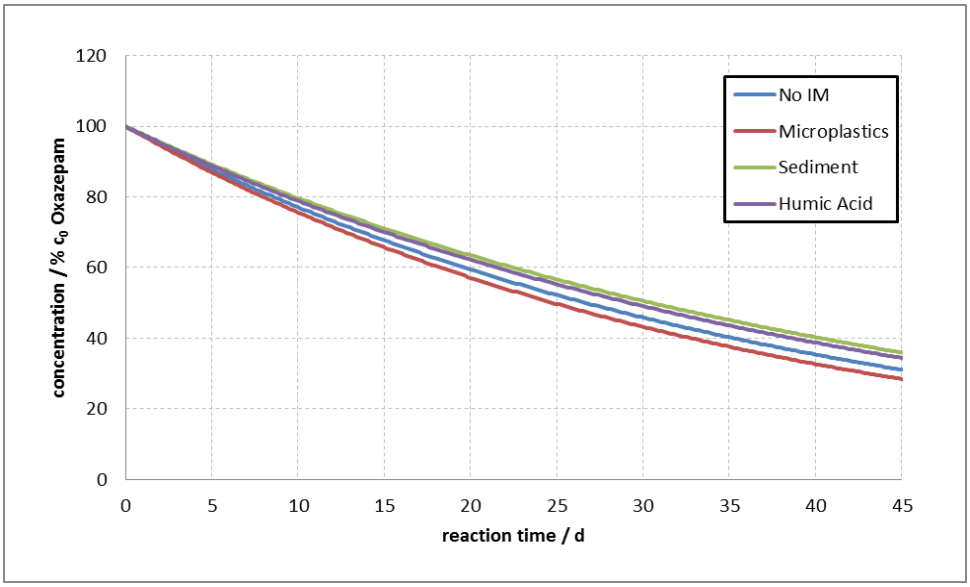
k and DT_{50} determined by non-linear regression of the untransformed data. k_{log} and $DT_{50,log}$ determined from the slope of a logarithmic plot of the concentration over time.

In absolute values, the measured starting concentration for all oxazepam experiments were very close to each other, although they were somewhat higher than the intended concentration, with values of 114.6 µg/L in the experiment without added IM, 117.4 µg/L in the microplastics experiment, 116.6 in the experiment with sediment, and 113.7 µg/L in the experiment with humic acid. Since these values are all in such good agreement, the explanation for the overquantification is almost certainly that the

concentration in the parent solution was too high or the concentration in the calibration curve was too low.

The differences from kinetic parameters derived from a logarithmic plot of the data (see Figure 34) and parameters obtained by non-linear regression were relatively small in all cases. However, they were large enough to cause a significant difference in the half-life without IM and with added humic acids. The mean value for the half-life with added sediment was even larger, but the difference in this was still not significant because of the larger uncertainty. A graphical comparison of the degradation curves for all oxazepam experiments is shown in Figure 33.

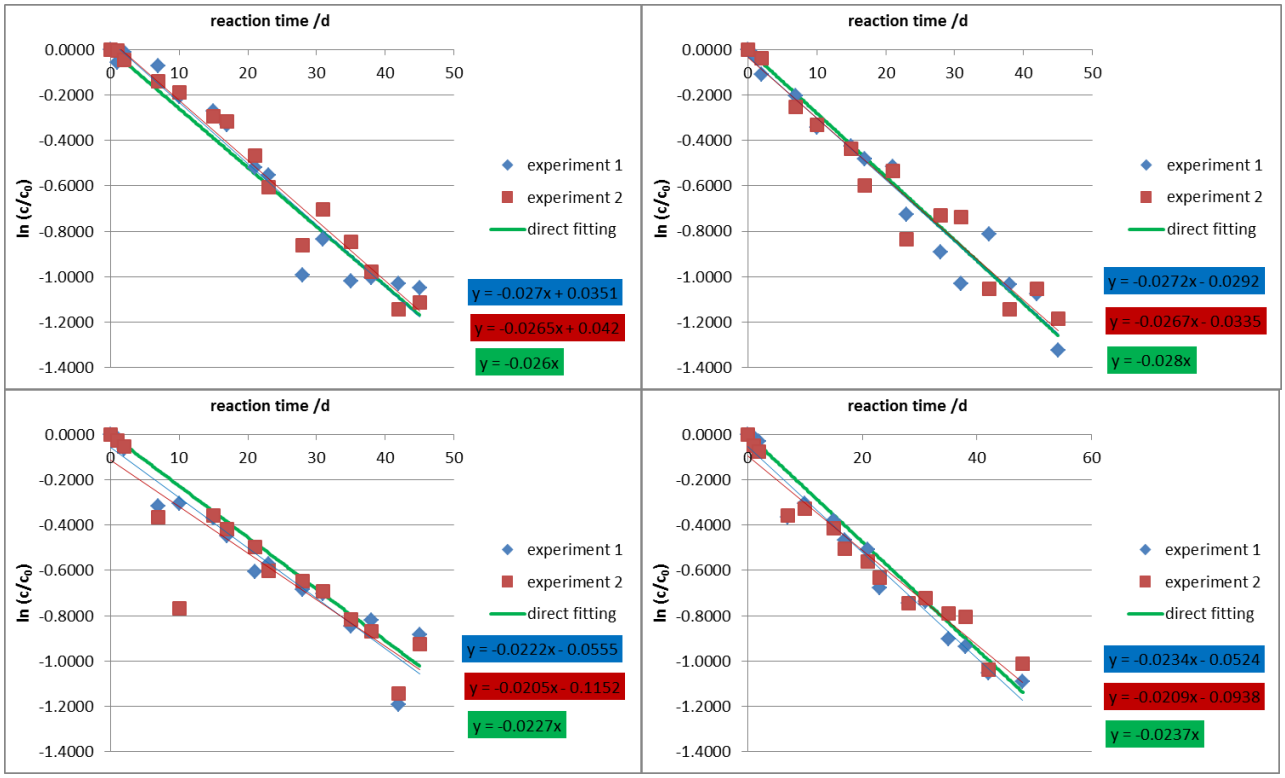
Figure 33: Direct comparison of degradation of oxazepam under different conditions.



Calculated curves based on the average of all experiments, based on non-linear regression of the untransformed data.

Parameters derived from non-linear regression should be more accurate, since it actually minimizes the errors between experimental data and modelled values rather than the logarithm of these errors. In this case, this probably means that the IMs had no effect on the rate of hydrolysis of oxazepam, although a small decreasing effect caused by humic acids or sediment seems possible.

Figure 34: Logarithmic plot of data from oxazepam experiments



Top row: no IM (left), microplastics (right). Bottom row: sediment (left), humic acids (right).

4.2 Losses by sorption

In order to determine sorbed quantities of the analytes, reaction vessels and filters in all experiments were extracted using methanol after filtering the reaction mixtures, as described in section 3.2. These methanol extracts were analyzed by the same detection methods as used for the filtrates. However, analytes and their hydrolysis products were only sporadically detected in these extracts, and their concentrations were very low in all experiments, never exceeding 1% of the original analyte concentration (data not shown).

We also conducted an experiment to estimate the minimum amount of IM at which sorption of the analytes would become problematic. To this end, we added increasing amounts of IM to solutions containing 100 µg/L analyte, and withdrew samples from the aquatic phase approximately after 30 min of vigorous stirring. At the end of the experiment, the IM (30 g/L) was removed from the remaining reaction mixture, washed with buffer solution, and subsequently extracted using ASE. The amount of analyte found in these extracts was compared with the amount that had disappeared from the water phase.

This test was conducted at acidic pH to slow down the hydrolysis of MCM-TPP (which is only catalyzed by OH^-, since the molecule itself is cationic) so that no significant hydrolysis would occur over the course of the experiment. This was verified by determining the amount of hydrolysis product in the samples, which never exceeded 0.81% of the starting amount of MCM-TPP.

Table 13: Recovery rates (%) of MCM-TPP in the aqueous phase with different amounts of IM

MCM-TPP	Sediment pH 4.0	Sediment pH 6.2	Microplastics pH 4.0	Microplastics pH 6.2
0 mg/L IM	99 - 101	97 - 103	100	89 - 111
30 mg/L IM	78 - 79	109 - 115	95 - 103	96 - 97
300 mg/L IM	18 - 20	75 - 82	103 - 104	96 - 104
3 g/L IM	8 - 9	3 – 4	103 - 104	91 - 101
30 g/L IM	3	<1	102 - 103	85 - 101

All numbers in % of the mean concentration found with no IM. For comparison: the main experiments were carried out at pH 6.2 with 30 mg/L IM.

Table 14: Recovery rates (%) of TFX in the aqueous phase with different amounts of IM

TFX	Sediment pH 7.0	Microplastics pH 7.0
0 mg/L IM	95 - 106	90 - 106
30 mg/L IM	94 - 111	91 - 117
300 mg/L IM	98 - 101	104 - 115
3 g/L IM	70 - 77	87 - 119
30 g/L IM	27 - 31	55 - 60

All numbers in % of the mean concentration found with no IM.

Table 15: Recovery rates (%) of oxazepam in the aqueous phase with different amounts of IM

Oxazepam	Sediment pH 7.0	Sediment pH 9.0	Microplastics pH 7.0	Microplastics pH 9.0
0 mg/L IM	97 - 103	94 - 110	97 - 105	95 - 103
30 mg/L IM	93 - 110	98 - 99	93 - 104	95 - 120
300 mg/L IM	98 - 104	90 - 101	83 - 103	95 - 103
3 g/L IM	92 - 94	95 - 90	86 - 90	100 - 106
30 g/L IM	56 - 66	45 - 50	85 - 97	95 - 109

All numbers in % of the mean concentration found with no IM.

The experiment was only carried out with sediment and microplastic, because the large amounts of IM would have made filtering the sample almost impossible when using humic acids. An overview of the results can be seen in Table 13-12.

The strongest sorption by far was observed with MCM-TPP, although even there it required IM amounts much larger than the ones used in the main experiments. With microplastics, it can be seen that both at pH 4.0 and at pH 6.2, almost the entire amount of MCM-TPP was found in the liquid phase. With sediment as IM, the effect was stronger – when 30 g/L of sediment were added, almost the entire amount of MCM-TPP was sorbed, regardless of pH. With the sediment concentration used in the main experiments however, sorption was only relevant at pH 4, while the main experiments were carried out at pH 6.2.

Using ASE, most of the MCM-TPP sorbed to the sediment could be recovered: 91.8% at pH 4.0 and 87.4% at pH 6.2. This was not tested for the other analytes, since they sorbed onto the IMs to a much smaller extent. During our project, extraction of absorbed analytes never became an important problem, but these data show that the ASE method could potentially be used in experiments in which this is the case. With microplastics, a different extraction method might be needed, because the one applied here involved temperatures of up to 90 °C, which is above the glass transition temperature of PET[139] and might cause permanent absorption of analytes by the PET fibers. In our experiment however, no significant sorption to the microplastic fibers occurred anyway, so no special extraction method was required.

It may seem surprising that so little sorption occurred in our experiments with analytes such as TFX, which has a relatively high log Kow of 4.5, or the permanently charged MCM-TPP. One must keep in mind however that the IMs were used in concentrations of only 30 mg/L – the amount of water thus is approximately 33,000 times larger than the amount of IM. Even if the concentration of the analyte is 100 times higher in the solid phase than it is in the water phase (Kd = 100), 99.7% of the analyte would still be present in dissolved state.

EPI suite estimates the log Koc of TFX as 6.48 (MCI method) or 3.35 (Kow method). The former value, combined with the organic carbon content of our used sediment (4.3%), would let us expect approximately 80% sorption onto the sediment:

$$10^{6.48} = \frac{Kd \cdot 100}{4.3}$$

$$Kd \sim 130{,}000 \;\; = \frac{concentration\ in\ the\ soil}{concentration\ in\ the\ water}$$

$$\frac{c\ (in\ soil)}{c\ (in\ water)} \frac{m_{soil}}{m_{water}} = \frac{130{,}000}{33{,}000} \sim \frac{4}{1}$$

If however the value of 3.35 is correct, less than 0.3 % of TFX should be sorbed onto the sediment.

$$\frac{c\ (in\ soil)}{c\ (in\ water)} \frac{m_{soil}}{m_{water}} = \frac{96}{33{,}000} \sim 0.0029$$

5 Conclusions

IMs used in this project had little influence on the rate of hydrolysis of the employed analytes. For TFX, no significant effect was observed with any IM. For MCM-TPP, a significant, but still limited effect on the hydrolysis was derived, resulting in slightly increased half-lives by about 25%. For oxazepam, no significant effect was observed, although a small decrease in the reaction rate with humic acids (and maybe even with sediment) cannot be totally ruled out.

In a previous study conducted by the German "Umweltbundesamt (UBA)", a significant effect on the degradation of Metilox (methyl 3-(3,5-di-tert-butyl-4-hydroxyphenyl)propanoate) was observed. This effect was quite large in the case of three specific IMs: activated sludge, carbon nanotubes (CNT), and an ion-exchange resin. In the former two cases, Metilox was degraded faster, while it was degraded much slower in the presence of the ion-exchange resin. The fast degradation in the presence of (non-sterile) activated sludge was convincingly attributed not to faster hydrolysis, but to biological degradation.

CNT and ion-exchange resin both have highly structured surfaces which apparently can influence the hydrolysis of trace compounds. However, both of them are not commonly found in the aquatic environment in appreciable amounts. With sediment and microplastic (polystyrene) fibers as well as with sterilized activated sludge, the study also found only minor effects of the IM on the hydrolysis of Metilox.

Based on the results of that study as well as those of this project, we cannot exclude that the hydrolysis of emerging contaminants *could* be affected by the presence of IMs, but to a rather minor extent for particles that are commonly found in the aquatic environment. In our study, the only analyte for which the IMs (sediment, humic acids) had an effect was a cationic phosphonium compound, which is likely for specific electrostatic interactions with materials/particles having negatively charged sites such as humic acids or sediments. It seems plausible that those ionic interactions might reduce the hydrolysis rates. This is supported by the fact that the hydrolysis of MCM-TPP was not influenced by the presence of PET microplastic fibers which do not have negatively charged sites.

In any case, commonly occurring natural particles seem to have a negligible effect on hydrolysis, while the effect of pH is orders of magnitude larger. Even the effect of the temperatures that changes the hydrolysis rate according to the Arrhenius equation is much more relevant.

5.1 Conclusions for future revisions of OECD guideline 111

The hydrolysis of chemicals is currently tested in purified water without the addition of any IM. The integration of a variety of IMs would significantly increase the complexity of the tests that need to be conducted as well as the interpretation of the results. Since the effect of IMs on the hydrolysis of micropollutants was very small in comparison to the effects of pH, we would not recommend including testing with IM into the official OECD 111 guideline.

OECD guideline 111 already demands that individual test samples should be used for each sampling point, rather than a single bulk sample from which aliquots are drawn. Our experiences confirm that this is an important provision. Another requirement stated in the guideline is that half-lives and other kinetic data should not be determined from a simple plot of the log-transformed data, but from "more accurate kinetic model calculations". In our experiments, we found that the difference between using log-transformed data and using non-linear regression generally lead to very similar results, but in one case actually made the difference between an effect being deemed significant or insignificant. We therefore agree that kinetic parameters should not be derived from a simple logarithmic plot, but from kinetic modelling of the untransformed data.

Demanding the inclusion of several IMs in the testing and registration process for chemicals would likely be excessive. If IMs are to be included, it would probably be sufficient to include them in sorption tests, which are easier to conduct than hydrolysis tests in which the transformation products and transformation pathways should be identified (see below). If no significant sorption of a chemical occurs to any IM at environmentally relevant concentrations, it seems unlikely that the IMs will affect the hydrolysis of that chemical. Further hydrolysis tests might then be considered for cases in which a significant percentage of the chemical will be sorbed to environmentally occurring particles.

However, other changes dealing with molecule transformation during hydrolysis to the current testing regime seem to be more useful.

1. The hydrolysis products need to be identified and quantified. It cannot be presumed that hydrolysis always leads to a detoxification of a compound. Thus, the hydrolysis pathway as well the hydrolysis products should be elucidated in order to assess the formation of potential stable hydrolysis products as well as estimate their ecotoxicological relevance.

2. Furthermore, the mass balances should be closed. Thus, the removed quantity should be explainable by the quantity of the formed hydrolysis products. Mass balances under OECD guideline 111 are only required when labelled test substances were used.

OECD guideline 111 already calls for the identification and quantification of hydrolysis products representing >10% of the applied substance; but does not require considerations as to whether the occurring transformations are significant. For instance, a substance is considered to be hydrolytically degradable even if only minor changes to its structure occur, e.g. the cleavage of a single methyl group. When the test substance is carcinogenic or mutagenic, these properties may not be much reduced by such a simple structural change. In such cases, it may be more appropriate to require the inclusion of stability tests for the transformation products as well, until complete mineralization or a significant structural degradation is achieved.

Overall, a revision of OECD guideline 111 to include the effect of interfering particles seems to be unnecessary based on our results and a literature review.

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