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Identification of Dufulin photolysis and hydrolysis products in water using a ^{13}C stable isotope assisted HPLC-HRMS strategy

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ABSTRACT

Dufulin is an efficient antiviral agent for plants, however, data on its environmental fate, particularly concerning its transformation products (TPs), remain scarce. The TPs formed during abiotic degradation may pose significant environmental risks due to potential toxicity. Therefore, this study systematically investigated the hydrolysis and photolysis kinetics of Dufulin in aqueous solutions across various pH conditions. By employing HPLC-HRMS and ^{13}C stable isotope labeling, we identified and quantified Dufulin and its degradation products. The results indicated that Dufulin undergoes accelerated photolysis under both acidic and alkaline conditions, while hydrolysis, although less influenced by pH, was comparatively slower than photolysis. Six TPs were identified and confirmed, five of which were reported for the first time. Moreover, abiotic transformation pathways of Dufulin in water were proposed. Predictions by the ECOSAR software indicated that the toxicity of two of the identified TPs was higher than that of the parent compound, meriting further assessment. These findings provide crucial insights into the environmental risks associated with Dufulin and underscore the scientific validity of using HPLC-HRMS combined with ^{13}C stable isotope labeling for TP identification.

1. Introduction

Large quantities of pesticides are being continuously introduced into the environment to maximize agricultural productivity (Fréneau et al., 2016; Suh et al., 2024; Tudi et al., 2021). However, our understanding of their environmental fate remains limited (Zhu et al., 2022). Both biological and abiotic processes transform pesticides in the environment, generating unknown transformation products (TPs) before complete mineralization (Mitra et al., 2024). Some of these TPs have been reported to exhibit higher toxicity than the parent compound and may also be more persistent (Anagnostopoulou et al., 2022; Chen et al., 2019; Ma et al., 2021; Man et al., 2023). This is why pesticide TPs are receiving substantial attention from researchers and regulators alike.

Dufulin is a recently developed antiviral compound designed to protect plants like rice, tomatoes, and cucumbers from viral infections (Shi et al., 2018; Zhang et al., 2013). The environmental behavior of Dufulin received considerable attention in the scientific community, but reports on its environmental TPs are virtually non-existent. Wang employed LC–MS techniques to identify the degradation products of Dufulin in soil, hypothesizing that 2-amino-4-methylbenzothiazole is one of its TPs, but did not provide further confirmation of this hypothesis (Wang et al., 2014). Therefore, it is urgently necessary to acquire more information to better understand the environmental behavior of Dufulin and its TPs.

High-performance liquid chromatography coupled with high-resolution mass spectrometry (HPLC-HRMS) in conjunction with non-targeted analysis can be employed for the preliminary identification of unknown chemicals, as it can provide accurate mass and mass spectral information (Fu et al., 2018; Jang et al., 2024; Mendes et al., 2024; van Herwerden et al., 2022). In real world samples, the complexity of the matrix often results in mass spectral data containing a significant amount of background and chemical noise. Additionally, since most TPs do not contain halogen atoms (Cl or Br), a lack of isotopic patterns in the mass spectral data, limits the rapid identification of TPs. To overcome these difficulties, we chose to use an identification strategy using stable isotope labeling combined with HPLC-HRMS (Bueschl et al., 2012; Geng et al., 2023). Since the vast majority of organic compounds contain carbon, the C atom is one of the commonly used labeled elements.

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Metabolites labeled with a small number of ^{13}C atoms (1 or 2 atoms) exhibit nearly identical physicochemical properties to their unlabeled counterparts, resulting in little to no separation on the chromatographic column. Labeled compounds can be distinguished by their mass-to-charge ratios (Geng et al., 2023; Klein and Heinzle, 2012) which enables the rapid identification of TPs.

In this study, the hydrolysis and photolysis of Dufulin in water were systematically investigated applying an analytical method coupling HPLC-HRMS with ^{13}C stable isotope labeling. Five identified TPs were further verified using standards, demonstrating the reliability and scientific validity of the method.

2. Materials and methods

2.1. Materials and reagents

The standards used in this study, Dufulin (purity 99.5 %) and [Thiazyl-2-^{13}C]-Dufulin (chemical purity 99.9 %, isotopic purity 98.6 %), were both purchased from Alta Scientific Co., Ltd., Tianjin. The newly identified transformation products (TPs) of Dufulin, including TP262 (purity 98.5 %), TP380 (purity 99.0 %), TP270 (purity 99.5 %), and TP286-2 (purity 99.0 %), were synthesized by Alta Scientific Co., Ltd., Tianjin. TP164 (purity 98 %) was purchased from Alta Scientific Co., Ltd. All stock solutions were prepared in acetonitrile (HPLC grade) and stored in the dark at 4 °C.

2.2. Hydrolysis and photolysis experiments

The pH value is a critical factor influencing the rate of pesticide hydrolysis and the formation of TPs (Liu et al., 2015). In this study, hydrolysis of Dufulin was simulated under different pH conditions (4, 7, and 9) using previously tested buffers (Chen et al., 2019). All solutions were autoclaved three times to eliminate biological interference. The stock solution of Dufulin was prepared by dissolving it in 40 mL of ultrapure water to achieve a final concentration of 5 mg/L. The hydrolysis reactions were conducted in brown plastic bottles with spiral caps, stored in the dark at 25 ± 1 °C in an incubator. Samples were collected at predetermined intervals (0, 3, 6, 10, 15, 22, 30, 40, 50, 60, 80, and 100 days) for subsequent analysis.

The initial solution for the photolysis experiment was identical to that used in the hydrolysis experiment. For photolysis, the buffer solutions (pH 4, 7, and 9) were transferred to stoppered quartz tubes. These tubes were then placed in a xenon lamp apparatus equipped with a cooling system (1000 W, emission range 290–800 nm, light intensity 4000 Lux, Xuezhongtan, Hangzhou, China) and maintained at 25 ± 1 °C. Quartz tubes covered with aluminum foil served as dark controls. Samples were collected at specified intervals (0, 2, 4, 8, 12, 24, 36, 48, 72, 96, 120, and 144 h) for analysis. Each experiment was conducted three times, and the results were calculated as an average.

2.3. Sample treatment

At specified time intervals, 2 ml of the sample were transferred into a 5 ml centrifuge tube, mixed with 2 ml of acetonitrile, and shaken for 5 min. Thereafter, 0.8 g of NaCl was added and the tube was shaken for an additional 2 min to completely dissolve NaCl. Finally, the tube was centrifuged at 4000 rpm for 5 min and the supernatant was transferred through a 0.22 μm organic membrane into a brown vial for subsequent analysis.

2.4. Analysis via UHPLC-Q exactive MS

The TPs of Dufulin were identified using UHPLC-Q Exactive MS. Separation was performed on a chromatographic column with a 5 μL injection volume and a column temperature of 40 °C. Mass spectrometry analysis utilized a HESI source, working in the positive ion mode. The source temperature was set to 320 °C, with an ion spray voltage of 3.5 kV, a lens voltage of 50 V, an auxiliary gas flow rate of 10 arb, and a sheath gas flow rate of 40 arb. The Full MS/ddMS2 (TOP10) mode was employed, with a first-level full scan resolution of 70,000 FWHM and a data-dependent second-level scan resolution of 17,500 FWHM. The instrument automatically selected the top 10 ion peaks for fragmentation in the second-level mass spectrometry. Compounds underwent collision-induced fragmentation with normalized collision energies (NCE) of 10, 30, and 50, generating fragment ion spectra at these three collision energies to provide a comprehensive view of the fragmented ions.

2.5. Data processing analytical strategy

The raw data were analyzed using Compound Discoverer 3.2 (Thermo Fisher Scientific). A combined approach of suspect screening and non-targeted screening was employed to identify Dufulin TPs. Subsequently, substances were synthesized for structural confirmation. A database of suspect compounds was first established, which included chemical structures, accurate molecular weights, and molecular formulas of TPs. This information was gathered through a literature review and transformation pathway predictions. The literature review involved consulting relevant articles on previously reported Dufulin compounds (Zhang et al., 2013; Zheng et al., 2023). Additionally, transformation pathways were predicted using the Biotransformer 3.0 platform https://biotransformer.ca/new.

We utilized ECOSAR, a widely adopted predictive model for compound toxicity, known for its accuracy in forecasting both acute and chronic toxicity to aquatic organisms (Graumans et al., 2024; Wu et al., 2023). ECOSAR's predictions are largely based on the structural similarity of compounds. For this study, we employed ECOSAR Version 1.11, developed by the US Environmental Protection Agency (EPA).

3. Results and discussions

3.1. Hydrolysis

As shown in Fig. 1, Dufulin exhibited a certain degree of resistance to hydrolysis under different pH conditions. The hydrolysis process predominantly followed pseudo-first-order (SFO) kinetics. As indicated in Table S2, the correlation coefficient (R^2) ranged from 0.8650 to 0.9413, reflecting an acceptable model fit. To more accurately quantify model uncertainty, Table S2 also reports the rate constant (k) along with its standard error and 95 % confidence interval. Under the tested conditions, the fastest degradation was observed at pH 4 with a half-life of 206.0 days. In pure water, at pH 7 and pH 9, the half-lives were 250 days, 340.0 days and 336.0 days, respectively. This trend could potentially be ascribed to the protonation of the phosphate ester moiety on Dufulin under relatively low pH conditions. The positive charge residing on the phosphorus atom endows it with enhanced reactivity towards water in an acidic milieu, thereby rendering it more prone to nucleophilic assault by water molecules and expediting the hydrolysis process (Harsági and Keglevich, 2021). It may also be attributable to protons (H^+) can interact with the nitrogen atom in amines, resulting in protonation and forming NH_3^+, thereby reducing the electron density at this site and making it more prone to nucleophilic attack, and finally facilitating the hydrolysis of Dufulin (Cismesia et al., 2017; Gattuso et al., 2022).

3.2. Photolysis

Dufulin photodegradation experiments were conducted using a xenon lamp as a simulated solar light source, investigating the impact of different pH values on photodegradation (Zhu et al., 2022). These findings indicate that the photodegradation of Dufulin generally follows single first-order (SFO) kinetics. The coefficient of determination (R^2) ranges from 0.8020 to 0.9922 (Table S3), demonstrating a good model

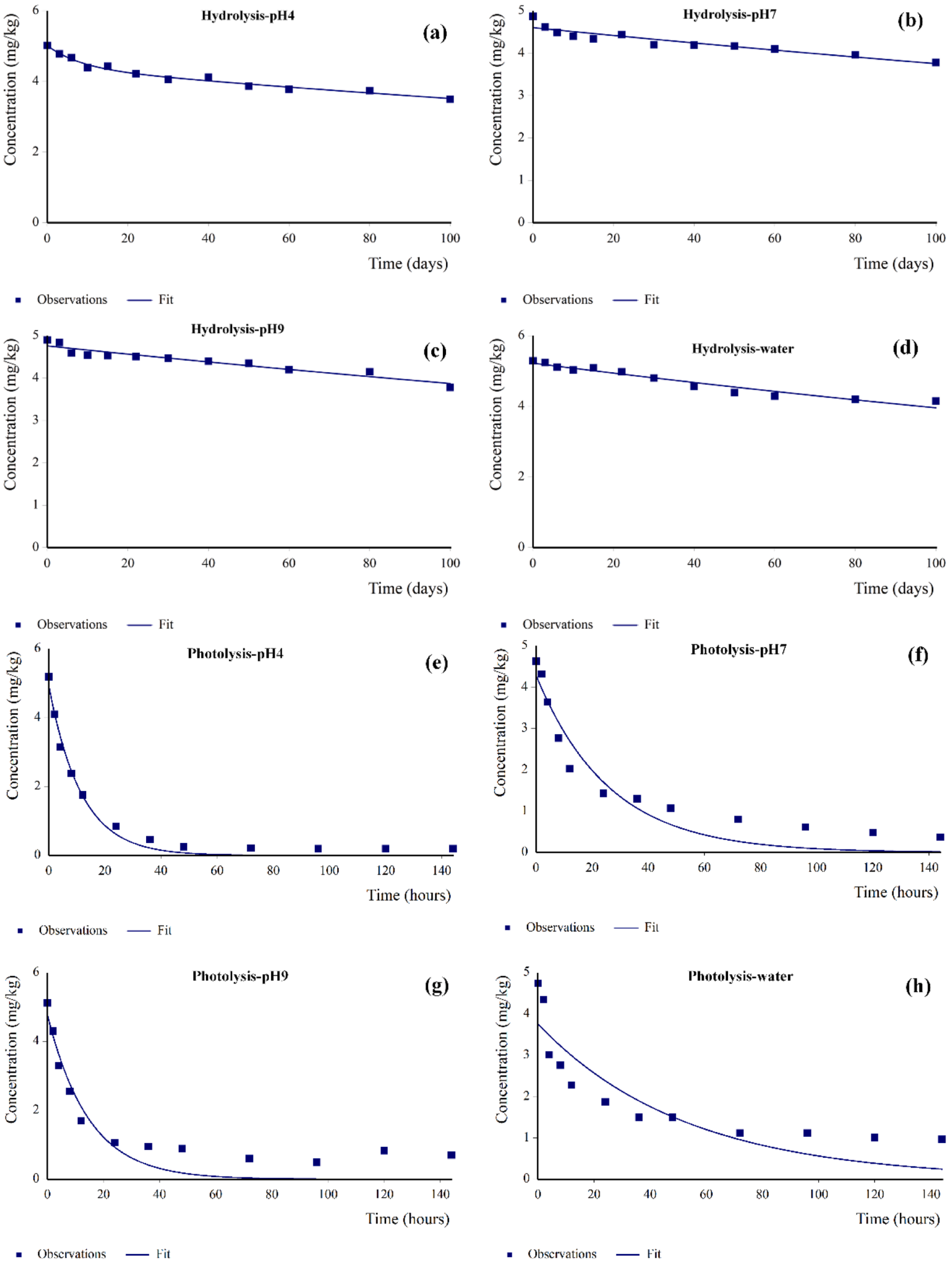


Fig. 1. The hydrolysis and photolysis kinetics curves (SFO) of Dufulin in aqueous solutions at different pH values.

fit. To quantify the uncertainty of the kinetic parameters, we report the rate constant (*k*), its standard error, and the 95 % confidence interval in Table S3. The photolysis half-life of Dufulin at pH 4 and pH 9 conditions is 7.9 and 10.2 h, while in ultrapure water and pH 7 conditions, the half-life is 36.5 and 18.0 h, respectively. The degradation rate of Dufulin is significantly faster under acidic and basic conditions compared to neutral conditions. Currently, no experimental p*K*a data are available for Dufulin. However, based on predicted p*K*a values using ChemAxon, Dufulin is neutral at all three pH values investigated (4, 7, and 9). This is because the basic p*K*a (1.98) is well below the lowest tested pH value of 4, and the acidic p*K*a (13.67) is well above the highest tested pH value of 9. Therefore, the pH dependence of Dufulin photolysis is likely not due to changes in its ionization state. The slow photolysis of Dufulin under neutral conditions might be due to the relatively low reactivity of water molecules as nucleophiles, which makes it difficult for them to effectively attack the phosphate ester bonds, thus slowing down the hydrolysis rate of Dufulin.

3.3. Structural elucidation of transformation products

Using the Biotransformer 3.0 platform, no TPs could be predicted for Dufulin hydrolysis, which is in line with the very slow hydrolysis observed. In contrast 22 TPs were predicted for photolysis. We here could experimentally confirm the formation of 6 TPs formed during photolysis. The identification of Dufulin TPs by HRMS was primarily based on the following criteria: (1) uniqueness (absent in controls, present only in the sample group), (2) time trend analysis, and (3) fragment ion matching (Jiao et al., 2022) which led to the identification of 6 TPs, one of which had been reported but not confirmed. Further confirmation for three TPs was then achieved by matching ^{13}C isotopic mass spectral information. Table 1 summarizes the information on Dufulin's TPs and identifies their confidence levels according to the scheme developed by Schymanski et al. (2014a) Finally, TP standards for confirmation were obtained commercially or via synthesis and injected into the LC-HRMS system at the same conditions. The chromatographic peaks and mass spectrometry fragmentation behavior of the standard and the sample were compared to confirm the TPs (Man et al., 2023).

3.3.1. TP164

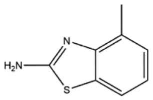
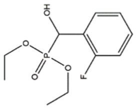
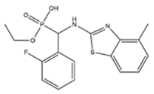
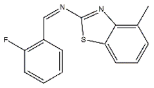
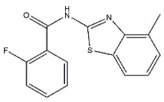
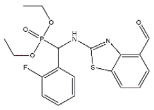
The accurate mass of TP164 is *m/z* 165.04819 with a molecular formula $C_8H_8N_2S$ and a mass error of −0.12 ppm. It exhibits a retention time of 4.94 min and is detectable during both hydrolysis and photolysis of Dufulin. Predictions using Mass Frontier for M164′s theoretical fragment ions revealed six consistent ions (Fig. S5): *m/z* 91.05459 $[C_7H_7]^+$, 106.06544 $[C_7H_8N]^+$, 123.02659 $[C_7H_7S]^+$, 138.03729 $[C_7H_8NS]^+$, 150.02473 $[C_7H_6N_2S]^+$, 165.04819 $[C_8H_9N_2S]^+$. Comparison with the parent compound's structure suggests likely C—N bond cleavage, yielding M164 (2-amino-4-methylbenzothiazole) and M262, as reported in literature, labeling M164 as a suspected compound (Wang et al., 2014).

During the experiment, ^{13}C-labeled and unlabeled Dufulin were added to water at a ratio of 1:1 for hydrolysis and photolysis, and then samples were taken at different times for mass spectrometry. From the mass spectrum, it can be seen that the signals at *m/z* 165.0477 and its (m + 1) ^{13}C stable isotope ion *m/z* 166.0510 display similar peak shapes and retention time (5.10 min), with a relative peak intensity ((m + 1)/m) around 1, and similar characteristic fragments (Fig. 2). These results strongly suggest TP164 as a TPs of Dufulin. A synthetic standard of TP164 was obtained, and its chromatographic and mass spectral characteristics matched those of the TP-samples, confirming TP164 as a Dufulin TPs.

3.3.2. TP262

The retention time of M262 is 8.07 min, with a accurate mass of *m/z* 263.0811 and a mass error of −0.63 ppm, suggesting a molecular formula of $C_{11}H_{16}FO_4P$. As shown in the Fig. S6, based on MS/MS spectrum information, 10 fragment ions were found to match theoretical fragment ions: *m/z* 64.97920 $[H_2O_2]^+$, 97.04500 $[C_6H_6F]^+$, 82.98965 $[H_4O_3P]^+$, 109.04495$[C_7H_6F]^+$, 125.03960 $[C_7H_6FO]^+$, 139.05148 $[C_4H_{12}O_3P]^+$, 169.00439 $[C_7H_6P]^+$, 173.01588 $[C_7H_7FO_2P]^+$, 217.04205 $[C_9H_{11}FO_3P]^+$, 245.07205 $[C_{11}H_{15}FO_3P]^+$. This indicates the parent compound underwent C—N bond cleavage, yielding 2-amino-4-methylbenzothiazole and M262. Due to the absence of ^{13}C-labeled carbon atoms, confirmation through stable isotope methods is not possible. By synthesizing a standard of M262 and injecting under the same chromatographic-mass spectrometric conditions, chromatographic peaks and mass spectral information of both sample and standard were obtained, as depicted in the Fig. S1, exhibiting identical retention times (RT = 8.07 min) and 10 identical secondary fragment ions with *m/z*: 245.07240, 217.04187, 191.02623, 173.01598, 135.06003, 125.03954, 64.09917, and similar fragment ion shapes, confirming M262 as a TPs of Dufulin.

Table 1
List of Dufulin TPs produced during hydrolysis and photolysis. Confidence classification follows the criteria outlined by Schymanski et al. (2014b): Level 1 indicates a confirmed structure verified by a reference standard, Level 2b suggests a probable structure supported by diagnostic evidence.

Proposed product	Structure	Retention time	Formula	Observed $[M+H]^+$	Mass error (ppm)	Confidence level*
TP164		4.94	$C_8H_8N_2S$	165.04819	−0.12	1
TP262		8.07	$C_{11}H_{16}FO_4P$	263.0811	−0.63	1
TP380		9.53	$C_{17}H_{18}FN_2O_3PS$	381.0826	−0.94	1
TP270		13.51	$C_{15}H_{11}FN_2S$	271.0691	−0.2	1
TP286-2		12.94	$C_{15}H_{11}FN_2OS$	287.06406	−0.3	1
TP422		12.27	$C_{19}H_{20}FN_2O_4PS$	423.09616	−0.4	2b

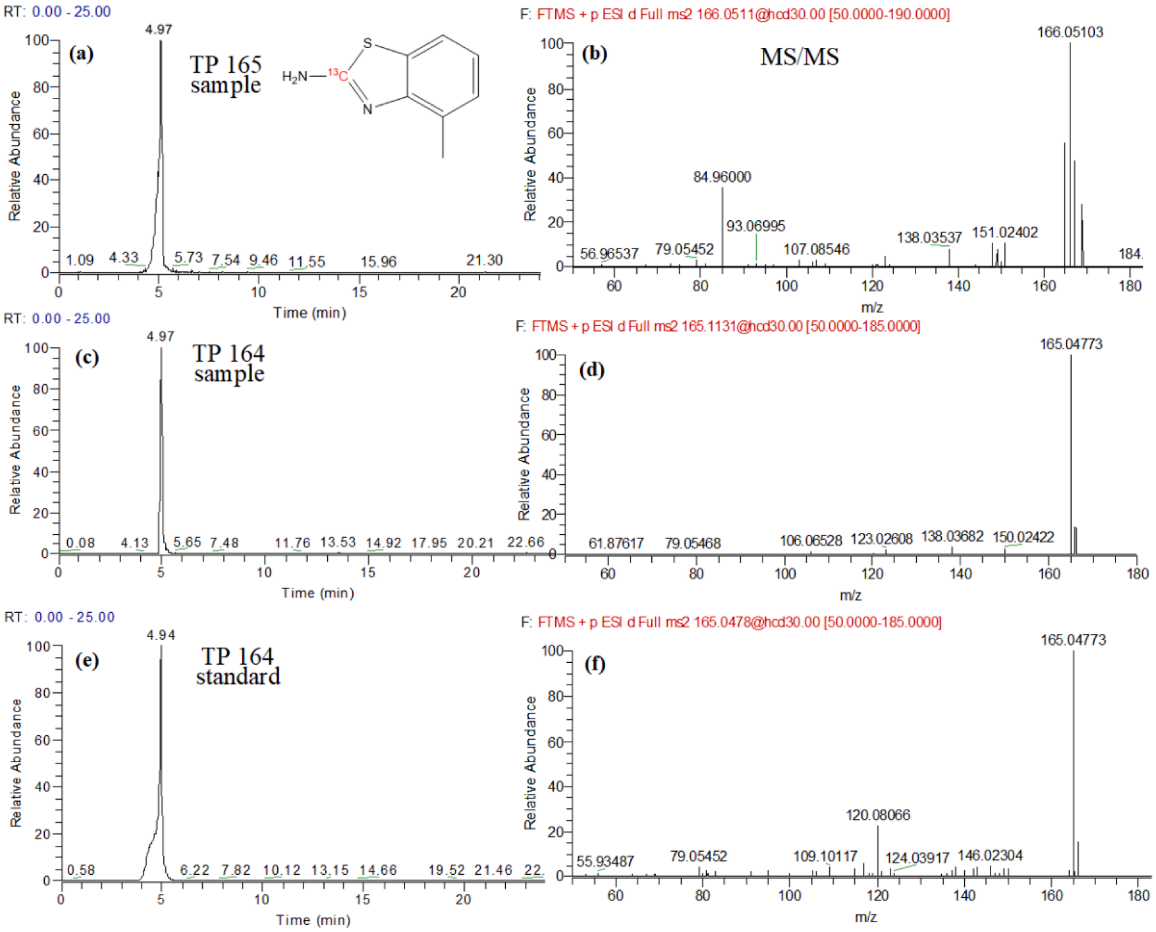


Fig. 2. Chromatogram (a) and mass spectrum (b) of the ^{13}C isotope peak of TP164 in the sample. Chromatogram (c) and mass spectrum (d) of TP164 in the sample. Chromatogram (e) and mass spectrum (f) of TP164 in the standard.

3.3.3. *TP270*

The exact molecular mass of M270 is *m/z* 271.06937, with a mass error of −0.2 ppm, suggesting a possible molecular formula of $C_{15}H_{11}FN_2S$. It exhibits a retention time of 13.51 min and is detectable during both hydrolysis and photolysis processes. It is likely a result of Dufulin undergoing hydrolysis, cleaving the C—P bond and eliminating diethyl phosphate. Analysis reveals seven major fragment ions consistent with theoretical ones, namely (Fig. S7): 122.03995 $[C_7H_5FN]^+$, 123.02628 $[C_7H_7S]^+$, 150.03716 $[C_8H_8NS]^+$, 148.02141 $[C_8H_6NS]^+$, 175.03217 $[C_9H_7N_2S]^+$, 251.06308 $[C_{15}H_{11}N_2S]^+$, 271.06937 $[C_{15}H_{12}FN_2S]^+$.

Signals at RT = 13.50 and RT = 13.52 respectively show peaks at *m/z* 271.06903 and its (m + 1) ^{13}C stable isotope, with a relative peak area intensity ((m + 1)/m) of approximately 1, along with similar characteristic fragments and proportions (Fig. S2). This confirms M270 as a likely TPs of Dufulin. Finally, a standard of M270 was synthesized, exhibiting consistent retention time and secondary mass spectral information, ultimately confirming M270 as a TPs of Dufulin.

3.3.4. *TP380*

The molecular mass of TP380 is *m/z* 381.08258, with a mass error of −0.94 ppm, suggesting a potential molecular formula of $C_{17}H_{18}FN_2O_3PS$. It exhibits a retention time of 9.53 min and is detectable during both hydrolysis and photolysis processes. Compared to the parent compound, TP380 lacks one ethyl group, indicating likely hydrolysis of the parent phosphorothioate group, leading to C—O bond cleavage. Analysis reveals four major fragment ions (Fig. S8): 148.02116 $[C_8H_6NS]^+$, 251.06310 $[C_{15}H_{11}N_2S]^+$, 271.0692 $[C_{15}H_{12}FN_2S]^+$, 381.08258 $[C_{17}H_{19}FN_2O_3PS]^+$, consistent with theoretical fragment ions.

Signals at RT = 9.53 and RT = 9.51 respectively show peaks at *m/z* 381.08258 and its (m + 1) ^{13}C stable isotope, with a relative peak area intensity ((m + 1)/m) of approximately 1. However, due to low response of stable isotope peaks, effective secondary mass spectral information was not obtained, hindering further analysis. Preliminary evidence suggests M380 may be a TPs of Dufulin based on similar retention times and peak intensity ratios. Finally, a standard of M380 was synthesized, and injection under the same chromatographic and mass spectrometric conditions yielded similar retention times (RT = 9.51 min and 9.53 min) and secondary mass spectral information (Fig. S3), confirming TP380 as a TPs of Dufulin.

3.3.5. *TP286-2*

TP286-2 has an exact molecular mass of *m/z* 287.06406, with a mass error of −0.3 ppm, suggesting a potential molecular formula of $C_{15}H_{11}FN_2OS$ and a retention time of 12.94 min. It possesses one additional oxygen atom compared to TP270, indicating likely hydrolysis of TP270, initially forming a hydroxyl group followed by oxidation to a ketone, resulting in TP286-2. Analysis reveals major fragment ions consistent with theoretical ones.

Signals at RT = 12.99 min and RT = 12.97 min respectively show peaks at *m/z* 287.06406 and its (m + 1) ^{13}C stable isotope, with a relative peak area intensity ((m + 1)/m) close to 1, along with similar characteristic fragment ions at *m/z* 116.02154 and 167.03436, 269.05325 and 270.05612, 287.06406 and 288.06729, and similar proportions (Fig. S4). This suggests M286-2 is likely a TPs of Dufulin.

Finally, a standard of M286-2 was synthesized, yielding similar retention times (RT = 12.97 and 12.94 min) and similar secondary mass spectral information, confirming TP286-2 as a TPs of Dufulin.

3.3.6. TP422

The exact molecular mass of M423 is *m/z* 423.09616, with a mass error of −0.4 ppm, suggesting a potential molecular formula of $C_{19}H_{20}FN_2O_4PS$. Relative to the parent compound, it has one additional oxygen atom and two fewer hydrogen atoms, indicating likely oxidation at the methyl group of Dufulin, forming a carbon-oxygen double bond. Four major fragment ions were identified (Fig. S9): 109.04512 $[C_7H_6F]^+$, 265.04236 $[C_{15}H_9N_2OS]^+$, 285.04916 $[C_{15}H_{10}FN_2OS]^+$, 423.09616 $[C_{19}H_{21}FN_2O_4PS]^+$, consistent with theoretical fragment ions. Due to the unavailability of a standard for TP423, further confirmation could not be conducted. According to Schymanski et al.'s classification, it falls under category 2b.

3.4. Quantitative analysis of TPs

Upon confirming the TPs of Dufulin, precise quantitative analysis was conducted to understand its degradation and TP formation in aquatic environments. Due to the slow hydrolysis of Dufulin and the low concentration of TPs, the analysis focused on photolytic TPs. As illustrated in Fig. 3, the newly identified TPs, TP262 and TP286-2, were detected at concentrations of up to 1.35 mg/L and 0.49 mg/L, respectively, after 96 h under acidic conditions. These values represent 27 % and 9.8 % of the initial Dufulin concentration, surpassing or nearing the 10 % threshold of the parent compound. Therefore, TP262 and TP286-2 are considered the main TPs due to their environmental risk. Interestingly, the concentration of TP380 continuously increased under pH 7, pH 9, and water conditions, while under pH 4 conditions, it exhibited a trend of initially rising and then decreasing. This could be due to the fact that Dufulin undergoes the fastest photodegradation under pH 4 conditions, with nearly all of it reacting within 40 h, and the generation of TP380 is also the fastest, as seen in the Fig. 3. However, in the acidic environment, the instability of the phosphate ester group in TP380 causes the carbonyl oxygen in the ester to become protonated, increasing its electrophilicity and making the phosphorus atom more susceptible to nucleophilic attack. As a result, TP380 is further degraded into other reaction products and consumed.

3.5. Deciphering the degradation pathways of Dufulin in aquatic environments

The possible transformation pathways of Dufulin in aquatic environments were proposed based on the structure and dynamic changes of its TPs (Fig. 4). Transformation reactions mainly involve cleavage, oxidation, elimination, and deethylation. The first pathway involves the cleavage reaction of Dufulin, resulting in Benzyl photodeamination to Carbonyl, forming TP164 and TP260. TP260 is unstable and undergoes further reduction to form TP262. This pathway has also been reported in the soil degradation of Dufulin (Hua et al., 2014). The second pathway involves the elimination reaction of Dufulin, where the $C{-}P$ bond is cleaved, leading to the removal of diethyl phosphate and the formation of an unsaturated $C{=}N$ double bond (TP270). Subsequently, the unstable $C{=}N$ double bond undergoes attack by water molecules, leading

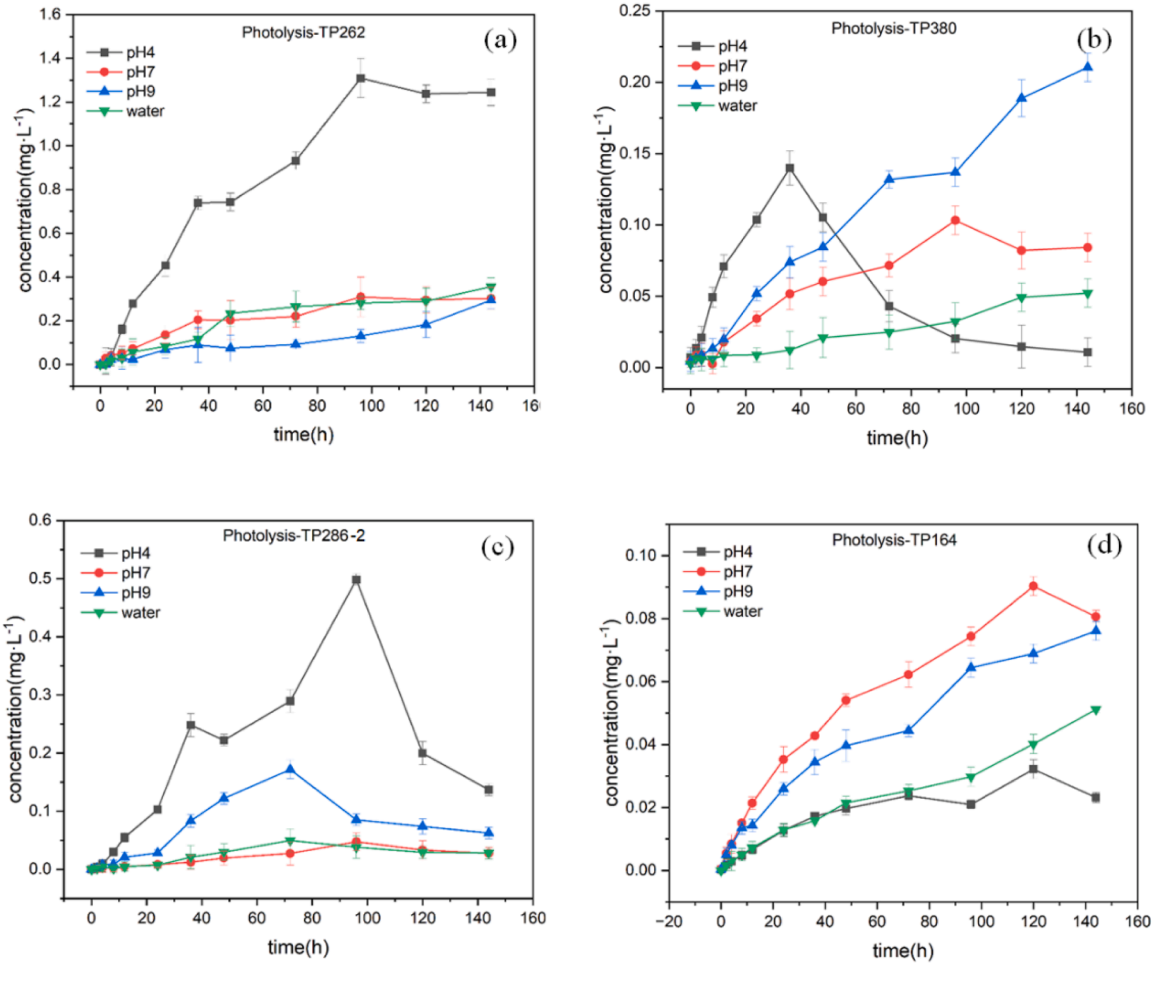


Fig. 3. Concentration trends of TP262 (a), TP380 (b), TP286-2 (c) and TP164 (d) in different pH aqueous solutions during photolysis.

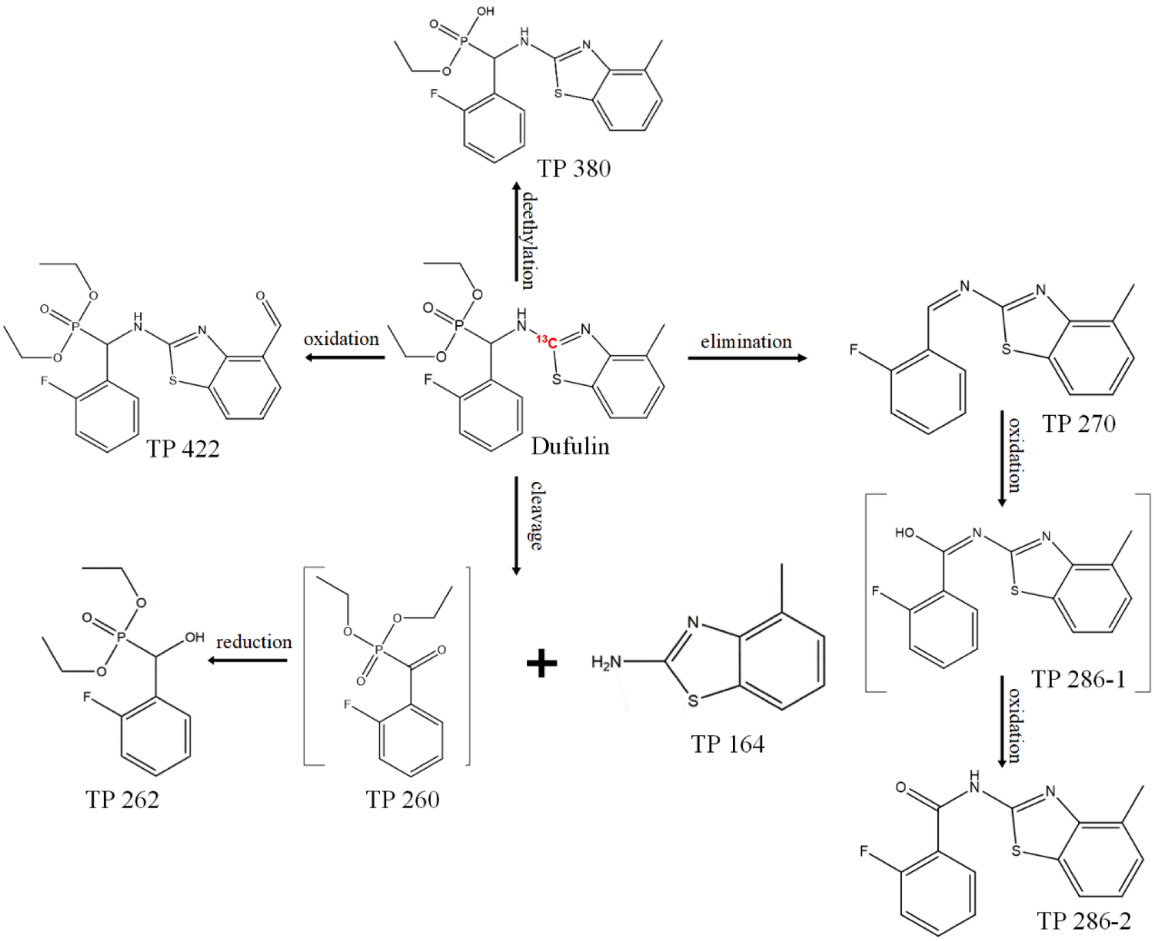


Fig. 4. Photolytic transformation pathways of Dufulin.

to oxidation and the formation of an intermediate, TP286-1. Finally, the hydroxyl group on TP286-1 is oxidized again to form a ketone, resulting in TP286-2. The third pathway involves direct dealkylation of the parent Dufulin, leading to the formation of TP380. Additionally, the oxidation of the methyl position on the benzothiazole moiety of Dufulin generates a ketone moiety (TP422).

3.6. Model based ecotoxicity assessment

Pesticide TPs products can exhibit higher mobility and persistence than the parent compound, increasing the likelihood of exposure to organisms (Escher et al., 2020, Hintze et al., 2020). Additionally, TPs may possess higher toxicity than the parent compound, underscoring the importance of toxicity assessment. This study employed the ECOSAR software to assess the acute and chronic toxicity of Dufulin TPs on fish, green algae, and daphnia, three aquatic organisms. As depicted in the Fig. 5, the predicted results indicate that Dufulin exhibits toxic effects on fish, green algae, and daphnia, with acute toxicity and chronic toxicity falling into the toxic range (1 mg/L < LC50/EC50 < 10 mg/L), and chronic toxicity for fish and green algae classified as highly toxic (LC50/EC50 < 1 mg/L). Predicted toxicities of TP262 and TP380 are reduced, while TP164′s toxicity closely resembles that of the parent compound. Notably, predicted toxicities of TP270 and TP286-2 are significantly higher than that of the parent compound, falling into the highly toxic range. This suggests a potential underestimation of Dufulin's environmental risk, emphasizing the need for greater attention to Dufulin's TPs in the environment.

4. Conclusions and perspectives

Our study demonstrated that Dufulin hydrolysis is significantly slow across various pH conditions, with half-lives extending beyond 200 days. In contrast, photolysis could transform Dufulin at much higher rates. Photolysis was most rapid under acidic and alkaline conditions, with half-lives of 7.9 and 10.2 h, respectively. Our approach utilizing a ^{13}C labeled parent compound allowed us to identify Dufulin TPs, including five compounds reported for the first time. Isotopic analysis preliminarily identified four TPs, while confirmation was achieved using standard substances for five TPs. Using ^{13}C labeled parent compounds in transformation experiments is a promising strategy for future investigations into the formation of unknown TPs from other parent compounds.

This study contributes to filling a crucial gap in understanding the environmental fate of Dufulin and its TPs in aquatic environments. Quantitative analysis revealed that under acidic conditions, TP262 and TP286-2 reached concentrations equivalent to 27 % and 9.8 % of the initial Dufulin concentration, respectively, warranting attention and inclusion in risk assessments.

Data availability

The authors are unable or have chosen not to specify which data has been used.

CRediT authorship contribution statement

Bochi Yu: Writing – review & editing, Writing – original draft,

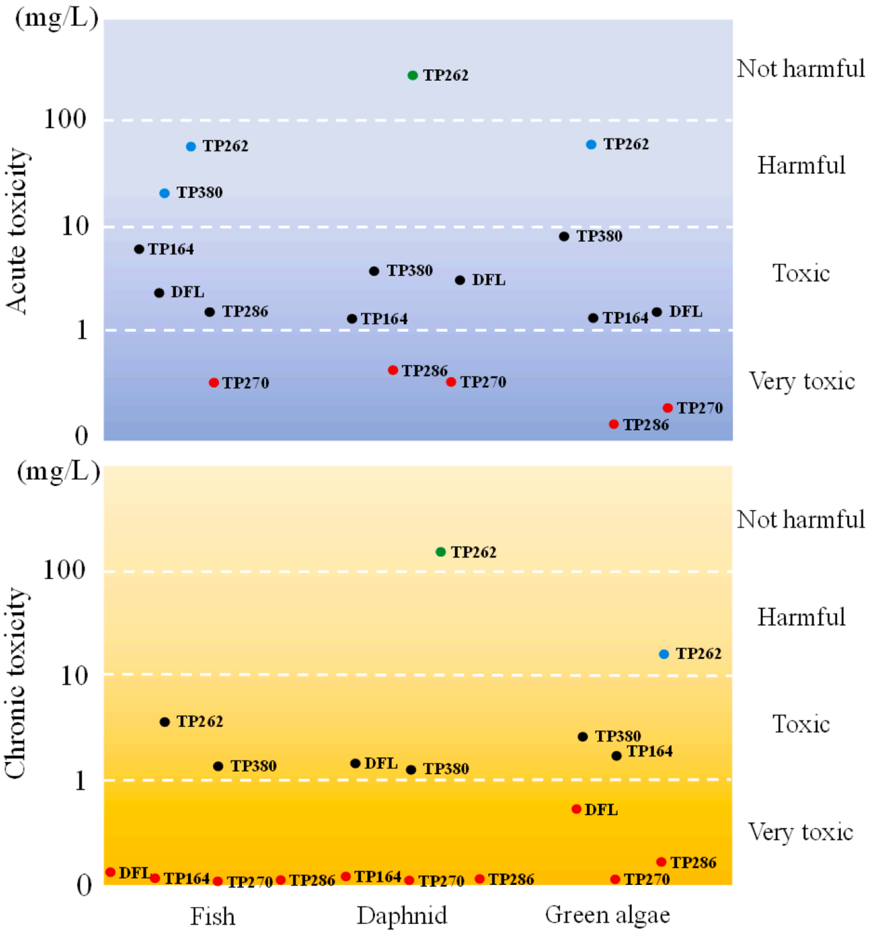


Fig. 5. Toxicity assessment of Dufulin and its TPs by ECOSAR program.

Visualization, Validation, Software, Methodology, Data curation, Conceptualization. **Yanli Man:** Writing – review & editing, Methodology, Data curation. **Gabriel Sigmund:** Writing – review & editing, Project administration, Conceptualization. **Pingping Wang:** Software, Methodology, Data curation. **Jun Xie:** Methodology, Formal analysis. **Wei Wang:** Software, Methodology, Data curation. **Lan Zhang:** Project administration, Formal analysis. **Liangang Mao:** Software, Methodology, Data curation. **Lizhen Zhu:** Supervision, Investigation, Formal analysis, Data curation. **Chi Wu:** Project administration, Methodology, Data curation. **Xingang Liu:** Writing – review & editing, Conceptualization.

Declaration of competing interest

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

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Supplementary materials

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