Environmental Science & Technology

Article

pubs.acs.org/est

Biocide Runoff from Building Facades: Degradation Kinetics in Soil

Ulla E. Bollmann,*[,†] David Fernández-Calviño,[‡,§] Kristian K. Brandt,[‡] Morten S. Storgaard,[†,∥] Hans Sanderson,[†] and Kai Bester*[,†]

[†]Department of Environmental Science, Aarhus University, Frederiksborgvej 399, 4000 Roskilde, Denmark

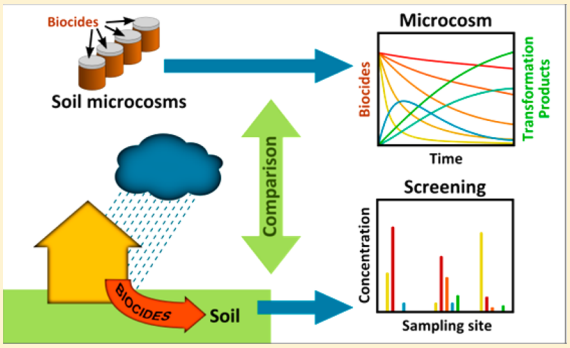
[‡]Department of Plant and Environmental Sciences, University of Copenhagen, Thorvaldsensvej 40, 1871 Frederiksberg, Denmark

[§]Department of Plant and Soil Science, University of Vigo, As Lagoas 1, 32004 Ourense, Spain

[∥]Department of Bioscience, Zoophysiology, Aarhus University, C.F. Møllers Allé 3, 8000 Aarhus, Denmark

Supporting Information

ABSTRACT: Biocides are common additives in building materials. In-can and film preservatives in polymer-resin render and paint, as well as wood preservatives are used to protect facade materials from microbial spoilage. Biocides leach from the facade material with driving rain, leading to highly polluted runoff water (up to several mg L^{-1} biocides) being infiltrated into the soil surrounding houses. In the present study the degradation rates in soil of 11 biocides used for the protection of building materials were determined in laboratory microcosms. The results show that some biocides are degraded rapidly in soil (e.g., isothiazolinones: $T_{1/2}$ < 10 days) while others displayed higher persistence (e.g., terbutryn, triazoles: $T_{1/2} \gg 120$ days). In addition, mass balances of terbutryn and octylisothiazolinone were determined, including nine (terbutryn) and seven (octylisothiazolinone) degradation products, respectively. The terbutryn mass balance could be closed over the entire study period of 120 days and showed that relative persistent metabolites were formed, while the mass balances for octylisothiazolinone could not be closed. Octylisothiazolinone degradation products did not accumulate over time suggesting that the missing fraction was mineralized. Microtox-tests revealed that degradation products were less toxic toward the bacterium *Aliivibrio fischeri* than their parent compounds. Rain is mobilizing these biocides from the facades and transports them to the surrounding soils; thus, rainfall events control how often new input to the soil occurs. Time intervals between rainfall events in Northern Europe are shorter than degradation half-lives even for many rapidly degraded biocides. Consequently, residues of some biocides are likely to be continuously present due to repeated input and most biocides can be considered as "pseudo-persistent"-contaminants in this context. This was verified by (sub)urban soil screening, where concentrations of up to 0.1 $\mu g\ g^{-1}$ were detected for parent compounds as well as terbutryn degradation products in soils below biocide treated facades.



INTRODUCTION

Facade paints and renders, especially those used on external thermal insulation composite systems, are susceptible for microbial deterioration. In order to protect the coatings, in-can and film preserving biocides are commonly added to polymer-resin render and paint. The use of biocides in Europe is regulated by the biocidal product regulation[1] for which each active ingredient needs to be approved and each formulated product needs registration. While low molecular weight isothiazolinones like methylisothiazolinone are usually used as in-can preserving bactericides, high molecular weight isothiazolinones as, for example, octylisothiazolinone are typically used as film preservatives against algae, fungi, and bacteria. Other film preservatives are phenylureas as well as triazines which are used against algae and carbamates against fungi. Additionally, triazoles are used as fungicides in wood protection.[2] Mecoprop (MCPP) is used to prevent larger plants from growing on bitumen sheets on flat roofs.[3,4] In coatings, typically a combination of different biocides is used with concentrations of about 0.1%.[5] However, it is known that these compounds leach out of the construction materials when they get in contact with rainwater.[6−8] Freshly treated facades emit several mg m^{-2} active ingredients if contacted with wind-driven rain and the respective runoff water contains about 1−5 mg L^{-1} single biocide.[6,7,9,10] In city centers the total facade runoff drains on paved surfaces like streets and terraces and further into the sewer system;[11] however, in suburban residential areas a large fraction drains directly to soil, for example, flowerbeds, gravel strips or the lawns surrounding the houses.[12] Consequently, the soil in areas with biocide-treated buildings is exposed to rain runoff water highly polluted with biocides and concentrations of several $\mu g\ g^{-1}$ can be expected in the receiving soils beneath the facade.

Received: November 1, 2016
Revised: March 13, 2017
Accepted: March 13, 2017
Published: March 13, 2017

DOI: 10.1021/acs.est.6b05512
Environ. Sci. Technol. 2017, 51, 3694−3702

The degradation kinetics of some of the active ingredients have already been assessed in agricultural soils as they are also used as pesticides (phenylureas, triazoles, phenoxy acids).[13−22] Others are only used in biocidal applications and, thus, very little data on degradation and degradation products in soils is available (e.g., isothiazolinones, iodocarb).[23−27] Biocide degradation half-lives vary from a few hours to more than a year.[13−27] In some cases it is known that accumulation of toxic compounds (e.g., 3,4-dichloroaniline from diuron) is possible.[13,16]

Many known biocides and pesticides on the marked are chiral, that is, they exist as two (or more) species that are mirror images of each other. Often the enantiomers show different toxicity as well as biodegradation rates and kinetics.[28] Considering mecoprop, only the *R*-enantiomer is biocidal active. Nonetheless, in biocidal applications often the racemic mixture is used, while the enantiopure *R*-mecoprop is used for agricultural and private gardening purposes.[3] The determination of the degradation kinetics for each mecoprop enantiomer may give valuable information on their fate and the risk of accumulating the more toxic enantiomer. Moreover, enantiomeric fractionation might give an indication on biological degradation processes and involved organisms.[29]

Biocides are usually used on buildings as combinations to address a wide range of target organisms and compounds used against similar target organisms are substitutable. Consequently, direct comparisons on the degradation and fate of the active substances in same soil types and under same conditions are important. Doing so, an environmentally friendly and effective combination could be identified. Not only the soil half-life is an important parameter to assess whether a compound is persistent in the environment ($T_{1/2}$ (soil) > 120 days[30]), also the degradation kinetics must be determined to establish which reaction order is really relevant and thus which mathematics can be used to interpret data as well as to predict field scenarios. However, close to the pollution source the time intervals between emissions ("time to exposure") is also of high importance as it might be shorter than the time that a compound needs to be degraded ("time to degrade") and, hence, the compounds might be constantly present,[31] also known as "pseudo-persistent".[32] Thus, as buildings are emitting biocides with each rainfall event, degradation half-lives need to be compared with rainfall-intervals to assess whether a biocide is degraded fast enough or if they have the potential to be continuously present or even accumulate close to the source. Additionally to the information on the degradation ability of the parent compound, information on the fate of degraded biocides is necessary to assess potential risks due to persistent or toxic degradation products.

In this contribution it was studied whether biocidal compounds are easily degradable or persistent in a loamy sand soil, as typical for urban regions in Northern Europe. In order to compare different compound groups, representatives of several groups were studied: four isothiazolinones [methylisothiazolinone (MI), benzisothiazolinone (BIT), octyl- (OIT), and dichloroctylisothiazolinone (DCOIT)], two phenylureas [diuron (DR) and isoproturon (IP)], one triazine [terbutryn (TB)], one carbamate [iodocarb (IPBC)], two triazoles [propiconazole (PPZ) and tebuconazole (TBU)], and one phenoxy acid [mecoprop (MCPP)]. The degradation products were elucidated and the mass balances calculated exemplarily for two compounds, that were moderately degradable (octylisothiazolinone and terbutryn) and were degradation data is completely lacking. As toxicity data is lacking for many degradation products, some compounds were tested using the Microtox bioluminescence inhibition test. Finally, a screening of urban soil was performed to verify the assessment based on the laboratory studies with field data.

MATERIALS AND METHODS

Soil Incubation. To gain soil material without previous biocide exposure, 10 soil samples were collected according to OECD guideline 217[33] at a depth of 0−20 cm from an agricultural field (Field 26; University of Copenhagen experimental farm, Tåstrup, Denmark) used for growing barley in November 2013. The field soil was fertilized exclusively with inorganic fertilizers (NPKS). The soil received a single summer application of propiconazole (2006) and tebuconazole was applied at recommended rates as single summer applications (2008−2013). None of the other biocides were applied on the field. The ten samples were mixed into one composite soil sample and stored at 4 °C until usage. The soil was of loamy sand texture, slightly acidic (pH 6.4), contained about 8% total moisture and an organic carbon content of 1.6%. Prior to the experiment the soil was sieved through 2 mm and preincubated at 22 °C for 2 weeks in darkness. Soil microcosms were prepared in 60 mL brown glass jars each containing 10 g ww of spiked soil. The soil was spiked with a biocide-mixture using sand as a carrier (100 mg per 10 g soil) following the OECD guideline 217:[33] the sand was spiked with a biocide multistandard dissolved in acetone and the solvent was evaporated overnight, before addition of the sand to the soil. Final concentrations in the soil were 2 μg g^{-1} for the isothiazolinones (methylisothiazolinone, benzisothiazolinone, octylisothiazolinone, dichloroctylisothiazolinone) and 10 μg g^{-1} for all other biocides (terbutryn, diuron, isoproturon, iodocarb, tebuconazole, propiconazole, mecoprop). The concentrations were chosen based on emission rates of several mg m^{-1} event^{-1}[6,7,10] and infiltration depths of 20 cm.

In total 51 soil microcosms were prepared to allow time-resolved measurements. The glass jars were covered with perforated aluminum foil to prevent contamination on one hand but allow free access of oxygen on the other hand. The samples were incubated at 22 °C for up to 120 days. Once a week, the weight of the microcosms was controlled and water was added to keep the initial water content of 8−10% ww. Three replicate samples (microcosms) were taken after specific time points throughout the incubation period (0, 1, 3, 5, 7, 10, 14, 22, 29, 36, 42, 55, 72, 87, 100, 118 days). The samples were transferred to the freezer and stored at −18 °C until further analysis.

To discriminate octylisothiazolinone and dichloroctylisothiazolinone degradation products single compound microcosms (50 μg g^{-1}, 25 g soil) were prepared as described above and incubated for 40 days. Samples were taken after 4 h and 40 days.

Soil Extraction and Analysis. A subsample of each microcosm (1 g soil), mixed with 1.5 g Hydromatrix (*Varian*, Palo Alto, CA, USA), was extracted using accelerated solvent extraction (ASE 200, *Dionex*, Sunnyvale, CA). Free space in the 11 mL cells was filled up with Ottawa sand. The cells were extracted at 80 °C and 1000 psi, using methanol (LiChrosolv gradient grade, *Merck*, Darmstadt, Germany) as a solvent. In total 2 extraction cycles were performed with the following settings: static time 5 min, preheating time 1 min, flush 60%, purge 60%. Successively, 50 μL of a surrogate standard (1 μg

DOI: 10.1021/acs.est.6b05512
Environ. Sci. Technol. 2017, 51, 3694−3702

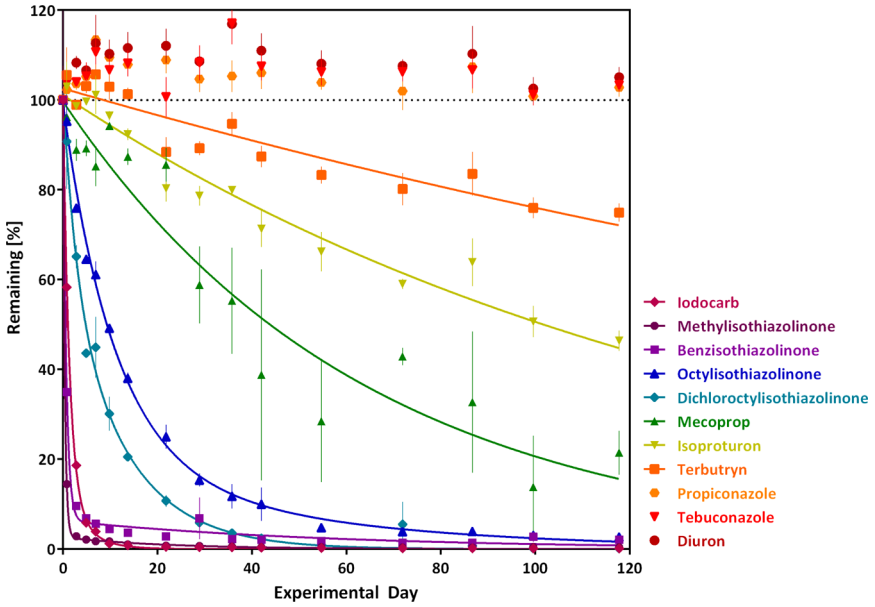


Figure 1. Degradation kinetics (error bars: standard error of mean (n = 3); single first order kinetics: terbutryn, isoproturon, mecoprop; two phase first order kinetics: methylisothiazolinone, benzisothiazolinone, octylisothiazolinone, dichloroctylisothiazolinone, iodocarb).

mL^{-1} carbendazim-D4, diuron-D6, iodocarb-D9, isoproturon-D6, methylisothiazolinone-D3, octylisothiazolinone-D17, tebuconazole-D6, terbutryn-D5 in methanol) were spiked to a 1 mL subsample of the primary extract and analyzed by high performance liquid chromatography coupled to tandem mass spectrometry (HPLC-MS/MS) according to Bollmann et al.[12] Relative extraction recoveries ranged between 67 and 112; the details are shown in the Supporting Information (SI) (Table S1–S3).

The measured concentrations for the respective compounds were plotted against the incubation time and fitted with GraphPad Prism 6 using single first order and two-phase first order kinetics. The determination on the phase-type was done graphically (SI Figure S1).

Besides the analysis of the parent biocides, several degradation products of terbutryn and octylisothiazolinone were analyzed according to Bollmann et al.;[7,10] Some of the terbutryn degradation products were analyzed with a neutral gradient (water/methanol), while others had to be analyzed using an acidified gradient (water/acetonitrile with 0.2% formic acid).[7] The octylisothiazolinone degradation products were analyzed with an acidified gradient.[10] Additionally, enantiomers of mecoprop were analyzed according to Frkovà et al.[29] using a Nucleodex α PM column (4 mm i.d. and 20 mm length, particle size 5 μm) from Macherey-Nagel (Düren, Germany) and a water/methanol (both acidified with 0.2% formic acid) multistep gradient.

Microtox Analysis. As little information is available on the toxicity of the degradation products, Microtox tests were performed for three terbutryn degradation products (desthiomethyl-terbutryn, desethyl-desthiomethyl-terbutryn, desethyl-desthiomethyl-terbutryn) and for three octylisothiazolinone degradation products (octylacetamide, octylpropenamide, octylthiazolinone). The two parent compounds were also tested. The Microtox test, determining the bioluminescence inhibition in cell suspensions of the bacterium *Aliivibrio fischeri* (lot. no. 14H4122 from Modern Water) dependent on the concentration, was performed following ISO 11348-3[34] and was previously described in.[35] Concentration profiles (0–8 mg L^{-1} in 2% NaCl) were prepared for each compound. As methanol stock solutions were used, the samples contained 4% of methanol, which was previously established as the maximum allowable concentration in Microtox tests.[36] All dose response-curves, showing light-inhibition, did fit logarithmic normal distribution (Goodness of fit <0.5). Effect concentrations at which 50% luminescence inhibition occurs compared to the control (EC_{50}s) and 95% confidence intervals were derived using a nonlinear dose–response regression. The test has been described by Christensen et al.[37]

(Sub)Urban Soil Screening. A total of 17 anonymized houses, suspected to be treated with biocides (e.g., painted wood, external thermal insulation system) and build or renovated within the last five years, were selected within the Greater Copenhagen area (Denmark) and sampled on 8./9. June 2016. Facade surfaces were flushed with water and analyzed for biocides using HPLC-MS/MS as described above. Soil samples (about 10 × 10 cm^2 and 10 to 20 cm depth) were taken with a metal shovel directly at the bottom of the facade. A subsample of 1 g of each sample was extracted with accelerated solvent extraction and analyzed by HPLC-MS/MS as described above.

RESULTS AND DISCUSSION

Degradation Kinetics. More than 97% of the isothiazolinones and iodocarb were degraded during 120 days at the applied soil incubation conditions, while only 78% of mecoprop, 54% isoproturon, and 25% of terbutryn were degraded (Figure 1). The isothiazolinones and iodocarb degraded rapidly with half-lives below 10 days (MI 0.28 days, BIT 0.52 days, IPBC 1.05 days, DCOIT 4.8 days, OIT 9.3 days) (Table 1). Their degradation followed 2-phase first order kinetics. Mecoprop, isoproturon, and terbutryn were degraded much slower (half-life MCPP 44 days, IP 100 days, TB 231 days) and followed single first order kinetics. Diuron, tebuconazole, and propiconazole were persistent under these conditions (half-lives ≫ 120 days).

The biodegradation kinetics of the two enantiomers of mecoprop separately followed first-order kinetics in both cases

DOI: 10.1021/acs.est.6b05512
Environ. Sci. Technol. 2017, 51, 3694–3702

Table 1. Degradation Rate Constants and Half-Lives Determined within This Study

group compound	degradation rate constants [d^{-1}] (percent fast[a])	half-life [d]
triazines		
terbutryn	0.003	231
carbamates		
iodocarb	0.75/0.22 (86.8)	1.05
isothiazolinones		
methylisothiazolinone	2.62/0.045 (97.4)	0.28
benzisothiazolinone	1.47/0.017 (93.7)	0.52
N-octylisothiazolinone	0.092/0.021 (80.2)	9.3
dichloro-*N*-octylisothiazolinone	0.35/0 079 (38.3)	4.8
phenylureas		
isoproturon	0.007	100
diuron		>2500[b]
triazoles		
tebuconazole		>2500[b]
propiconazole		>2500[b]
phenoxy acids		
mecoprop	0.016	44

[a]Percent Fast: fraction of the span accounted for by the faster of the two constants. [b]estimated based on 6% standard deviation first-order kinetic (SI Note S1).

(SI Figure S2). However, the *R*-enantiomer was degraded considerably faster ($T_{1/2}$ 30 days) than the *S*-enantiomer ($T_{1/2}$ 90 days). While *R*-mecoprop was below detection limits after 50 days, 40% of the initial *S*-mecoprop was still present after 120 days of incubation. The preferential degradation of the *R*-enantiomer under aerobic conditions agrees with the finding by Frková et al.[29] as well as other studies. Accordingly, in the aerobic soil surface, the more toxic *R*-enantiomer is degraded. This is indicating that the microbial community was performing as typical for this soil and no influence on the soil microbial community by the adding of the biocides can be detected.

For iodocarb and the isothiazolinones the fast degradation in the beginning of the two phase degradation kinetics was followed by a phase with nearly constant residual concentrations of 0.005−0.094 $\mu g\ g^{-1}$. While the residual concentration of methylisothiazolinone and dichloroctylisothiazolinone is ten times below the predicted no-effect concentration for soil organisms ($PNEC_{soil}$),[38,39] the residual concentration of iodocarb (0.013 $\mu g\ g^{-1}$) lies above the $PNEC_{soil}$ (0.005 μg a.i./g dwt).[27] Consequently, iodocarb might cause a risk to soil organisms even though being fast degraded.

These results are in accordance with the little literature that is available on the isothiazolinones (i.e., methyl- and dichloroctylisothiazolinone).[23,24] Although being the most important film-preserving isothiazolinone biocide in paints and renders, no comparison data is available for octylisothiazolinone. The present study shows, that it is degrading slower than dichloroctylisothiazolinone and, thus, is the isothiazolinone slowest in degrading. This goes along with activated soil filter experiments, in which octylisothiazolinone showed the lowest removal rates of the tested isothiazolinones.[40] Both terbutryn and mecoprop were degraded about four times slower in the present study than experienced previously,[21,22] although similar conditions and soil type was used in the terbutryn experiment.[21] Also iodocarb was degraded considerably slower in the present than in previous studies.[26,27] Information on previous exposure to those compounds is not stated in the studies; hence, acclimatization of the soils cannot be excluded. The determined half-lives of isoproturon and diuron were comparable to previous studies.[13−15] In previous studies tebuconazole showed biphasic degradation kinetics,[17] and a half-life of 800 days[18] and also for propiconazole half-lives of 200 days to more than a year have been determined previously,[19,20] while the two triazoles showed no degradation within the time frame of the present study.

As the different biocides belong to different substance groups (isothiazolinones, triazines, phenylureas, carbamates, triazoles, and phenoxy acids) and those used as pesticides are devoted to contrasting agricultural applications, the direct comparison of these compounds in a single experiment has never been performed before. However, within the present application as biocides in paints and render the compounds are used in combination. Additionally, compounds used against similar target organisms are substitutable. In this respect the direct

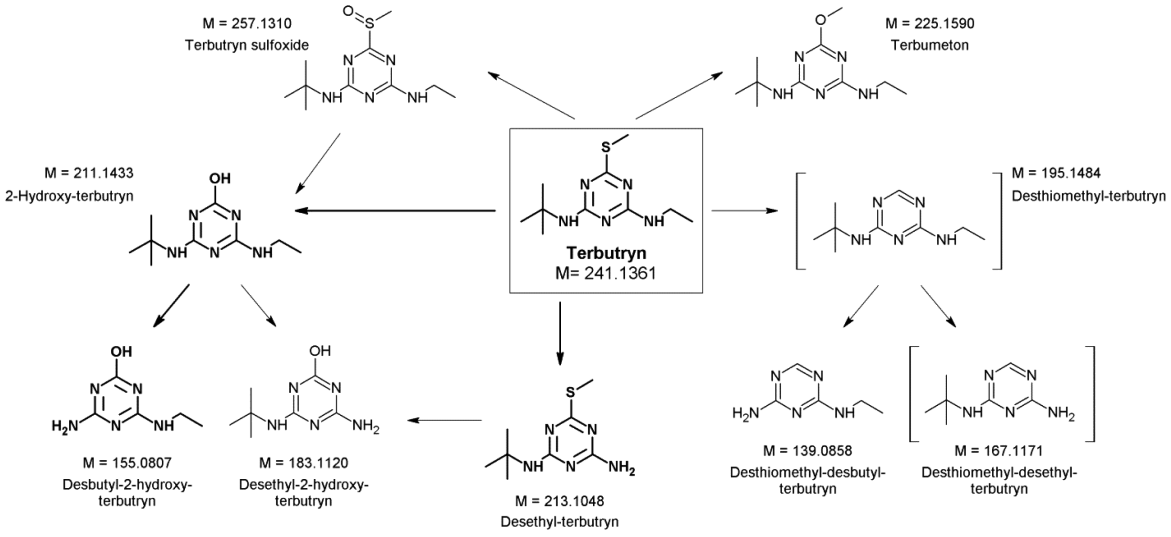


Figure 2. Proposed degradation pathway for terbutryn in soil (Compounds in bracket: below detection limits) based on their presence in the incubated soil samples.

DOI: 10.1021/acs.est.6b05512
Environ. Sci. Technol. 2017, 51, 3694−3702

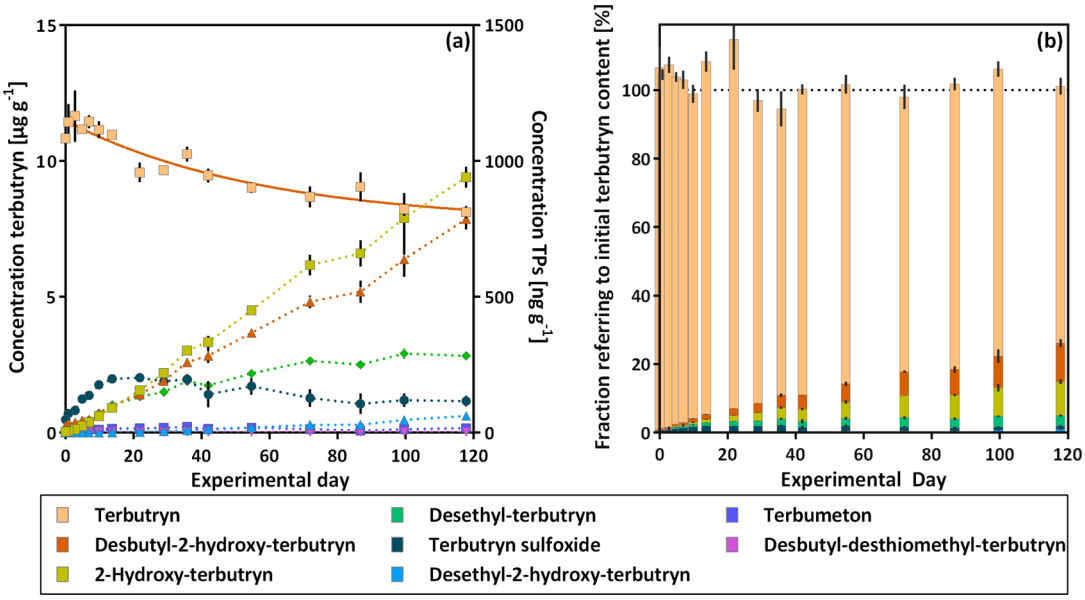


Figure 3. (a) Concentrations of terbutryn and its degradation products (TPs) over time and (b) total mass balance of terbutryn; error bars: standard error of mean (n = 3).

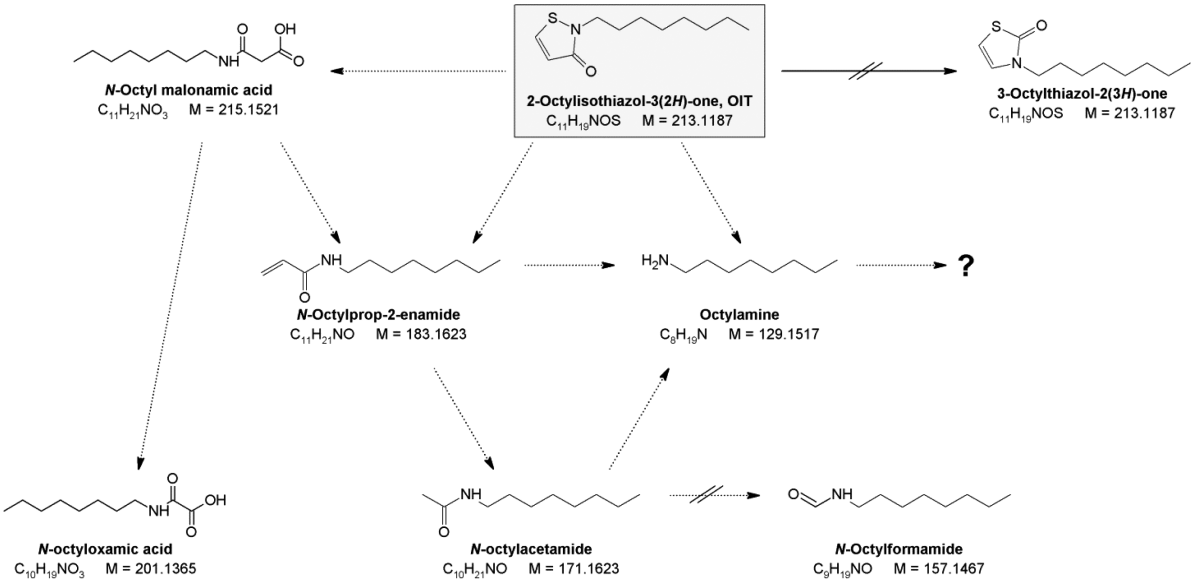


Figure 4. Proposed degradation pathway for octylisothiazolinone in soil based on their presence in the incubated soil samples.

comparison is of great interest, showing that some biocides are degrading within a few days, whereas others are more persistent.

Based on these results and considering rain intervals of a few days in Northern Europe,[41] it seems clear that methyl- and benzisothiazolinone as well as iodocarb will not lead to accumulation of residues of the parent compound in soils as emission intervals are longer than degradation half-lives. Nonetheless, the relatively high temperature during the soil incubation might have accelerated the degradation and, additionally, very slow degradation in the later phase of incubation resulted in low residual concentrations in the incubated soils. Hence, the above-mentioned assessment might be overestimating the degradation of these fast-degrading biocides and residues might be present anyway. The probability of accumulating residues of octyl- and dichloroctylisothiazolinone is small. However, residues are expected following a steady state equilibrium emissions-degradation as emission intervals are similar to degradation half-lives. However, the degradation of mecoprop, isoproturon and terbutryn is so slow that accumulation of residues in soils around treated buildings is likely. The worst case scenario, that is, all leached biocide will be accumulated in the soil into which the runoff water is infiltrated, is realistic for diuron, tebuconazole and propiconazole if transport processes to deeper soil layers are neglected.

Metabolites and Mass Balances. Octylisothiazolinone degradation products have previously not been reported in the literature and information on degradation products of terbutryn is very rare. Hence, these two compounds were studied in more detail. Degradation can be either complete (mineralization) or incomplete (primary degradation). It was thus tested to which extent degradation products accumulated from these two compounds.

DOI: 10.1021/acs.est.6b05512
Environ. Sci. Technol. 2017, 51, 3694−3702

Figure 2 depicts the predicted degradation scheme for terbutryn. The mass balance of terbutryn including several degradation products could be closed during the whole incubation period of 120 days (Figure 3). Hence, mineralization of terbutryn was negligible within this time frame. After 120 days 26% of terbutryn was recovered as degradation products. Main degradation products were desbutyl-2-hydroxy terbutryn (11%), 2-hydroxy-terbutryn (10%), and desethyl-terbutryn (often referred to as M1; 3%) (Figure 3b). While the concentration of desethyl-terbutryn was stabilizing at around 250 ng g^{-1} after 50 days, desbutyl-2-hydroxy terbutryn, and 2-hydroxy-terbutryn concentrations were continuously increasing up to 1000 ng g^{-1}, indicating that these degradation products were persistent or at least their production rate higher than their degradation rate. Terbutryn-sulfoxide, which has previously been described as intermediate product in biological wastewater treatment,[42] showed a fast increase in concentration within the first 20 days, whereupon it was degraded again and concentrations decreased to 150 ng g^{-1}. Hence, terbutryn-sulfoxide was not a persistent degradation product in soil (Figure 3a). Harada et al.[43] found that the *Bacillus cereus* strain JUN7 is able to degrade methylthio-*s*-triazines via sulfoxides to the corresponding 2-hydroxy-derivative, a pathway already proposed by Kaufman and Kearney in the 1970s.[44] Transformation of terbutryn sulfoxide to 2-hydroxy-terbutryn easily explains the fast increase of 2-hydroxy-terbutryn and at the same time decreasing concentrations of terbutryn sulfoxide after about 20 days of incubation. In further reactions the 2-hydroxy-terbutryn was degraded. While photodegradation processes predominantly lead to side chain losses of the ethyl-group,[7] in the soil processes the loss of the butyl-side chain was favored or more persistent and not further degraded. This applies at least to those pathways where the initial reaction takes place at the thiomethyl-group (2-hydroxy-terbutryn, desthiomethyl-terbutryn). Hence, desbutyl-2-hydroxy-terbutryn and desbutyl-desthiomethyl-terbutryn were formed in higher amounts than the corresponding desethyl-derivates (Figure 2).

Octylisothiazolinone is a commonly used film-preserving biocide in facade coatings.[2] Based on previous biodegradation studies on chloromethyl- and dichloroctylisothiazolinone[25,45] as well as a photodegradation study on octylisothiazolinone[10] a set of possible degradation products was established and analyzed (Figure 4). Out of the seven studied degradation products, four were detected in the soil samples: octylamine (up to 40 ng g^{-1}), octylmalonamic acid (up to 12 ng g^{-1}), octylacetamide (up to 3 ng g^{-1}), and octylpropenamide (up to 1.3 ng g^{-1}). Octylamine, octylmalonamic acid, and octylpropenamide were formed in the beginning of the experiment (Figure 5). None of the degradation products were persistent as maximum concentrations were reached between 10 and 20 days. After 60 days all degradation product concentrations were below 1 ng g^{-1}, that is, below 0.01% of the spike concentration of the parent compound. Single compound degradation studies showed that most of the degradation products might result from both octyl- and dichloroctylisothiazolinone degradation; solely octylmalonamic acid derived predominantly from octylisothiazolinone degradation (SI Figure S2). Due to the overlap it was not possible to calculate the total mass balance for octylisothiazolinone alone. Nevertheless, it can be concluded that none of the studied degradation products accumulated in the soil. The formed degradation products were lost from the system, that is, degraded further or eventually evaporated into the air (e.g., vapor pressure of octylamine is about 20 000 times higher than

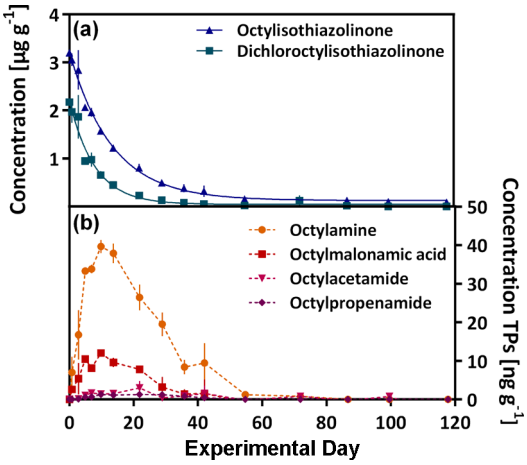


Figure 5. Concentrations of octylisothiazolinone and dichloroctylisothiazolinone (a) and their degradation products over time (b); error bars: standard error of mean (n = 3).

octylisothiazolinone). Additionally, incorporation into soil organisms or formation of bound residues might have resulted in a nondetectable fraction.

Environmental Toxicological Assessment. In total, eight compounds were tested by Microtox (two parent compounds, the algaecide terbutryn and the bactericide octylisothiazolinone and three degradation products associated with each parent compound). EC_{50} values were successfully determined for three compounds (octylisothiazolinone, octylpropenamide, octylthiazolinone) and are shown in Table 2.

Table 2. EC_{50} Values (With Respective 95% Confidence Intervals in Brackets) Obtained with the Microtox Test [Test Species: *Aliivibrio fischeri*; End Point: Inhibition of the Luciferase Activity (Bioluminescence)]. Parent Compounds Are Marked in Bold

compound	CAS-No	EC_{50}
octylisothiazolinone (OIT)	26530−20−1	0.05 mg L^{-1} (0.04−0.06)
3-octylthiazol-2(3*H*)-one	1600599−28−7	1.09 mg L^{-1} (0.79−1−51)
N-octylacetamide	7462−62−6	>8.36 mg L^{-1}
N-octylprop-2-enamide	10124−68−2	4.51 mg L^{-1} (3.43−5.94)
terbutryn (TB)	886−50−0	>8.13 mg L^{-1}
desbutyl-desthiomethyl-terbutryn	30368−49−1	>7.96 mg L^{-1} (No inhibition)
desethyl-desthiomethyl-terbutryn	73956−52−2	>7.60 mg L^{-1} (No inhibition)
desthiomethyl-terbutryn	73956−51−1	>6.54 mg L^{-1} (No inhibition)

The other compounds are listed according to the maximum tested concentration. Individual treatment-response curves are available as SI (Figure S3). The most toxic compound was the parent compound octylisothiazolinone (EC_{50} = 0.05 mg L^{-1}), followed by its two degradation products octylthiazolinone (EC_{50} = 1.09 mg L^{-1}) and octylpropenamide (EC_{50} = 4.51 mg L^{-1}). None of the three degradation products of terbutryn (desthiomethyl-terbutryn, desethyl-desthiomethyl-terbutryn, desethyl-desthiomethyl-terbutryn) did show inhibition at all. Terbutryn itself and octylacetamide showed too little inhibition to calculate an EC_{50} at the concentration level tested (EC_{50} > 6.5 mg L^{-1}).

DOI: 10.1021/acs.est.6b05512
Environ. Sci. Technol. 2017, 51, 3694−3702

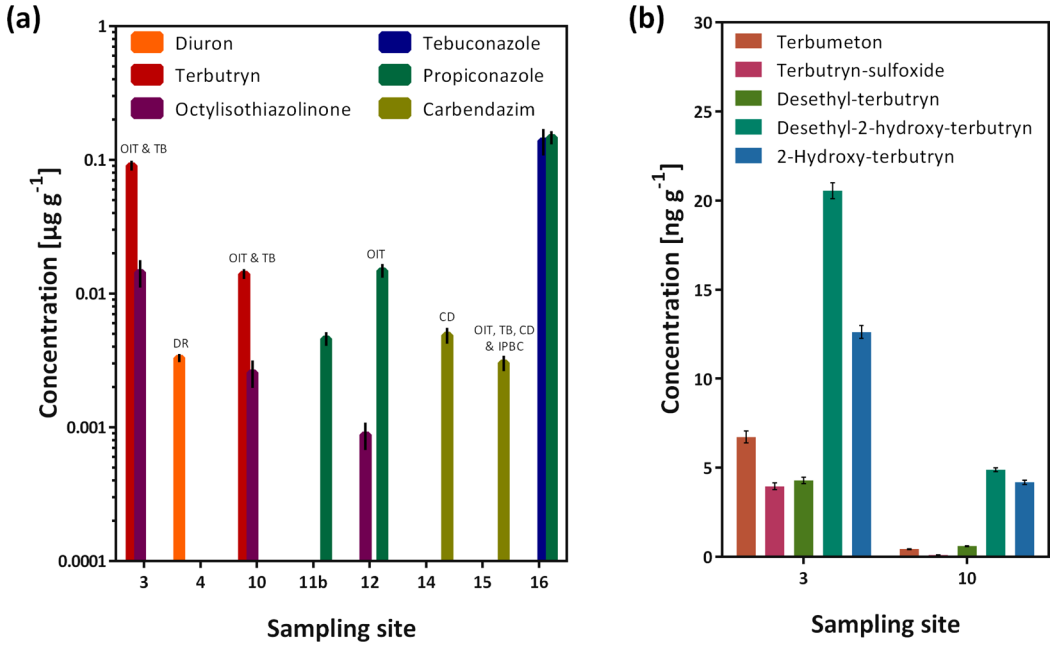


Figure 6. Urban soil concentrations of (a) biocides and (b) terbutryn degradation products (only sampling site 3 and 10) next to treated facades (Error bars: methodological relative standard deviation; Abbreviated biocides above sampling sites indicate detection in leachate samples).

Octylisothiazolinone and terbutryn were more toxic than their respective degradation products (Table 2; SI Table S4). Especially in the case of terbutryn, where degradation products were accumulating over time, the effects of the degradation products are of high interest. However, terbutryn is inhibiting the photosynthesis II system and, hence, is more toxic against algae than bacteria. If similar effects of its degradation products are expected, other test systems than Microtox might be more sensitive. However, linear correlations between algae toxicity and Microtox tests[46] allow relative assessments of the degradation products in comparison to their parent compounds. Additionally, it has to be mentioned that for most compounds only aquatic toxicity data is available for the comparison, while toxicity data for soil organisms is rare. Hence, the Microtox tests can be seen as first tier screening, while the final toxicity assessment should rely on data from several tiers.

(Sub)Urban Soil Screening. Five of the 11 analyzed biocides were detected in the leachate of the facade from 8 out of the 17 studied houses: terbutryn, diuron, carbendazim, iodocarb and octylisothiazolinone. Usually, a combination of one or two biocides was detected for a specific facade. Although being detected in two facade leachates, iodocarb was below detection limits in all soil samples. For the other biocides concentrations ranging from 0.001−0.1 $\mu g\ g^{-1}$ were detected in the soils surrounding those houses (Figure 6a). Additionally, up to 0.15 $\mu g\ g^{-1}$ tebuconazole and propiconazole were detected in some soil samples, while not being detected in runoff water from the respective building facades. However, in one case the sampled location might have received some runoff from a wooden surface close by, which might be treated with triazoles fungicides. However, the leachate was not analyzed. Another location received leachate from a wooden surface, which could have been painted with propiconazole or tebuconazole containing paint previously.

The measured concentrations in the soils where ten to 100 times lower than the concentrations estimated based on semifield studies with artificial walls. This might be caused by the fact that transportation process into deeper soil layers where neglected during the estimation, as well as overestimations of runoff water amount due to wind-driven rain.[47,48] Additionally, the sampling days were preceded of warm (up to 28 °C) and dry summer days.

Nonetheless, although being assessed as nonaccumulative in soil when used as wood or film preservative due to dissipation half-life of less than 77 days in field experiments,[49] tebuconazole was measured at concentrations slightly above the predicated no effect concentrations for soil organisms ($PNEC_{soil}$(TBU, earthworm) = 0.1 μg a.i./ g ww).[49] Also propiconazole concentrations were up to seven times above the $PNEC_{soil}$(PPZ, earthworm) = 0.02 μg a.i./ g ww.[50] Hence, tebuconazole and propiconazole may pose a risk to soil biota. However, it is noteworthy that fungal growth rates and other microbial activity endpoints were only significantly inhibited by propiconazole at concentrations above 25−50 $\mu g\ g^{-1}$ in a recent study performed in the same soil as used in the present study.[51]

The determination of octylisothiazolinone and terbutryn degradation products in the soil samples is consistent with the assessment based on the degradation studies. Terbutryn degradation products could be detected in concentrations ranging from 0.1 to 20 ng g^{-1} in soil samples receiving terbutryn polluted runoff water (Figure 6b), whereas octylisothiazolinone degradation products were below detection limits in all samples.

COMPARISON LABORATORY TO FIELD DATA

In general, the soil screening was consistent with the results of the laboratory experiments, as particularly those compounds, which showed slow degradation in the laboratory study as terbutryn, diuron and the triazoles, were detected in the field samples. Additionally, terbutryn degradation products were detected while octylisothiazolinone degradation products were below detection limits. However, although being relatively

DOI: 10.1021/acs.est.6b05512
Environ. Sci. Technol. 2017, 51, 3694−3702

rapidly degraded in laboratory experiments, octylisothiazolinone could be detected in soil samples at three sampling sites. In all cases, the facades were treated recently (<3 years); hence, emissions from the facades were expected to be very high. The reoccurring emission through repeated wash-off during rain events prevents the dissipation of octylisothiazolinone. Hence, even readily degradable biocides used in building materials can constitute "pseudo-persistent" or "continuously present"[31] contaminants in soil close to the buildings when considering the Northern European weather conditions.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.est.6b05512.

extraction recoveries, limits of detection, mass spectrometric data and suppliers of the used standards, graphical determination of the degradation kinetics, half-live estimation of PPZ, TBU, and DR, degradation products and mass balances in DCOIT/OIT single compound incubations, treatment-effect curves from the Microtox-tests, and ecotoxicological data for terbutryn and octylisothiazolinone degradation products found in the literature (PDF)

■ AUTHOR INFORMATION

Corresponding Authors

*(U.E.B.) Phone: +45 8715 8462; e-mail: ueb@envs.au.dk.
*(K.B.) Phone: +45 8715 8552; e-mail: kb@envs.au.dk.

ORCID

Ulla E. Bollmann: 0000-0001-7834-1364
Kristian K. Brandt: 0000-0001-7886-9708

Author Contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

We acknowledge funding obtained from the Danish Environmental Protection Agency (Project MST-667-00103) as well as funding by the AUFF grant "Center for Advanced Water Purification". Additionally, We thank Sidra Ilyas for her help with the sampling and extraction of the soil screening.

■ REFERENCES

(1) European Parliament and Council. Regulation (EU) No 528/2012 concerning the making available on the market and use of biocidal products. *Off. J. Eur. Communities: Legis.* **2012**, *L167*, 1−122.

(2) Paulus, W. *Directory of Microbicides for the Protection of Materials: A Handbook*; Springer: Dordrecht, Netherlands, 2005.

(3) Bucheli, T. D.; Müller, S. R.; Voegelin, A.; Schwarzenbach, R. P. Bituminous roof sealing membranes as major sources of the herbicide (R,S)-mecoprop in roof runoff waters: Potential contamination of groundwater and surface waters. *Environ. Sci. Technol.* **1998**, *32* (22), 3465−3471.

(4) Burkhardt, M.; Kupper, T.; Hean, S.; Haag, R.; Schmid, P.; Kohler, M.; Boller, M. Biocides used in building materials and their leaching behavior to sewer systems. *Water Sci. Technol.* **2007**, *56* (12), 63−67.

(5) Burkhardt, M.; Zuleeg, S.; Vonbank, R.; Schmid, P.; Hean, S.; Lamani, X.; Bester, K.; Boller, M. Leaching of additives from construction materials to urban storm water runoff. *Water Sci. Technol.* **2011**, *63* (9), 1974−1982.

(6) Burkhardt, M.; Zuleeg, S.; Vonbank, R.; Bester, K.; Carmeliet, J.; Boller, M.; Wangler, T. Leaching of Biocides from Facades under Natural Weather Conditions. *Environ. Sci. Technol.* **2012**, *46* (10), 5497−5503.

(7) Bollmann, U. E.; Minelgaite, G.; Schlüsener, M.; Ternes, T.; Vollertsen, J.; Bester, K. Leaching of Terbutryn and Its Photodegradation Products from Artificial Walls under Natural Weather Conditions. *Environ. Sci. Technol.* **2016**, *50* (8), 4289−95.

(8) Schoknecht, U.; Gruycheva, J.; Mathies, H.; Bergmann, H.; Burkhardt, M. Leaching of Biocides Used in Facade Coatings under laboratory Test Conditions. *Environ. Sci. Technol.* **2009**, *43* (24), 9321−9328.

(9) Breuer, K.; Hofbauer, W.; Krueger, N.; Mayer, F.; Scherer, C.; Schwerd, R.; Sedlbauer, K. Wirksamkeit und Dauerhaftigkeit von Bioziden in Bautenbeschichtungen (Effectiveness and durability of biocidal ingredients in façade coatings). *Bauphysik* **2012**, *34* (4), 170−182.

(10) Bollmann, U. E.; Minelgaite, G.; Schlüsener, M.; Ternes, T. A.; Vollertsen, J.; Bester, K. Photodegradation of octylisothiazolinone and semi-field emissions from facade coatings. *Sci. Rep.* **2017**, *7*, 41501.

(11) Bollmann, U. E.; Tang, C.; Eriksson, E.; Jönsson, K.; Vollertsen, J.; Bester, K. Biocides in urban wastewater treatment plant influent at dry and wet weather: Concentrations, mass flows and possible sources. *Water Res.* **2014**, *60*, 64−74.

(12) Bollmann, U. E.; Vollertsen, J.; Carmeliet, J.; Bester, K. Dynamics of biocide emissions from buildings in a suburban stormwater catchment - Concentrations, mass loads and emission processes. *Water Res.* **2014**, *56*, 66−76.

(13) Giacomazzi, S.; Cochet, N. Environmental impact of diuron transformation: A review. *Chemosphere* **2004**, *56* (11), 1021−1032.

(14) Sørensen, S. R.; Bending, G. D.; Jacobsen, C. S.; Walker, A.; Aamand, J. Microbial degradation of isoproturon and related phenylurea herbicides in and below agricultural fields. *FEMS Microbiol. Ecol.* **2003**, *45* (1), 1−11.

(15) Alletto, L.; Coquet, Y.; Benoit, P.; Bergheaud, V. Effects of temperature and water content on degradation of isoproturon in three soil profiles. *Chemosphere* **2006**, *64* (7), 1053−1061.

(16) Tixier, C.; Bogaerts, P.; Sancelme, M.; Bonnemoy, F.; Twagilimana, L.; Cuer, A.; Bohatier, J.; Veschambre, H. Fungal biodegradation of a phenylurea herbicide, diuron:structure and toxicity of metabolites. *Pest Manage. Sci.* **2000**, *56* (5), 455−462.

(17) Herrero-Hernández, E.; Andrades, M. S.; Marín-Benito, J. M.; Sánchez-Martín, M. J.; Rodríguez-Cruz, M. S. Field-scale dissipation of tebuconazole in a vineyard soil amended with spent mushroom substrate and its potential environmental impact. *Ecotoxicol. Environ. Saf.* **2011**, *74* (6), 1480−1488.

(18) US-EPA. *Memorandum: Ecological Risk Assessment of Tebuconazole*; Washington, D.C., 2000.

(19) Kim, I. S.; Shim, J. H.; Suh, Y. T. Laboratory studies on formation of bound residues and degradation of propiconazole in soils. *Pest Manage. Sci.* **2003**, *59* (3), 324−330.

(20) Bromilow, R. H.; Evans, A. A.; Nicholls, P. H. Factors affecting degradation rates of five triazole fungicides in two soil types: 1. Laboratory incubations. *Pestic. Sci.* **1999**, *55*, 1129−1134.

(21) Brandhorst Daho, M. *Ecotoxicological Evaluation of the Herbicide Terbutryn*; Uppsala University: Uppsala, 1994.

(22) Paszko, T.; Muszyński, P.; Materska, M.; Bojanowska, M.; Kostecka, M.; Jackowska, I. Adsorption and degradation of phenoxyalkanoic acid herbicides in soils: A review. *Environ. Toxicol. Chem.* **2016**, *35*, 271−286.

(23) Jacobson, A.; Williams, T. M. The environmental fate of isothiazolinone biocides. *Chim. Oggi* **2000**, *18* (10), 105−108.

(24) Krzeminski, S. F.; Brackett, C. K.; Fisher, J. D. Fate of microbicidal 3-isothiazolone compounds in the environment: Modes and rates of dissipation. *J. Agric. Food Chem.* **1975**, *23* (6), 1060−1068.

DOI: 10.1021/acs.est.6b05512
Environ. Sci. Technol. 2017, 51, 3694−3702

(25) Krzeminski, S. F.; Brackett, C. K.; Fisher, J. D.; Spinnler, J. F. Fate of microbicidal 3-isothiazolone compounds in the environment: Products of degradation. *J. Agric. Food Chem.* **1975**, *23* (6), 1068−1075.
(26) Juergensen, L.; Busnarda, J.; Caux, P. Y.; Kent, R. Fate, behavior, and aquatic toxicity of the fungicide IPBC in the Canadian environment. *Environ. Toxicol.* **2000**, *15* (3), 201−213.
(27) European Parliament and Council. *Assessment Report IPBC (PT6 in-Can Preservative)*, 2008.
(28) Garrison, A. W.; Gan, J.; Liu, W. *Chiral Pesticides: Stereoselectivity and Its Consequences*; American Chemical Society: Washington DC, 2011.
(29) Frková, Z.; Johansen, A.; de Jonge, L. W.; Olsen, P.; Gosewinkel, U.; Bester, K. Degradation and enantiomeric fractionation of mecoprop in soil previously exposed to phenoxy acid herbicides − New insights for bioremediation. *Sci. Total Environ.* **2016**, *569−570*, 1457−1465.
(30) ECHA, Guidance on Information Requirements and Chemical Safety Assessment. Chapter R.11: PBT/vPvB assessment. In Helsinki, Finland, 2014.
(31) Mackay, D.; Hughes, D. M.; Romano, M. L.; Bonnell, M. The role of persistence in chemical evaluations. *Integr. Environ. Assess. Manage.* **2014**, *10*, 588−594.
(32) Daughton, C. G. "Emerging" Chemicals as Pollutants in the Environment: a 21st Century Perspective. *Renew. Resour. J.* **2005**, *23* (4), 6−23.
(33) OECD, *Guidelines for the Testing of Chemicals, No. 217 "Soil Microorganisms: Carbon Transformation test"*; OECD Publishing: Paris, France, 2000.
(34) International Organization for Standardization, *ISO standard 11348-3 "Water Quality - Determination of the inhibitory effect of water samples en the light emission of Vibrio fischeri (Luminescent bacteria test)"*. 2007.
(35) Christensen, I. M. A.; Storgaard, M. S.; Fauser, P.; Hansen, S. F.; Baatrup, E.; Sanderson, H. Acute toxicity of sea-dumped chemical munitions: Lumnating the environmental toxicity of legacy compounds. *Global Security: Health, Science and Policy* **2016**, *1*, 39−50.
(36) Kwan, K. K.; Dutka, B. J. Simple 2-step sediment extraction procedure for use in genotoxicity and toxicity bioassays. *Toxic. Assess.* **1990**, *5* (4), 395−404.
(37) Christensen, E. R.; Kusk, K. O.; Nyholm, N. Dose-response regressions for algal growth and similar continuous endpoints: calculation of effective concentrations. *Environ. Toxicol. Chem.* **2009**, *28*, 826−835.
(38) European Parliament and Council. *Assessment Report DCOIT (PT21 Antifouling)*, 2014.
(39) European Parliament and Council. *Assessment Report MIT (PT13 Metalworking-Fluid Preservatives)*, 2014.
(40) Bester, K.; Benzhaf, S.; Burkhardt, M.; Janzen, N.; B, N.; Scheytt, T. Activated soil filters for removal of biocides from contaminated run-off and waste-waters. *Chemosphere* **2011**, *85*, 1233−1240.
(41) European Environment Agency. *Simulated Land Average Maximum Number of Consecutive Dry Days for Different European Regions (1860−2100)*; Copenhagen, Denmark, 2009.
(42) Luft, A.; Wagner, M.; Ternes, T. A. Transformation of Biocides Irgarol and Terbutryn in the Biological Wastewater Treatment. *Environ. Sci. Technol.* **2013**, *48* (1), 244−254.
(43) Harada, N.; Takagi, K.; Fujii, K.; Iwasaki, A. Transformation of methylthio-s-triazines via sulfur oxidation by strain JUN7, a Bacillus cereus species. *Soil Biol. Biochem.* **2006**, *38* (9), 2952−2957.
(44) Kaufman, D. D.; Kearney, P. C., Microbial transformations in the soil. In *Herbicides: Physiology, Biochemistry, Ecology*; Audus, L. J., Ed.; Academic Press: London, UK, 1976; Vol. 2.
(45) Williams, T. M.; Jacobson, A. H. *Paper No. 303: Environmental Fate of Isothiazolone Biocides*, Corrosion, 1999; NACE International: Houston, TX, 1999.
(46) DeZwart, D.; Slooff, W. The Microtox as an Alternative Assay in the Acute Toxicity Assessment of Water Pollutants. *Aquat. Toxicol.* **1983**, *4* (2), 129−138.
(47) Blocken, B.; Derome, D.; Carmeliet, J. Rainwater runoff from building facades: A review. *Build. Environ.* **2013**, *60*, 339−361.
(48) Coutu, S.; Wyrsch, V.; Rossi, L.; Emery, P.; Golay, F.; Carneiro, C. Modelling wind-driven rain on buildings in urbanized area using 3-D GIS and LiDAR datasets. *Build. Environ.* **2013**, *59*, 528−535.
(49) European Parliament and Council. *Assessment Report Tebuconazole (PT 8 wood preservative)*; 2007.
(50) European Parliament and Council. *Assessment Report Propiconazole (PT8 wood preservative)*; 2007.
(51) Fernández-Calviño, D.; Rousk, J.; Bååth, E.; Bollmann, U. E.; Bester, K.; Brandt, K. K. Ecotoxicological assessment of propiconazole using soil bacterial and fungal growth assays. *Appl. Soil Ecol.* **2017**, in press. DOI: 10.1016/j.apsoil.2017.03.009.

DOI: 10.1021/acs.est.6b05512
Environ. Sci. Technol. 2017, 51, 3694−3702