

Assessment of quinoxifen phototransformation pathways by liquid chromatography coupled to accurate mass spectrometry

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Abstract Quinoxifen has been recently identified as a priority hazardous substance in the field of the European water policy. In this work, its fate in aqueous samples and solid supports under UV and solar radiation is investigated. Diverse degradation experiments were carried out, at lab scale, using spiked aliquots of different aqueous matrices (ultrapure, treated wastewater and river water) irradiated at different wavelengths ($\lambda = 254$ nm, $\lambda = 365$ nm and solar light). Half-lives of quinoxifen (2–26 min) depended on the wavelength and the intensity of radiation whilst the nature of the aqueous matrix did not play an important role in degradation kinetics. Moreover, experiments under solar radiation of doped silicone tubes were performed to simulate degradation when quinoxifen is adsorbed on plant leaves or soil. As the compound is not completely mineralized, the identification of quinoxifen transformation products (TPs) was performed by liquid chromatography quadrupole time-of-flight mass spectrometry (LC-QTOF-MS) injection of different irradiated time aliquots. The full-fragment ion spectra, at different collision

energies, allowed the elucidation of the chemical structure of TPs formed by hydroxylation, cyclization or cleavage reactions. Five out of seven identified TPs have not been reported previously. The ecotoxicity simulation by software (TEST and ECOSAR) for TPs revealed that some of them could cause harmful effects to organisms such as *Daphnia magna* or *Fathead minnow* in a similar extent to the precursor; moreover, the time course profiles of major TPs (TP1 and TP2) revealed a much higher resistance to further photodegradation than quinoxifen.

Keywords Quinoxifen · Photodegradation · Transformation products · Liquid chromatography · Time-of-flight mass spectrometry

Introduction

Quinoxifen (QUI) is a systemic fungicide used mainly to control powdery mildew in cereals and grapes. It has been introduced in several countries of Europe since 1996, and it is commercialized as active species in products such as Apres, Excelsior, Fortress and Orka [1–3]. As regards harmful effects, a high acute toxicity for aquatic invertebrates such as *Daphnia magna* and moderate acute toxicity for some aquatic plants such as *Lemna gibba*, fish such as *Oncorhynchus mykiss* or sediment-dwelling organisms such as *Chironomus riparius* have been reported. In humans, it is a possible liver, kidney, blood toxicant and skin sensitizer [1–3].

According to the above-described potential effects, QUI has been included recently in the Directive 2013/39/EU [4], which calls the attention to the important role of measuring emerging pollutants not regularly considered in monitoring programs, but which can have ecotoxicological and toxicological effects. In this vein, QUI has been identified as a priority

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hazardous substance in the field of water policy [5]. This document also establishes the maximum allowable concentration (MAC) of QUI in continental surface waters ($2.7 \mu\text{g L}^{-1}$).

Consequently, there are several authors that have already incorporated this compound in water monitoring programs based on liquid chromatography coupled to mass spectrometry analysis (LC-MS) [6–10], although, as far as we could trace, no data about its concentration is reported. This could be explained because this chemical is a non-volatile quinoline substance with a log K_{ow} of 4.66. This lipophilic character makes feasible its particle bound transport and its introduction in all environmental compartments with preference for solid hydrophobic matrices [11, 12]. Thus, concentrations in both particulate matter ($1.13\text{--}2.16 \text{ pg m}^{-3}$) [6] and sediments (up to $170 \mu\text{g kg}^{-1}$) have been already reported [13], although concentration found in sediments are often in contradiction with QUI physicochemical properties, models or laboratory results [14, 15]. QUI has been also detected in different fish species such as *Leuciscus cephalus*, *G. gobio*, *Cobitis taenia* and *A. alburnus alburnella* ($3.77\text{--}6.69 \mu\text{g kg}^{-1}$) [14] pointing out to a high potential for bioaccumulation.

Beyond these studies, the research about the fate of QUI in the environment is scarce. A few laboratory reports suggest that in aqueous media, hydrolysis is pH dependent, being stable at $20 \text{ }^\circ\text{C}$ and pH 7, whereas photolysis is rather fast [1–3]. In laboratory-scale aerobic soil degradation studies, half-life ($t_{1/2}$) values range from 224 to 508 days which confirms an important persistent character [1–3]. The predominant mechanism for breakdown is microbial which produces two transformation products at low levels, the 2-oxo-quinoxyfen and the 5,7-dichloro-4-hydroxyquinoline [16]. Volatilization from plants is another reported removal route of QUI [17] which shows a higher tendency to volatilization (approx. 15%) compared to other pesticides (6% for fenpropimorph).

It cannot be dismissed that some compounds as QUI are not completely eliminated (mineralized), but transformed in different environmental by-products (TPs). Sometimes, these TPs are more persistent and/or toxic than their own precursor [18]. For complete removal of these pollutants, and therefore to avoid the emission of the contaminant to the water environment, UV radiation can be routinely used in sewage treatment plants (STPs). The photochemical dissipation of a given compound will depend on many factors, such as the own nature of the compound, the intensity of radiation and the reception matrix (environmental water, airborne particulate matter, soil, sediment, plants, etc.).

In this work, we investigate the behaviour of QUI under UV radiation in both aqueous matrices and solid supports. For this latter purpose, silicone [19, 20] is used to incorporate the analyte and to simulate its degradation in the real environment, where it can be adsorbed to soil particles or plants (leaves). LC-MS, using a hybrid quadrupole time-of-flight

(QTOF) MS instrument was used (1) to follow the time-course of the parent fungicide and (2) to detect and elucidate the potential TPs.

Experimental

Standards, solvents and supports

Acetonitrile, methanol and ethanol, HPLC grade, were provided by Merck (Darmstadt, Germany). Ultrapure water was obtained from a Milli-Q system (Millipore, Billerica, MA, USA). The standard of QUI (99.9%) was purchased from Aldrich (Milwaukee, WI, USA). Stock solutions were prepared in methanol and stored at $-18 \text{ }^\circ\text{C}$. Diluted solutions were made in water and kept in amber vessels, at $4 \text{ }^\circ\text{C}$. Aqueous solutions of the precursor were prepared at the beginning of each phototransformation experiment.

Silicone was acquired from Goodfellow (Bad Nauheim, Germany) in a tubular format, with internal and external diameters of 2 and 3 mm, respectively. The tube was cut in 1-cm-length pieces which were conditioned as reported elsewhere [19]. QUI was incorporated in the silicone supports using 10 mL ultrapure water solutions previously spiked with this fungicide (1 mg L^{-1}). The QUI-loaded silicone pieces were dried with a lint-free tissue and kept in amber vessels at $-20 \text{ }^\circ\text{C}$, until being used in degradation experiments. Each support was used for an only test. Surface and wastewater samples were filtered through glass fibre filters before being spiked.

Degradation experiments

The stability of QUI in water experiments was evaluated in open quartz tubes (o.d. 30 mm), acquired from Afora (Barcelona, Spain). Laboratory studies were carried out using an in-house built photoreactor described elsewhere [18]. Experiments were performed using two different UV sources: a low-pressure Hg lamp (Philips reference G8T5) emitting at 254 nm and a 8-W black light fluorescence lamp (Philips reference F8T5/BLB) with a maximum emission band centred at 365 nm. Both sources provided nominal emission intensities of 2 mW/cm^2 at a distance of 5 cm. Lamps were stabilized for 15 min before the beginning of each experiment. Then, the quartz vessel, containing 25 mL of a spiked ($200 \mu\text{g L}^{-1}$) water solution (either Milli-Q, surface or treated wastewater) was inserted in the reactor and aliquots (ca. 0.5 mL volume plus 0.1 mL of added methanol) were withdrawn at different times. These aliquots were stored in amber autosampler vessels, at $4 \text{ }^\circ\text{C}$, for a maximum of 2 days before analysis. Dark control tests were performed wrapping the quartz tube with aluminium foil for the duration of the experiment. Series of

experiments were carried in different reaction times for the 254 and the 365 nm emission sources, respectively.

The silicone tubes were inserted through a stainless steel wire (1 mm diameter) previously wrapped with Teflon tape, to prevent catalytic effects of metals, or their oxides, in the transformation of precursor fungicides. After a given radiation time, they were stripped (two tubes at each time) from the Teflon-covered wire, with tweezers, and desorbed.

Outdoors studies were carried out at the University campus, in Santiago de Compostela (Northwest Spain) during summer of 2015, with silicone supports maintained at a height of 0.9–1 m from soil surface. See Electronic Supplementary Material (ESM), Table S1, to check meteorological conditions. Control experiments outdoors ($n = 3$ replicates) were performed with supports wrapped with aluminium foil for the duration of the corresponding experiments, and silicone tubes desorbed and analysed as in the rest of experiments. Compounds were recovered from silicone tubes (two tubes at each time) soaking them with 0.5 mL of acetonitrile (desorption recoveries higher than 96%), within a glass insert (0.6 mL capacity) for 15 min. After removing the support, the acetonitrile extract was injected in the LC-MS/MS system without further treatment.

Determination conditions

The time-course degradation experiments of QUI and also the TPs formation and evolution were monitored using a LC-ESI-QTOF-MS system acquired from Agilent Technologies (Wilmington, DE, USA). The instrument consisted of an Agilent 1200 Series LC system furnished with a binary high-pressure mixing pump, an autosampler and an oven for the LC column. The QTOF mass spectrometer was an Agilent 6520 model, equipped with a Dual-Spray ESI source and a hexapole collision cell situated between the quadrupole and the TOF analysers. Compounds (QUI and its TPs) were separated in a Zorbax Eclipse XDB C18 column (100 × 2 mm, 3.5 μm) acquired from Agilent Technologies, under gradient programme and at a constant flow of 0.2 mL min^{-1} . The column, connected to the binary pump after a C18 (4 × 2 mm) guard cartridge from Phenomenex (Torrance, CA, USA), was thermostated at 30 °C within the chromatographic oven. The mobile phases consisted of water (A) and methanol (B), both containing 5 mM of ammonium acetate, and the gradient programme was as follows: 0–2 min, 5% B; 2–4 min, 50% B; 10 min, 100% B, 10–18 min, 100% B; 19 min, 5% B; and 19–27 min, 5% B. The sample injected volume was 20 μL .

QUI and its TPs were determined operating the ionization source in the positive mode (ESI+). The ESI source counted with a secondary nebuliser, which was constantly infusing a mass reference solution (Agilent calibration solution A). Thus, recalibration was continuously performed considering the ions

121.0509 and 922.0098 Da, and the accuracy of m/z assignments guaranteed. Voltages of 4500 V in the capillary and 150 V for the fragmentor were used. The Mass Hunter Workstation software was performed to control all the acquisition parameters of the LC-ESI-QTOF-MS system and also to process the obtained data. The peaks of the potential TPs of QUI were noticed either in the TIC chromatogram (major TPs), by searching for a predicted exact mass using the EIC (extracted ion chromatogram) function and using automated search routines (e.g. Find by Molecular Feature) integrated in the Mass Hunter software. The retention times and accurate masses of $[M + H]^+$ for TPs were obtained and their full scan accurate fragment ion (MS/MS) spectra recorded in further injections. Different collision energies (10, 20, 30, 35, 40, and 45 eV) were tested in order to attain enough spectral information to characterize the structure of photoproducts. Additionally, a few experiments were carried out in order to detect the formation of a specific potential QUI metabolite, 4-fluorophenol operating the spectrometer in the negative mode.

Table S2 in the ESM summarizes some features of the LC-ESI(+)-MS procedure for QUI determination.

Ecotoxicity assessment

The Toxicity Estimation Software Tool (TEST) [21] and ECOlogical Structure-Activity Relationship Model (ECOSAR) (<https://www.epa.gov/tsca-screening-tools/ecological-structure-activity-relationships-ecosar-predictive-model>), developed by the US Environmental Protection Agency (EPA), were used to predict the toxicity of the selected TPs. These tools calculate the toxicity of an organic compound from physical characteristics of its structure, basing the results on mathematical models derived from quantitative structure activity relationships (QSARs). Both the 48-h *D. magna* 50% lethal concentration (LC_{50}) test and the fish (*Fathead minnow*) (LC_{50}) were selected because they are the most frequently used ecotoxicological endpoints.

Results and discussion

Degradation kinetics in water samples

The influence of the type of radiation on the degradation of QUI was evaluated using spiked Milli-Q water exposed to different light sources ($\lambda = 254$ nm, $\lambda = 365$ nm and solar light). Obtained half-life times ($t_{1/2}$) are compiled in Table 1.

Irradiation with two lamps at $\lambda = 254$ nm produces the fastest elimination with $t_{1/2}$ shorter than 2 min in comparison with solar irradiation, which requires almost half an hour $t_{1/2}$ for dissipation of quinoxifen. QUI possesses a maximum UV absorption at 298 nm [1–3] and therefore, radiation at

Table 1 Half-life values ($t_{1/2}$) and first-order rate constants (k) of QUI in different aqueous matrices

	$t_{1/2}$	k	R^2	Time interval	Points
Ultrapure water					
1 lamp, $\lambda = 254$ nm	2.24 min	0.310 min^{-1}	0.993	0–10 min	5
2 lamps, $\lambda = 254$ nm	1.45 min	0.467 min^{-1}	0.997	0–5 min	4
2 lamps, $\lambda = 365$ nm	3.73 h	0.186 h^{-1}	0.991	0–8 h	9
Solar light ^a	0.43 h	1.59 h^{-1}	0.993	0–3 h	10
Treated wastewater					
1 lamp, $\lambda = 254$ nm	2.27 min	0.305 min^{-1}	0.995	0–15 min	10
2 lamps, $\lambda = 254$ nm	1.40 min	0.494 min^{-1}	0.996	0–10 min	9
Surface water					
2 lamps, $\lambda = 254$ nm	1.68 min	0.412 min^{-1}	0.998	0–10 min	9

^a 15 July 2015. See meteorological conditions in Supporting Information

$\lambda = 365$ nm has a low potential for the molecule degradation showing a $t_{1/2}$ value of 3.73 h.

The effect of intensity radiation on the photolysis time was also evaluated. An experiment was carried out by irradiating spiked samples of either Milli-Q water or sewage using either one or two lamps. Results presented in Table 1 are very similar for both matrices showing a slightly faster degradation using two lamps. Thus, whilst the amount of irradiation plays an important role in the degradation rate, the type of matrix seems not to have a relevant influence in the time of photolysis in this particular case. This finding is in agreement with the previously reported behaviour of other pollutants, e.g. the nitrogenated species fluconazole and climbazole [18]. In order to confirm this hypothesis, a different aqueous matrix, a spiked sample of river water, was also investigated obtaining a half-life time in the same order as for the other water matrices, Table 1.

Transformation products detection

In order to detect potential transformation products during the photolysis process, a Milli-Q water sample was spiked with QUI (10 mg L⁻¹ to allow enough concentration of TPs to be determined) and irradiated during 30 min at $\lambda = 254$ nm using just one lamp to allow longer TPs life times.

The detection tool was the Mass Hunter software using the Find by Molecular Feature function. This function performs a spectral deconvolution, grouping ions with the same retention time which are compatible with the same empirical formula. Then molecular features in control experiments (zero time and dark controls) and irradiated samples were compared. Figure 1 shows the LC chromatogram observed after 30 min of irradiation. Depicted chromatograms correspond to their $[M + H]^+$ ions extracted within a mass window of 20 ppm. The code used to name each TPs is simply TP followed by a number from 1 to 4. In the case that the TP is suspected to be originated from another TP instead from QUI, TP with the number or the precursor and a second number is utilized. As it can be

noticed, the peak of QUI is still observed after 30 min of UV irradiation. All of the TPs appear at shorter retention times than QUI, pointing out to possible more polar structures, and they were neither present before irradiation nor in the dark control assays.

With the help of the Mass Hunter software, the empirical formula of TPs was obtained from both the exact mass of the $[M + H]^+$ species in the extracted ion chromatogram and also the relative intensities ions in the $[M + H]^+$ cluster of signals. Most TPs are chlorinated compounds, so the mass defect existing between ³⁵Cl and ³⁷Cl was checked to be present in their pseudo-molecular ($[M + H]^+$) ions.

Table 2 compiles the generated empirical formulas. The mass error is also shown, being in all cases lower than 6 ppm, which reveals an elevate coincidence of the experimental mass for $[M + H]^+$ with the theoretical exact mass. This is also corroborated by the score (Table 2) higher than 90 for most TPs, considering 100 as the perfect match between accurate masses and isotopic profiles predicted for the proposed formulas and the experimental MS data. In the case of TP22, despite the relatively low mass error, the calculated score was rather poor. In this particular case, the low intensity of this TP disturbed the isotopic profile of the $[M + H]^+$ cluster of ions. Double bond equivalents (DBE) for detected TPs are also compiled in Table 2. This data is useful to elucidate potential molecular structures for the identified TPs. It must be noted that TP1 and TP2 were also present in wastewater and TP1-4 in river water with a QUI spiked level of 200 ng mL⁻¹.

Transformation products structure investigation

Figure 2a–e shows the accurate production mass spectra of QUI and the main four tentatively identified TPs, detected in the water samples. The QUI MS/MS fragmentation pattern consists of loss of HCl followed by cleavage of the ether bond to render the fragment ions at m/z 95.0300 and 213.9828. Another characteristic ion is that at m/z 196.9799 corresponding to the chlorinated quinoline structure, Fig. 2a. However, in the

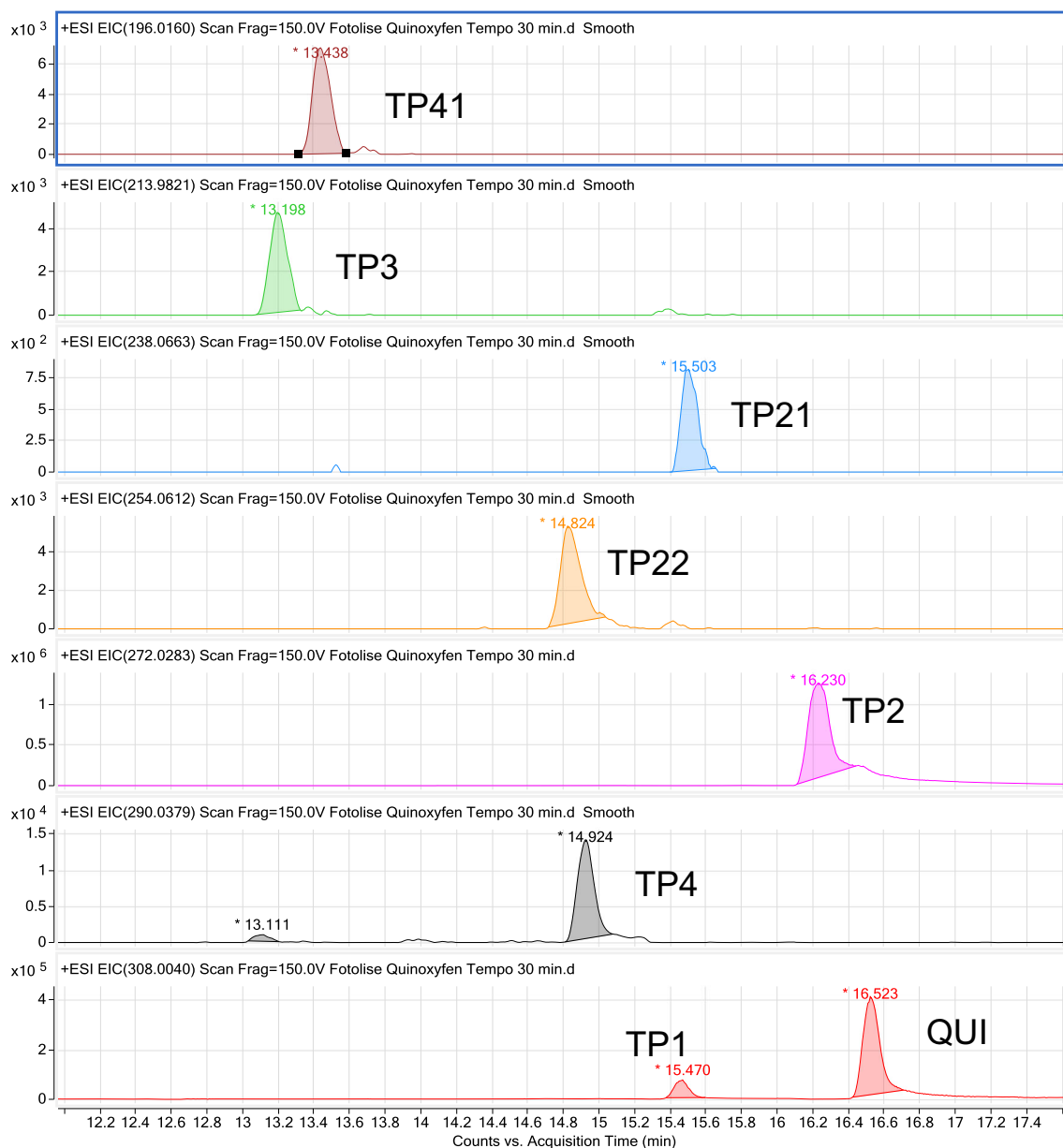


Fig. 1 Selective LC-MS chromatograms (mass window ± 20 ppm) for QUI and its TPs in an irradiated (254 nm) ultrapure water sample

Table 2 Identification by LC-ESI(+)-QTOF-MS of quinoxifen and its TPs

Compound code	Empirical formula (M)	Retention time (min)	DBE (double bonds equivalents)	Exact mass [M + H] ⁺	Experimental mass [M + H] ⁺	Mass error (ppm)	Score (MS)
QUI ^a	C ₁₅ H ₈ Cl ₂ FNO	16.523	11	308.004	308.0046	-1.94	98.25
TP1	C ₁₅ H ₈ Cl ₂ FNO	15.470	11	308.004	308.0037	0.97	98.83
TP2	C ₁₅ H ₇ ClFNO	16.230	12	272.0283	272.0273	3.67	99.87
TP21	C ₁₅ H ₈ FNO	15.400	12	238.0663	238.0656	2.94	80.06
TP22	C ₁₅ H ₈ FNO ₂	14.830	12	254.0612	254.0600	4.72	65.62
TP3	C ₉ H ₅ Cl ₂ NO	13.198	7	213.9821	213.9815	2.80	95.94
TP4	C ₁₅ H ₉ ClFNO ₂	14.924	11	290.0379	290.0362	5.86	83.51
TP41	C ₉ H ₆ ClNO ₂	13.438	7	196.0160	196.0152	4.08	94.6

^a Initial concentration: 10 mg L⁻¹, 1 lamp $\lambda = 254$ nm, 30 min

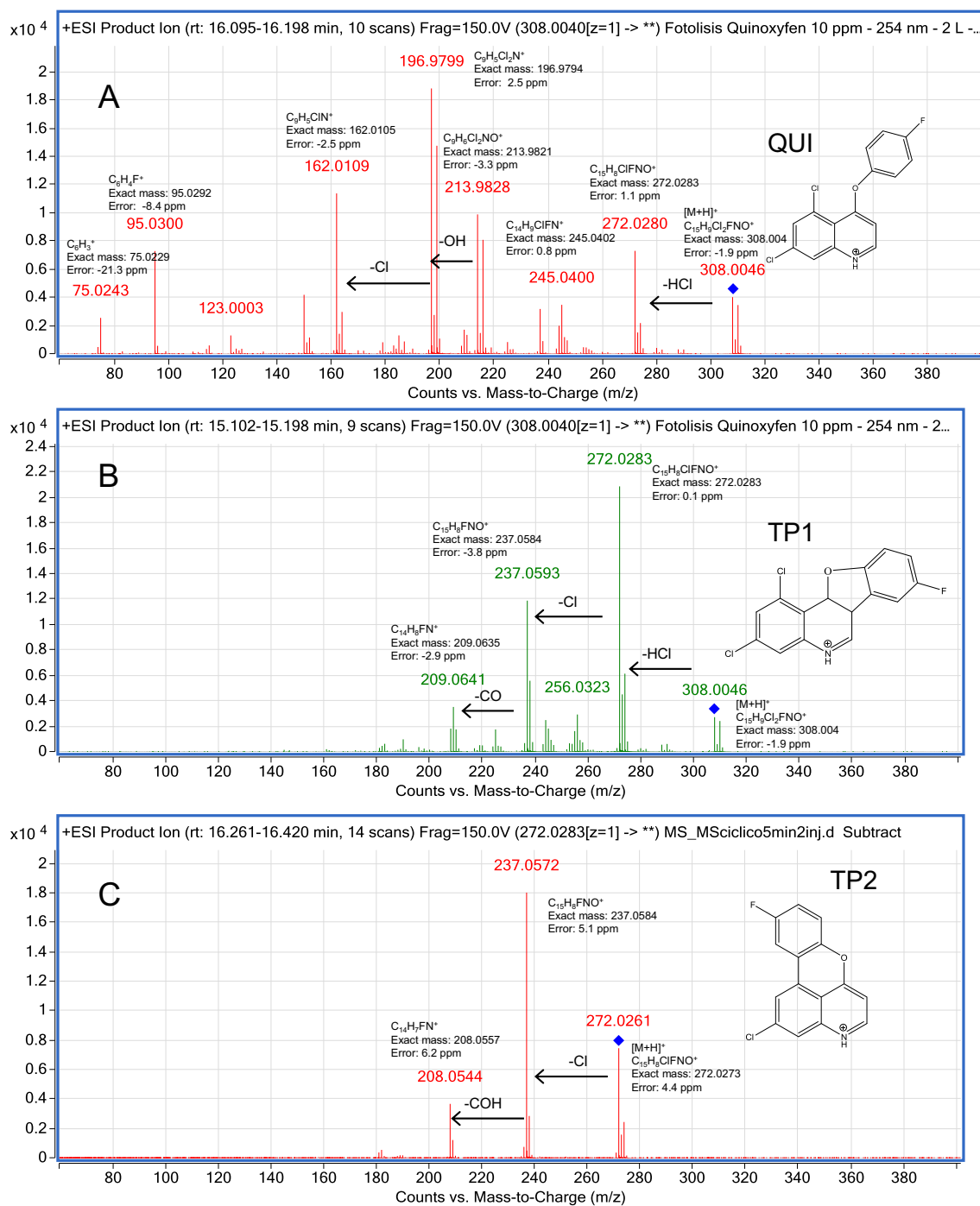


Fig. 2 Fragment ion scan (MS/MS) spectra and structures of QUI (a), TP1 (b), TP2 (c), TP3 (d) and TP4 (e)

case of TP1, which possesses exactly the same formula of QUI, main transitions are the losses of HCl, Cl and CO, being the latter the result of the ring opening, Fig. 2b. No characteristic masses of the ether bond cleavage are present in the scan MS/MS spectrum which points to a cyclic structure. TP1 is suspected to result from the hydroxylation of QUI in the quinoline structure and further loss of a water molecule with simultaneous cyclization. To the best of our knowledge, this compound is described as a QUI photoproduct for the first time.

TP2 is the most abundant TP and arose from the loss of HCl and simultaneous intramolecular cyclization of QUI. It has been described before as a QUI photoproduct [1]. The fragmentation pattern consists of mainly chlorine and COH losses, Fig. 2c.

TP3 corresponds to the chlorinated hydroxyquinoline structure which is originated from cleavage of the ether bond in the molecule of QUI. It must be noticed that, other molecular species resulting from this cleavage could be 4-

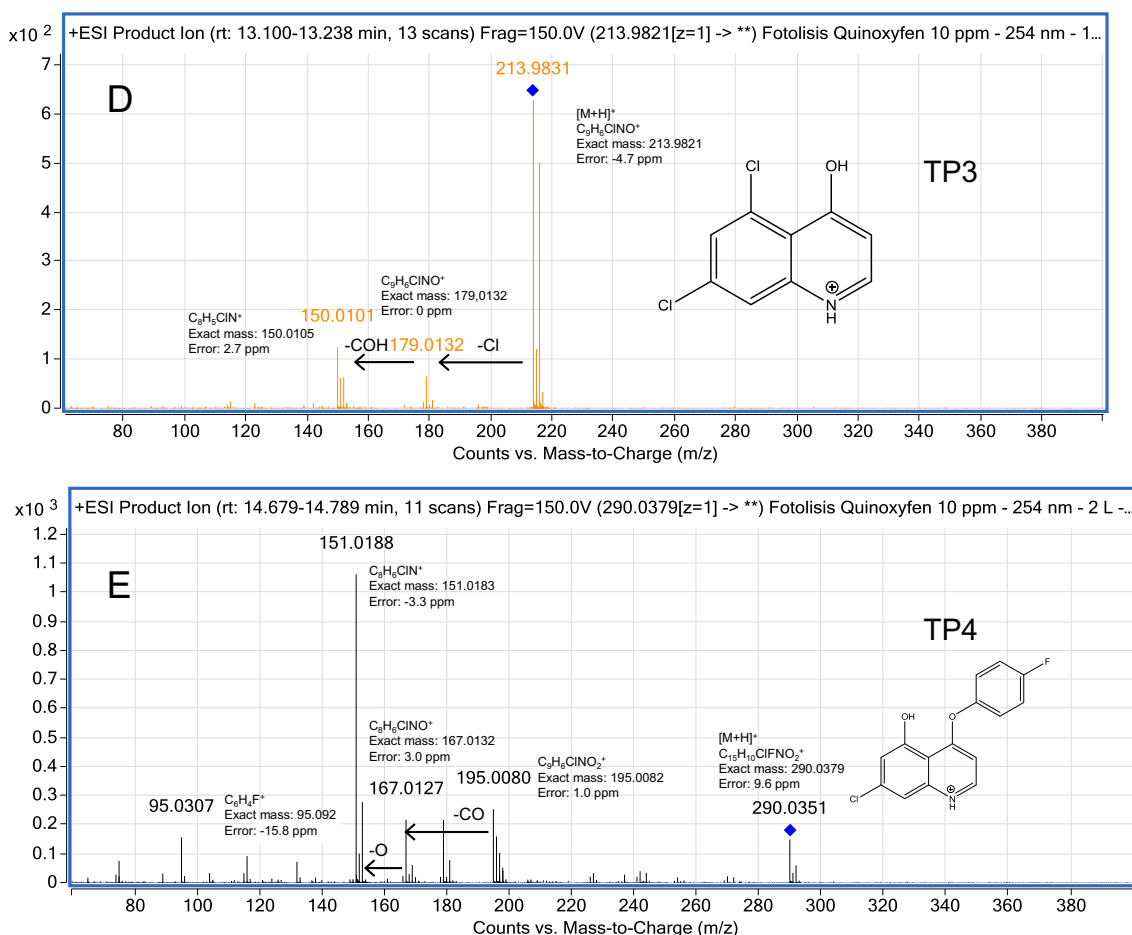


Fig. 2 (continued)

fluorophenol. However, although this compound has been described as a quinoxifen metabolite in plants and animals [1], it could not be detected in any water extracts either using the positive or negative ESI modes.

TP3 fragmentation pattern shows the loss of Cl and COH with the consequent opening of the quinoline structure, Fig. 2d. TP3 has already been described as a photodegradation product of QUI as well as a hydrolysis product at acidic pH. It is also a QUI metabolite in animals and plants [1].

TP4 consists of the substitution of a chlorine atom by a hydroxyl group in the structure of QUI, Fig. 2e. To the best of our knowledge, this compound has not been previously reported in the literature. Its mass spectrum is basically represented by the cleavage of the ether bond and losses of O and CO. The fragment ion at m/z 95 (nominal value) corresponds to the fluorobenzene ion and it confirms the break of the ether bond. The small peak observed at 13.1 min (Fig. 1) could correspond to the substitution of the other chlorine atom in the molecule, but due to the weakness of the signal, the MS/MS spectrum was not obtained.

TP21 (see Fig. S1 in the ESM) would correspond to a chlorine loss of the TP2 and TP22 to the hydroxylation of

TP21. TP41 would be the quinoline structure generated by the cleavage of the ether bond in TP4. Neither of these three TPs has been previously reported in the literature. Obviously, they have to be considered as secondary TPs.

Figure 3 represents the predicted photodegradation pathway followed by QUI in aqueous matrices. In summary, there are four possible vias for transformation: hydroxylation, loss of water and cyclization (TP1), dechlorination and cyclization (TP2), cleavage of the ether bond (TP3) and dechlorination and hydroxylation (TP4). The compound named ITP1 could be a potential intermediate in the formation of TP1, because 3-hydroxyquinoxifen ($C_{15}H_8Cl_2FNO$, exact mass 323.9989) has been described as a QUI metabolite in animals and plants [1]; however, its presence could not be detected in any of the analysed samples. TP2 could follow further transformation by dechlorination (TP21) and hydroxylation (TP22). Either TP3 or TP4 could originate TP41. TP3 would undergo dechlorination followed by the addition of a hydroxyl group. The cleavage of the ether bond in TP4 would generate TP41. Major routes of degradation would be those generating more stable cyclic TPs.

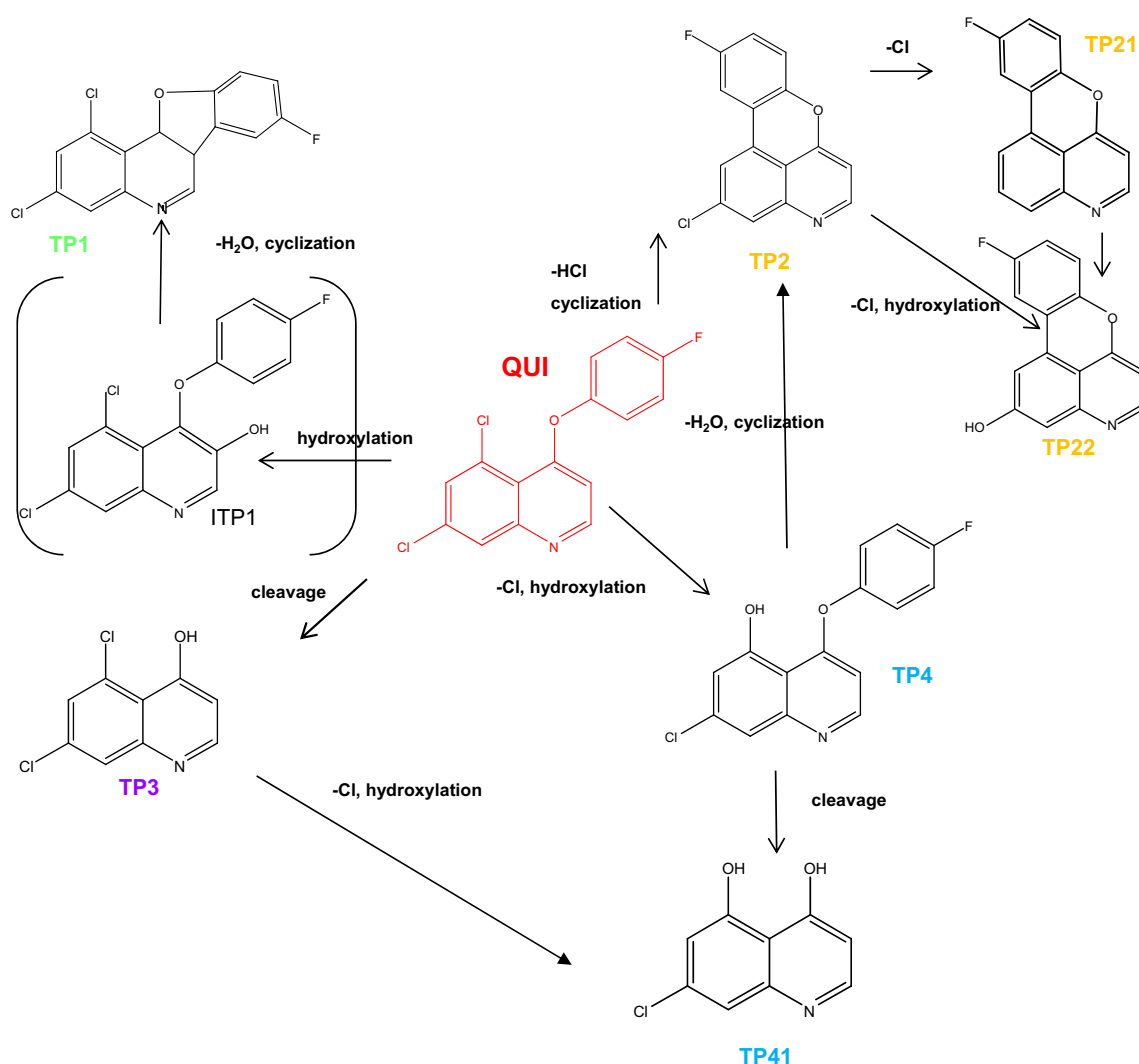


Fig. 3 Proposed phototransformation routes of QUI

Time-course of TPs

Figure 4 represents the time-course of QUI and its major photoproduct TP2. The evolution of the TP formation versus QUI dissipation in two different real water samples: river and sewage, under UV radiation, is shown. Responses in the y-axis are the normalized ratios between the peak area for the $[M + H]^+$ ion of each TP at the considered irradiation time and that measured for QUI at zero time. This cyclic compound is much more stable in real water samples than its precursor which disappears in a few minutes (Fig. 4a, b). Regarding solar radiation, the same pattern is obtained for a spiked ultrapure water sample, but kinetics is much slower. QUI completely disappears in 2 h, whilst more than 50% of TP2 is still present after 8 h of radiation, Fig. 4c (see *ESM* for meteorological conditions (15th July)).

Figure 5 provides the time-course of the minority TPs. Under UV radiation of a spiked ultrapure sample, all of them appear with a normalized response relative to that of

QUI at zero time equal or lower than 3%. Just TP1 and TP4 are not detected after 2 h; the rest of TPs are still present in the water sample after this period, Fig. 5a. Under solar radiation, apart from TP1, just TP3 and TP4 are detected, Fig. 5b. The time-course profile of TP3 presents a maximum between 1 and 2 h and after that the signal decreases to disappear after 4 h. TP4 is formed in a minor amount, but it is still present after 8 h of radiation. Despite the low responses of these TPs, it must be noticed the high stability shown compared to QUI. Under solar irradiation, no secondary TPs (TP 21, TP22 and TP 41) were noticed.

Transformation in solid matrices

Apart from environmental water matrices, QUI applied in agriculture can reach other environmental compartments such as soils or plant leaves. There is almost no data about the photodegradation of QUI over these matrices. It has

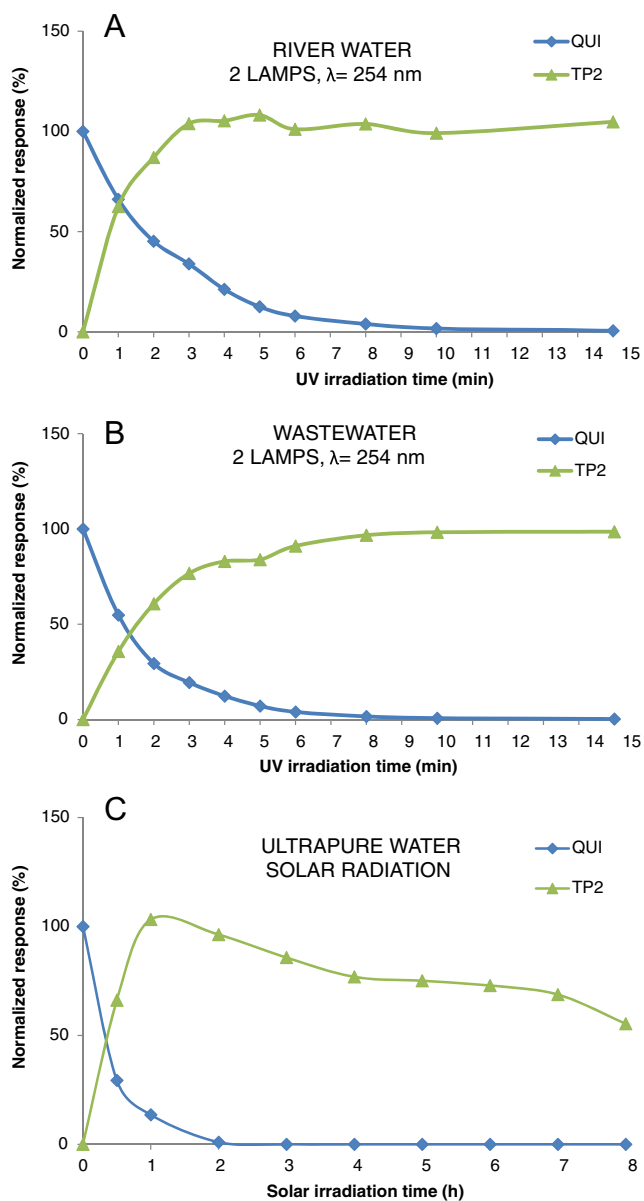


Fig. 4 Time course of QUI and its major phototransformation product in different aqueous matrices under both UV (river (a) and wastewater (b)) and solar radiation (ultrapure water (c))

only been described the formation of TP2 via surface photolysis in plants [1] and of TP3 during aerobic soil metabolism [1].

In this work, we used silicone tubes as solid supports to emulate real solid environmental matrices such as soil or plants [19]. In a first series of experiments, tubes were doped with QUI, as explained in the experimental section and exposed to UV radiation ($\lambda = 254$ nm). The dissipation of QUI followed a pseudo first-order kinetics until 20 min, showing a $t_{1/2}$ of 15.2 min ($k = 0.046 \text{ min}^{-1}$, $R^2 = 0.993$). After that, it become rather stable and its concentration decreased much slower, still existing 20% of the compound after an hour (data not shown). This kind of time-course profile (fast initial

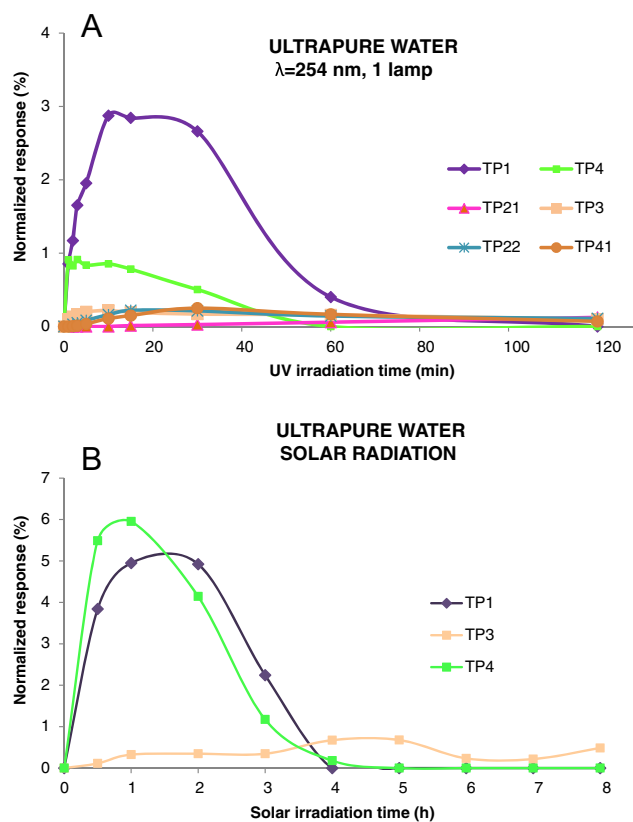


Fig. 5 Time-course of minority TPs of QUI in ultrapure water under both UV (a) and solar radiation (b)

degradation followed by a second slow removal step) has been reported as characteristic during photodegradation of many pesticides from soil surface [22]. The only TP detected was TP2 which was correlated to the precursor dissipation arising more than 20% of the QUI signal after 1 h. Thus, it seems obvious that QUI photolysis and the formation of this main TP are much slower if they are performed over solid matrices instead of aqueous matrices.

Figure 6 shows the time course of QUI dissipation under outdoors environmental conditions. The meteorological conditions are reflected in the ESM (Table S1) and correspond to both a foggy and a sunny day during summer 2015. As it can be noticed, QUI $t_{1/2}$ approaches 3–4 h (foggy day: $k = 0.156 \text{ h}^{-1}$, $t_{1/2} = 4.4$ h, $R^2 = 0.990$; sunny day: $k = 0.166 \text{ h}^{-1}$, $t_{1/2} = 4.2$ h, $R^2 = 0.991$), but after that time, signal becomes almost constant. This could be due to some difficulty of radiation to access the QUI molecules adsorbed in the silicone. As it can be also observed in the figure, both cyclic derivatives TP1 and TP2 are detected under solar exposure of QUI doped silicone tubes. Moreover, the intensity of radiation plays an important role in the time and degree of transformation of the compound, being higher during the sunny day; but even in a foggy day, the photodegradation of QUI and the formation of its major TPs keep on happening.

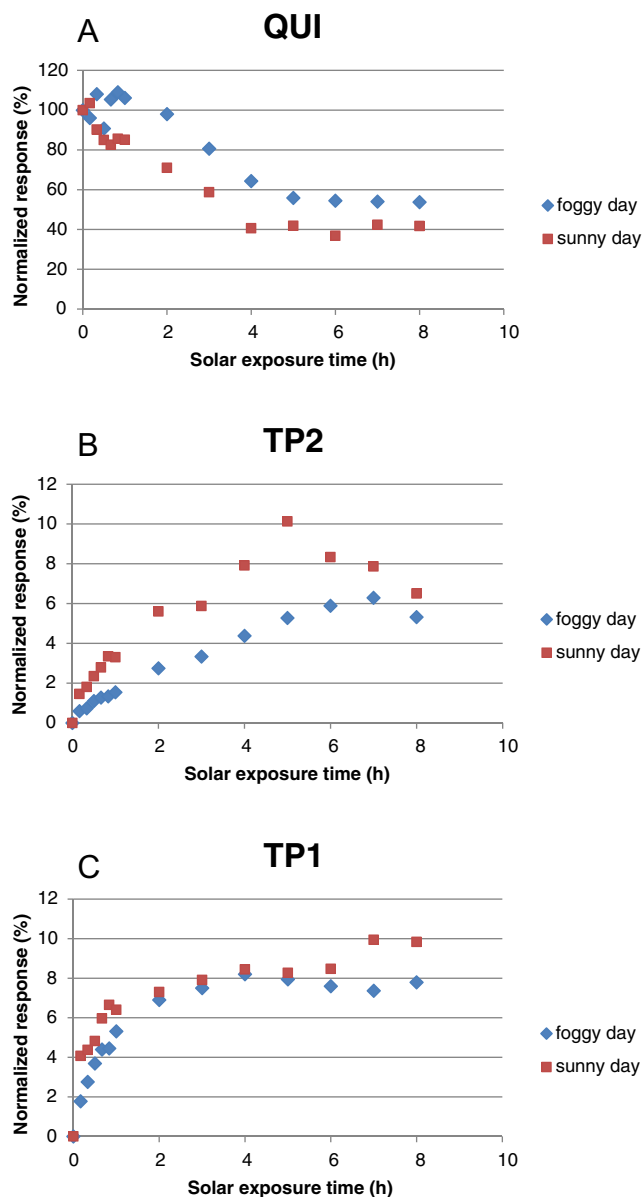


Fig. 6 Time-course of QUI (a) and its major photoproducts (b, c) on silicone supports observed under exposure to environment conditions

Ecotoxicity

The EPA TEST and ECOSAR softwares were employed to predict the toxicity of the TPs either to *D. magna* or *Fathead minnow*. Table 3 compiles the results obtained. As it can be elucidated, major TPs of QUI (TP1 and TP2) present toxicities in the same order of magnitude as QUI when TEST is used; however, the ECOSAR predicted value for TP1 is more conservative (one order of magnitude higher). LC₅₀ for *D. magna* were lower, as expected, than those for fish using both programs (just TP41 shows higher LC₅₀ for *D. magna* than for fish using ECOSAR). This fact is very important, because it has been demonstrated that these cyclic TPs of QUI are much more stable than their precursor against phototransformation.

Table 3 Predicted 50% lethal concentrations (LC₅₀)

Compound	TEST (LC ₅₀) (mg/L)		ECOSAR (LC ₅₀) (mg/L)	
	<i>D. magna</i> (48 h)	<i>Fathead minnow</i> (96 h)	<i>D. magna</i> (48 h)	<i>Fathead minnow</i> (96 h)
QUI	0.075	0.18	0.098	0.12
TP1	0.091	0.15	0.84	1.16
TP2	0.18	0.19	0.41	0.31
TP21	1.59	1.24	0.97	1.36
TP3	3.05	3.56	3.24	7.03
TP4	0.12	0.34	0.53	0.57
TP41	6.08	7.10	111.5	13.3

TP4 presents also a high toxicity but it is predicted to be formed in lesser extent. Therefore, QUI presents rather short $t_{1/2}$ under different photolysis conditions, but it has been proved that the compound is not completely mineralized but transformed mainly in more stable cyclic, with a similar toxicity, compounds.

Conclusions

The investigation of the transformation routes of QUI upon exposure to different light sources in both aqueous and solid matrices, including the structural elucidation of the generated TPs from their accurate scan MS spectra after LC analysis was carried out. QUI was found to be rapidly dissipated from aqueous and solid matrices with $t_{1/2}$ of several minutes, whilst it was transformed to two major cyclic TPs which reported much higher stability and similar toxicity to that of the precursor. Therefore, although QUI seems to be quickly dissipated by photolysis, the formation of toxic and photostable TPs could be a serious concern for the environment, and this must be taken into account when photolysis is employed during sewage treatment.

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Compliance with ethical standards In this article, no research with human participants and/or animals was performed.

Conflict of interest The authors declare that they have no conflict of interest.

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