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Ecotoxicity and photodegradation of Montelukast (a drug to treat asthma) in water

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ABSTRACT

The present work focuses on the ecotoxicological effects of montelukast sodium (MTL) and its photoproducts, obtained under environmentally-like conditions. Despite of the potential presence in surface waters and the common use of MTL as asthma drug, limited data has been published for its photodegradation, while no information is available for its ecotoxicity. Light-induced degradation is an effective way for drugs to degrade in aquatic environments, and MTL is highly photosensitive, even by exposure to sunlight. In this study, solar-simulated irradiation of the drug in water was investigated. The drug was quickly converted into a series of photoproducts that were spectroscopically characterized. The possible photoreaction pathways were proposed. Ecotoxicity tests were performed on parent compound and mixture of photoproducts towards two bioindicators (*Raphidocelis subcapitata* and *Daphnia magna*). Results evidenced that effects of MTL on *D. magna* (EC50 = 16.4 mg/L) were greater than effects on *R. subcapitata* (EC50 = 195.7 mg/L). Microscopy observations revealed that MTL had mainly accumulated in the gut of daphnia. Toxicity data on photolysed solutions highlighted the presence of residual toxicity in all samples, evidencing that no complete mineralization occurred. Future research should focus on monitoring of MTL concentrations in the environment and study its effects in bioaccumulation tests.

1. Introduction

In the past three decades, the great concern about the occurrence of pharmaceutically active compounds (PhACs) in aquatic environments is due to their potential subtle effects on key sensitive non-target organisms (Barenboim and Kozlova, 2018; Caracciolo et al., 2015; Mejías et al., 2021; Sanderson, 2011; Sangion and Gramatica, 2016; Watanabe et al., 2016).

Due to long environmental half-lives and hydrophilicity many PhACs remain in the environment and this results in their persistence and accumulation (Fontes et al., 2020; Lofrano et al., 2018). Most of these compounds are physiologically active and may adversely affect aquatic organisms by causing morphological and metabolic changes (Grzesiuk et al., 2020; Lofrano et al., 2016, 2018; Maselli et al., 2017; Sabir et al., 2019; Siciliano et al., 2019). Some other drugs are degraded by biological processes and/or abiotic factors (hydrolysis or redox or light-induced reactions) and lead to transformation products that can be potentially more persistent or toxic than the parent compounds (Calza et al., 2021; DellaGreca et al., 2014; Ibáñez et al., 2021; Khan et al., 2020).

Commonly, drug monitoring and ecotoxicological studies select compounds relying on ease of detection, brand recognition, or, in many cases, few categories of interest like antibiotics (Dong et al., 2013). Information is available only for a small proportion of the 3000 PhACs currently in use (Dong et al., 2013; Hickmann et al., 2016; Patel et al., 2019; Taylor and Senac, 2014). As a consequence, developments of monitoring and prioritization methodologies are key to screen compounds with potential environmental hazard data (Burns et al., 2018; Desbiolles et al., 2018; Durig et al., 2019; Zhou et al., 2019). Thus, an increasing number of studies supports these approaches to rank or prioritize PhACs in the environment using different criteria and models (Dong et al., 2013; Emara et al., 2019; Gaston et al., 2019; Patel et al., 2019; Sorell, 2016).

Montelukast sodium (MTL), sodium 1-(1(R)-(3-(2-(7-chloro-2-

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quinolinyl)-(*E*)-ethenyl)phenyl)-3-(1-hydroxy-1-methylethyl)phenyl) propyl)thio)methyl)cyclopropane)acetate (**1**) (Fig. 1), is classified in a class of asthma drugs called leukotriene blockers (Nayak, 2004) and is considered high priority pharmaceutical, neverthelessits occurrence in the environment and behavior in aquatic biota are still lacking of data (Daughton, 2014; Dong et al., 2013; Ji et al., 2016; Roos et al., 2012).

Identification of MTL as high priority candidate is based on its consumption and/or calculations of environmental loads, distribution coefficient or aquatic toxicity calculated using ECOSAR (Dong et al., 2013; Eichhorn, 2013; Roos et al., 2012; Silva L.J.G., 2011). In particular, Dong et al. (2013) combining the numbers of prescriptions, metabolism, WWTP removal, and multiple toxic endpoints, ranked MTL in the highest priority scores, primarily for its high rankings in aquatic endpoints, and listed the drug among top-20 ecotoxicologically most relevant compounds in a risk prioritization.

MTL has been approved in over 100 countries worldwide for prescription treatment of asthma and in over 50 countries for prescription treatment of allergic rhinitis (FDA, 2014).

However, few data dealing with the presence of MTL in natural waters are available in literature. In 2015, MTL was studied in wastewater effluents of treatment plants located in Portugal, but it was detected under its limit of quantification or undetected (Ribeiro et al., 2015). The drug has been added to the class of pharmaceuticals with high production volume that are not detected in the environment even if estimated to be persistent and/or bioaccumulative (Daughton, 2014; Howard and Muir, 2011). So, although studies demonstrating the occurrence of MTL as pollutant in treatment plant effluents or in surface waters are not available, its presence in water bodies cannot be excluded.

Chronic and acute ecotoxicity data on MTL were only collected from the Swedish FASS database of pharmaceuticals (www.fass.se), which reported EC50 (median effective concentration) above 0.07 mg/L for *Daphnia magna*, above 0.08 mg/L for *Pimephales promelas* and 1000 mg/L for *Raphidocelis subcapitata*. The estimated NOEC (No Observed Effect Concentration) for *P. promelas* was 0.07 mg/L (FASS, 2011).

Roos et al. (2012) stated that the environmental risk, based on the ratio between predicted environmental concentration of the substance (PEC) and the highest concentration of the substance that does not have a harmful effect on the environment (PNEC) was 0.7. All these values indicate that this drug should be considered harmful to the aquatic environment (Roos et al., 2012).

Regarding its environmental behavior, there are several studies describing MTL as highly photolabile (Smith et al., 2004), even under normal laboratory light (Tiwari et al., 2018), or by exposure to daylight either in solution or as powder. Its photochemical behavior has been generally examined in alcohols (Hernandez Duran et al., 2015; Maafi and Maafi, 2014; Roman et al., 2010, 2011) and MTL *cis*-isomer and MTL S-oxide **2** (See Scheme 1) are the main light-induced products (Al Omari et al., 2007). Predicted physicochemical, absorption,

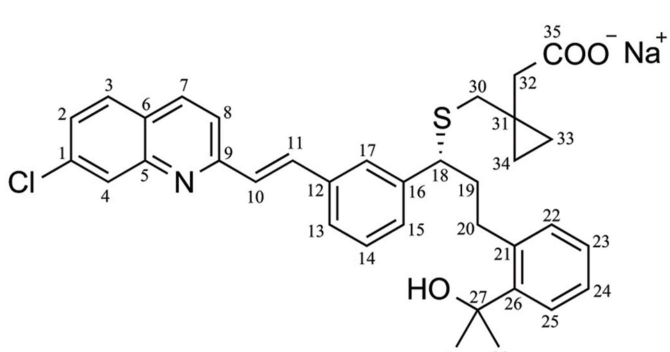

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Fig. 1. Montelukast (1) chemical structure.

distribution, metabolism, excretion and toxicity (ADMET) properties of some degradation products have been also reported (Tiwari et al., 2018), as well as the biological effects of some derivatives via *in silico/in vitro* genotoxicological assessments (Emerce et al., 2015). Indeed, it is clear that attention should be paid also to the environmental metabolites as light-induced degradation products, since they could retain the biological activity of the parent compound. To the best of our knowledge, there are no studies that have examined the toxicity of MTL photoproducts on aquatic organisms. Because of the high photosensitivity of the drug, these products have more potential to appear in the aquatic environment.

Based on the above, the aim of the present study was to contribute to a better understanding of the fate of MTL in aquatic environment and its ecotoxicity. In particular, the work investigated the behaviour of MTL in water under sunlight or solar-simulated UVA irradiation and tested the ecotoxicity of the drug and its photodegradation products on the algae *Raphidocelis subcapitata* and the crustaceans *Daphnia magna* in order to estimate the potential negative effects of MTL before and after photodegradation.

2. Materials & methods

2.1. Chemicals

Montelukast (>98%) sodium was purchased by Kemprotec. Solvents were of HPLC grade and were purchased from Sigma Aldrich. Water was of Milli-Q quality and was obtained from a Milli-Q gradient system (Millipore).

2.2. Chemical analysis

Analytical and preparative TLCs were made on Kieselgel 60 F254 plates with 0.2 mm and 0.5 mm layer thickness, respectively (Merck). Solid Phase extraction was performed with Sep-Pak cartridges (Waters).

NMR spectra were recorded on a Varian Inova-500 instrument operating at 499.6 and 125.6 MHz for ^{1}H and ^{13}C, respectively, and referenced with deuterated solvents ($CDCl_3$). The carbon multiplicity was evidenced by DEPT experiments. The proton couplings were evidenced by ^{1}H–^{1}H COSY experiments. The heteronuclear chemical shift correlations were determined by HMQC and HMBC pulse sequences.

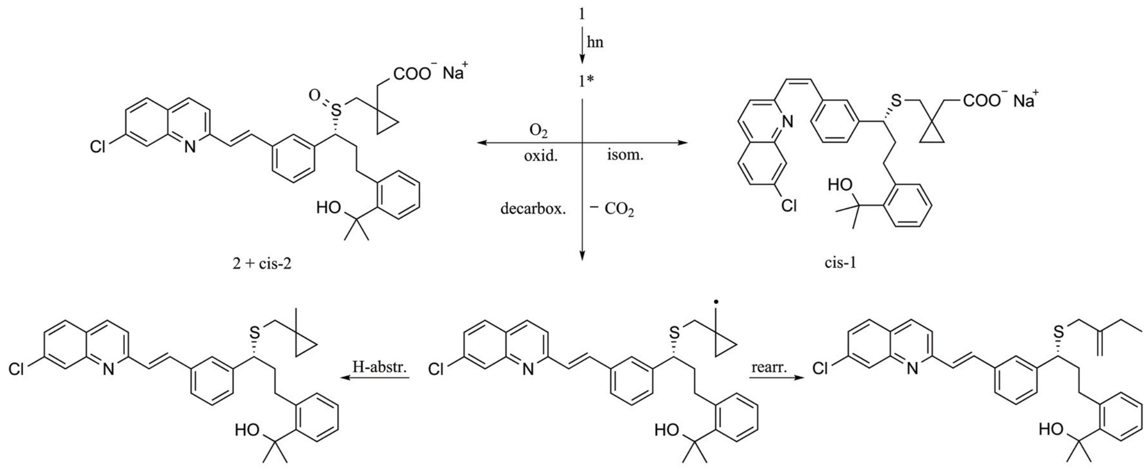
UV–Vis spectra were recorded with a Jasco V730 spectrophotometer.

HPLC experiments were carried out on an Agilent 1100 HPLC system, equipped with an UV detector set at 280 nm, using a RP-18 column (Gemini, 5 μm, 110 A, 250 mm × 4.6 mm) at a flow rate of 0.4 mL min^{-1}. HPLC analysis was performed under isocratic conditions and a mixture A of CH_3OH/H_2O (0.1% formic acid) or a mixture B of CH_3CN/H_2O (0.1% TFA acid) in 90:10 v/v was used as eluent. For preparative experiments a RP-18 column (LUNA, 10 μm, 100 A, 250 mm × 10 mm) was used with mixture A as eluent and a flow rate of 2 mL min^{-1}.

LC-MS experiments were carried out on an Agilent 1100 HPLC system equipped with a quadrupole MS spectrometer detector, using a RP-18 column (Gemini, 5 μm, 110 A, 250 mm × 4.6 mm) at a flow rate of 0.4 mL min^{-1} using the same solvents in the same proportions as used for HPLC analysis. LC-MS analyses of photoproducts were performed using a positive mode.

Irradiations were performed using a Multirays photochemical reactor (Helios Italquartz). The apparatus is constituted by an airy radiation chamber composed from one carousel with two rotating disks that support quartz test tubes and UV lamps fixed in a special lampholder. Four UVA lamps (15 W; 55 V) with a maximum at 366 nm and quartz tubes (20 cm × 1 cm x 25 mL) were used.

A 150-W solar simulator equipped with a Xenon lamp and a filter to simulate irradiation at the earth surface (Oriel instrument) was also used.



Scheme 1. Photoproducts and their formation pathways.

2.3. Experimental plan

MTL solutions (200 mg/L, 3.3×10^{-4} M to have clear solutions) were prepared in MilliQ water and then stored at 4 °C in the dark until being analyzed.

Dark experiments were carried out at room temperature and the MTL solutions were analyzed by HPLC at different times (1, 2, 5, 8 and 14 days).

MTL solution was exposed to sunlight in a quartz tube in October 2019 in Naples (Italy) and analyzed after 15, 30, 45, 60, 120, 240 min using mixture A as eluent.

In the photolysis experiments, the MTL solution (40 mg in 200 mL of water) was irradiated in the photoreactor by UVA lamps, using quartz tubes, and monitored by HPLC. After 5 min of irradiation, the solvent was evaporated under vacuum and the residue was analyzed by ^{1}H NMR and separated by Sep-Pak using, in succession, 10 mL of CH_2Cl_2, CH_2Cl_2/ethyl acetate (AcOEt) (1:1 v/v), AcOEt, CH_3OH. Each fraction was separated by HPLC and the products identified by NMR spectra.

The fraction eluted with CH_2Cl_2 (13.5 mg) was further separated by preparative HPLC to give compounds **3** and **4** (Scheme 1). Compound 4 was identified by comparison of its NMR data with those reported in literature (Gandhi et al., 2016). The structure of compound 3 was assigned on the basis of spectroscopic data. The numbering of the protons and carbons in the NMR spectra of **3** is reported in Fig. 1.

(*E*)-2-(2-(3-(3-(2-(7-chloroquinolin-2-yl)vinyl)phenyl)-3-((1methyl-cyclopropyl)-methylthio)-propyl)phenyl)propan-2-ol (**3**): LC-MS m/z 542 $[M-H]^+$; ^{1}H-NMR (CD_3OD) δ 8.31 (1H, d, H-7), 8.01 (1H, d, H-4), 7.91 (1H, d, H-3), 7.82 (1H, d, $J = 16$ Hz, H-11), 7.52 (1H, d, H-2), 7.61 (1H, d, H-17), 7.44 (1H, d, $J = 16$ Hz, H-10), 7.35–7.40 (3H, m, H-13, H-14 and H-15), 7.25 (1H, d, H-25), 7.13–7.18 (4H, m, H-22, H-23 and H-24), 4.00 (1H, m, H-18), 3.11 (1H, m, Hb-20), 2.90 (1H, m, Ha-20), 2.51 (1H, d, Hb-30), 2.42 (1H, d, Ha-30, 2.20 (1H, m, Hb-19), 2.15 (1H, m, Ha-19), 1.54 (3H, s, H-28), 1.53 (3H, s, H-29), 1.10 (3H, s, H-32), 0.4 (2H, m, Ha-33 and Ha-34), 0.2 (2H, m, Hb-33 and Hb-34); ^{13}C-NMR (CD_3OD) δ 149.8, 141.7, 138.7, 138.3, 137.9, 137.4, 132.9, 131.0, 130.5, 129.6, 129.3, 128.7, 128.6, 128.3 (overlapping), 128.0, 127.8, 127.0, 121.3, 74.4, 52.0, 43.3, 41.7, 34.0, 32.3, 23.4, 16.6, 14.4, 14.8.

The fraction eluted with CH_3OH (11 mg) was separated by repeated preparative TLC using hexane/AcOEt and gave **1** and *trans*-sulphoxide **2**. The latter was identified by comparison of its NMR data with those reported (Dufresne et al., 1996).

For all products HPLC chromatograms evidenced the presence of two peaks due to *trans*- and *cis*-isomers in ca. 8:2 ratio.

2.4. Ecotoxicity analysis

Toxicity tests were performed to determine the effect of MTL and its photolysis products on *R. subcapitata* and *D. magna*. Artificial freshwater, containing $CaCl_2 \cdot 2H_2O$ (294 mg/L), $MgSO_4 \cdot 7H_2O$ (123 mg/L), $NaHCO_3$ (64.8 mg/L) and KCl (5.75 mg/L), was used for the preparation of test solutions and for the control medium. Temperature, conductivity, dissolved oxygen and pH were measured in the test media before and after exposure.

Measured concentrations 1–200 mg/L (dilution ratio 1:2) were used for toxicity tests. Toxicant stock solution (potassium dichromate: 1000 mg/L) was prepared with distilled water and was diluted was diluted with different media according to the specific test procedures of each bioassay.

An ISO protocol 2012 was used to measure the algal growth inhibition with *R. subcapitata* (ISO, 2012). For each sample, six replicates were inoculated with 10^4 algal cells·L^{-1} in 6-well plates with 10 mL of each treatment solution to the respective well plate. The plates were incubated for 72 h at 24 ± 2 °C under continuous illumination (in the irradiance range of 120–60 μein· m^{-2} s^{-1}) on an orbital shaker (70 rpm).

The toxicity of the samples on *D. magna* was assessed by viability, morphological alterations and immobilization toxicological endpoints. Four replicates of five daphnids were exposed to different concentrations of samples in the darkness to ensure no MTL photolysis in aqueous solution during exposure time. All details of the acute toxicity test using daphnids were in accordance with ISO 6341 (ISO, 2012a).

All solutions of Montelukast in the artificial freshwater used as medium for the toxicity tests (see below) (1–200 mg/L) were periodically analyzed during toxicity test by HPLC using mixture B as eluent. In detail, total MTL concentrations were measured at the start of the toxicity bioassay (0 h) as well as at the conclusion of the assay (48 h or 72 h) to verify the concentrations in exposures. All exposure scenarios had a corresponding dark control without organisms.

Results were expressed as the mean (±standard deviation) of the percentage effect. Whenever possible, ecotoxicity data were expressed as the EC50 (median effect concentration) values and its 95% confidence intervals.

Immobility was quantified at 24 and 48 h and daphnids that were unable to swim within 15 s after gentle shaking, were considered immobile.

The specific growth rate of *R. subcapitata* in each replicate was calculated from the logarithmic increase in cell density in the interval 0–72 h as follows:

$$\mu = \frac{\ln N_i - \ln N_0}{t_i - t_0}$$

where N_i represents the cell concentration at time t_i. This parameter was used to calculate the percentage of growth inhibition compared to the negative controls.

The significance of the differences between the mean values of different tests and controls was assessed by Student's test, analysis of variance (ANOVA) with a 0.05 significance level and *post-hoc* analysis carried with Tukey's test. Statistical analyses were performed using Graphpad Prism software and XLSTAT.

3. Results and discussion

3.1. Photochemical behaviour of MTL

The UV-spectrum of montelukast in water (Fig. 2) evidences that the drug absorbs in a wide range of wavelengths up to 400 nm and it is highly photosensitive, as shown by the evolution of the spectra of the solution when exposed to simulated sunlight. Preliminary control experiments confirmed that as reported in the literature (Al Omari et al., 2007), MTL was recovered unchanged by keeping it in the dark in water after 15 days.

The drug was then exposed to sunlight. HPLC analysis evidenced that the drug was photochemically degraded and led to a series of photoproducts. Fig. 3 reports the HPLC profile obtained after 60 min. Similar chromatographic profile in reduced time (5 min) was obtained by irradiating solutions of the drug by UV-A lamps.

The products were identified by comparison of their retention times with those of pure samples. The latter were obtained by HPLC and TLC chromatography and spectroscopically characterized (structures in Scheme 1). HPLC chromatogram of each fraction showed a main peak accompanied by a smaller one with identical mass attributable to the transand *cis* forms, respectively, due to the presence of a double bond in the structure. Attempts to separate them failed due to the efficient light-induced *trans* and *cis* isomerization.

The product at R_t 18.03 min exhibited the same molecular ion at *m/z* 586 as MTL and was identified as *cis*-**1**. The mass of the product at R_t 15.43 min was *m/z* 602 ($mass_{drug}$ + O) attributable to sulphoxide **2**, and, particularly to *trans*-isomer by comparison of NMR data with those reported (coupling constant between H-10 and H-11 equal to 16.1 Hz; Dufresne et al., 1996). Compound *cis*-**2** was eluted at R_t 13.58 min.

The photoproducts at R_t 39.91 min (and 30.92 min) and 43.82 min (and 32.70 min) exhibited the same molecular ions (*m/z* 542), indicating a removal of a 44 mass unit from the drug. This depicted the decarboxylation (loss of CO_2) in both compounds *trans*-**3** (and *cis*-**3**) and

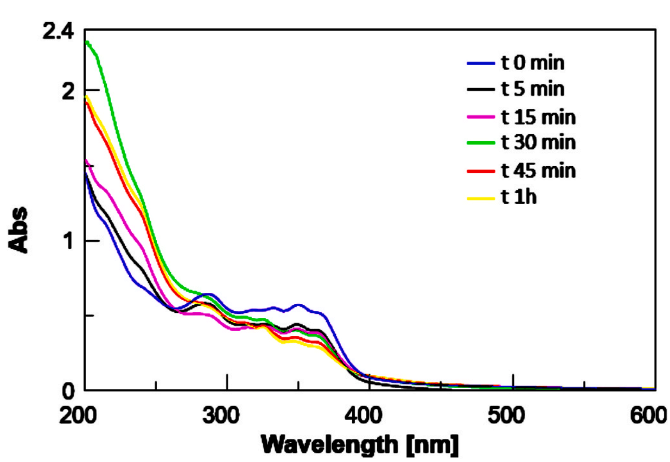


Fig. 2. Evolution of the UV spectra of 3.3 x 10-5 M solution of MTL in water when exposed to a simulated sunlight.

trans-**4** (and *cis*-**4**), respectively. Compound *trans*-**4** was identified by comparison of NMR data with those reported by Gandhi et al. (2016). This product was evidenced by exposing the drug to sunlight and spectroscopically characterized (Gandhi et al., 2016).

Structure *trans*-**3** was assigned to photoproduct at R_t 39.91 min on the basis of NMR data. In the proton spectrum due to the presence of methyl group linked to cyclopropane ring a singlet at δ 1.10 integrated for three protons is present and is related to a carbon signal at δ 23.4. This signal correlates in the HMBC spectrum to cyclopropane protons. Formation of decarboxylated product **3** has been recently evidenced in a solid state sample of the drug that was kept in oxygen-purged desiccator (Tiwari et al., 2018). The authors assigned the structure on the basis of HRMS and multi-stage tandem spectrometric data. Here, NMR data allow to complete spectral characterization.

Scheme 1 summarizes all the photoproducts isolated (**2**–**4**) and their suggested mechanistic pathways. In addition to the well-known light-induced sulphide oxidation and photoisomerization (Turro et al., 2012), the excited drug undergoes a decarboxylation reaction. It is probable that the α-breakage to the carboxylic group leads to a radical intermediate that can add hydrogen to give product **3** (Tiwari et al., 2018) or rearrange to **4**.

The photodecarboxylation reaction is likely favored by the presence of the acetic group at a tertiary carbon and by the aqueous medium. Indeed, it is reported that photodecarboxylation is significant when the carbon linked to the carboxylic group is secondary or tertiary and the reaction does not occur in organic solvents (Isobe et al., 1992). This suggests a key role of water as solvent in the photochemical routes for compounds 3 and 4, previously detected only in small amounts (Gandhi et al., 2016; Tiwari et al., 2018).

All compounds obey a *trans*–*cis* photoisomerization mechanism whereby (*E*)-ethenyl moiety rotates to the (*Z*)-geometry.

3.2. Acute and chronic ecotoxicity

Results from quality assurance and quality control procedures with potassium dichromate were in agreement with the relative toxicity test protocols. In this study, tests were performed with MTL (**1**) and, also, with the mixture of photoproducts obtained by UV-A irradiation of the drug in water after 5 min.

The MTL toxicity tests revealed that daphnids were more sensitive to MTL with EC50 of 32.51 mg/L (30.25–34.94 mg/L) and 16.4 mg/L (13.40–20.10 mg/L) for 24 h and 48 h of exposure respectively, while algae were less sensitive, with an EC50 of approximately 195.7 mg/L (170.1–225.2 mg/L) (Fig. 4).

These EC50 values of MTL could only be compared, as previously mentioned, with those supplied in Swedish FASS database and a discrepancy was observed for *D. magna*. This could probably depend on different test conditions (these previous tests were conducted using solvent).

When compared to algae, *D. magna* proved to be more sensitive towards MTL. The mechanism of toxicity remained unknown but microscopical observations showed that daphnids at 48 h under static conditions revealed the presence of clear morphological changes as well as a comparable growth in all groups of exposure (*t*-test) (Fig. 5).

Under MTL exposure (48 h), *D. magna* retained the drug in the gut (Fig. 5). Changes were observed in the gut filling: an orange terminal gut under 13 mg/L, 25 mg/L and 50 mg/L of MTL was observed (Fig. 5 c, d and e). The uptake in the gut increased as the exposure concentration increased. This hypothesis was strongly supported by the HPLC measurement of the MTL in exposure media in which 50% of the initial concentration decreased at all tested concentrations, in contrast to aqueous MTL concentrations that remained stable over the 48 h in the dark and in the absence of organisms. Previous experimental evidence demonstrated that the reduction of ferric ion into ferrous ion by MTL in the presence of some ligand formed a highly stable complex (ShanmukhaKumar et al., 2010). Therefore, it is plausible that the orange gut

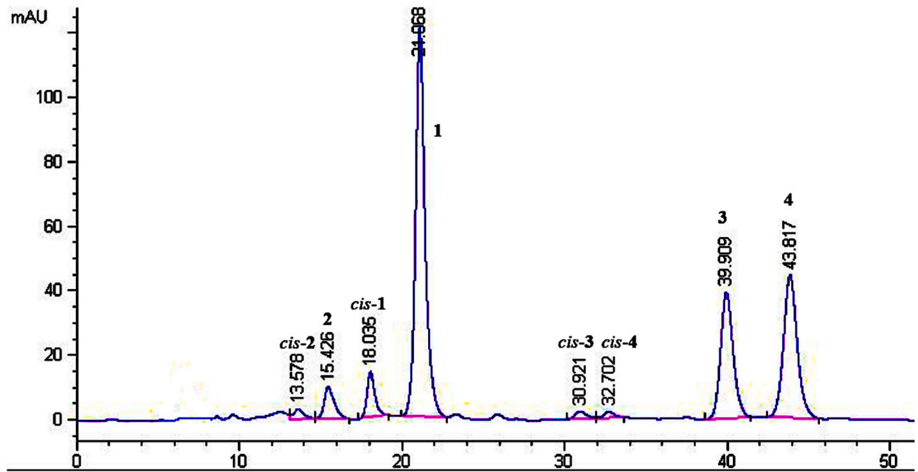


Fig. 3. HPLC chromatogram of the mixture from Montelukast (MTL) in water (3.3 x 10-4 M) after 60 min sunlight exposure (October 2019, Naples, Italy).

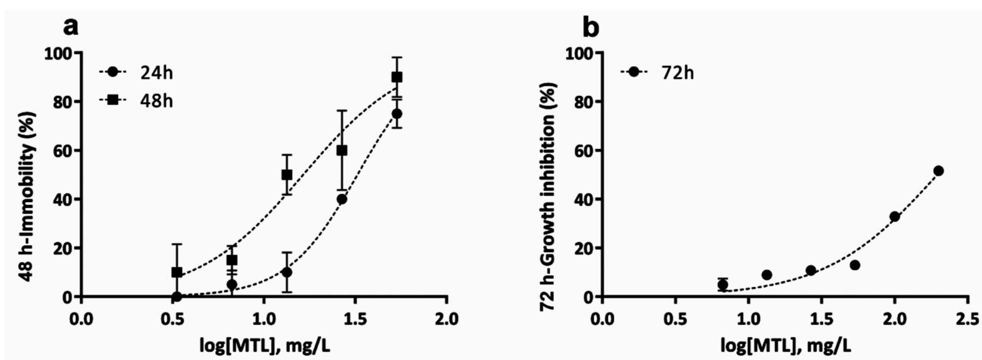


Fig. 4. Toxic effect of Montelukast (MTL) on viability of crustaceans D. magna (a) and growth of algae R. subcapitata (b): a concentration-effect curve.

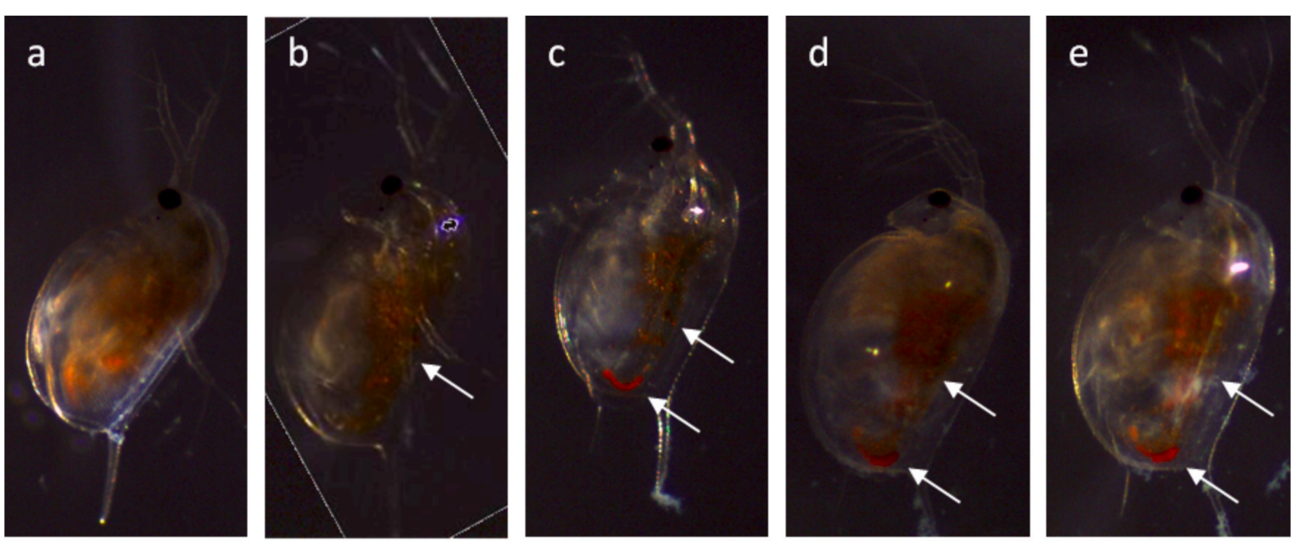


Fig. 5. D. magna exposed to Montelukast (MTL) samples at different concentration: Control (a), 6 mg/L (b), 13 mg/L (c), 25 mg/L (d), 50 mg/L (e). The arrows indicate the presence of lipid droplet and the different colour of gut.

filling of *D. magna* exposed to MTL is due to this complex for the presence of iron loosely bound in the gut wall (Steeves, 1968).

Generally, pharmaceuticals are designed to be metabolised and excreted to minimise accumulation, but the internal coloured gut suggested a slow turnover of MTL and/or its transformation products after 48 h of exposure. It has been reported that the daphnid digestive tract filled with food source excreted within 48 h (Freese and Schink, 2011). The persistence of MTL in the gut may bring to significant implications with respect to toxicity and the lower excretion rate may be correlated with potential bioaccumulation. Our results confirmed what had already been estimated in previous studies (Daughton, 2014; Howard and Muir, 2011).

Similar to a previous study with other xenobiotic (Yang et al., 2017), these results suggested that MTL could cause the dysfunction of the digestive system and, paired with the reduced food uptake, restricting the energy uptake of daphnia. Under longer-term exposure, this damage would be expected to cause decreased physiologic performance and viability, such as adopting "the toxic anorexia strategy".

The microscopical observations also determined that *D. magna* treated with MTL contained more lipid droplets than the control group. The fat droplets increased in a concentration related manner. These reserves of maternal origin represent an adaptive mechanism developed by organisms and changes in the accumulation of lipids is a sensitive endpoint that reveals negative environmental conditions such as exposure to toxic compound or starvation. A lot of substances have been demonstrated to increase the lipid droplets accumulation in *D. magna* and the accumulation of lipids in *D. magna* has been suggested to be similar to vertebrates (Brun et al., 2017; Favorito et al., 2017; Fuertes et al., 2018; Khoshnamvand, 2020; Spasiano et al., 2016).

The occurrence of lipids in their body could represent in many cases a resistance to pollutant (Fuertes et al., 2018). The mechanism has been linked to the ecdysteroid receptors enzymes that are involved in fatty acid uptake and catabolism and regulate growth and reproduction in arthropods. Consequently, the increase of storage lipid levels dramatically reduced reproductive performance on *D. magna* (Fuertes et al., 2018). These outcomes suggest that MTL may affect key physiological processes in the crustacean, such as lipid homeostasis or endocrine disruption that regulate the fitness traits. In order to follow the ecotoxicity evolution of MTL after simulated sunlight degradation, the "effect-driven approach" (Escher and Fenner, 2011), in which a compound is degraded and analyzed employing bioassays, was used to follow the ecotoxicity evolution of MTL after simulated sunlight degradation. For this purpose, 13 mg/L concentration was chosen as sublethal level for 24 h- *D. magna* test and for 72 h- *R. subcapitata* test as displayed in Fig. 6.

The results of 24 h and 48 h immobility tests with *D. magna* in contact with MTL and MTL photodegradation mixture are available in Fig. 6A. It was observed that the mixture notably showed almost the same toxicity as the sample without treatment on *D. magna* at 24 h, while the toxicity was significantly reduced when daphnids were exposed for 48 h, demonstrating that the photoproducts did not affect the mobility of *D. magna*.

As for MTL toxicity assays on *R. subcapitata*, slightly different results from *D. magna* experiments were obtained. As evidenced in Fig. 6B, the samples deriving from UVA treatment of MTL solutions showed a toxicity higher than MTL before treatment. An increase in toxicity of photoproducts compared to their parent drugs has often been observed (DellaGreca et al., 2014), such as in the case of photoproducts from the anti-virus acyclovir towards *Photobacterium phosphoreum* T3 spp (Jia et al., 2019), or of a derivative with mutagenic properties from the nonsteroidal anti-inflammatory drug etodolac (Passananti et al., 2015).

4. Conclusions

The direct photolysis of MTL in water by sunlight or solar simulated irradiations induced fast MTL transformations. In addition to the well-known MTL sulfoxide and MTL cis-isomer the main photoproducts were two decarboxylated products, likely favored by the aqueous medium, and one of them was completely characterized. The ecotoxicological tests evidenced that the growth of the algae *R. subcapitata* was not significantly influenced during the MTL exposure, while the immobility of crustaceans *D. magna* was more sensitive. After photodegradation, the algae growth inhibition increased, while immobility of *D. magna* was not significantly affected by photoproducts.

The fast light-induced degradation of MTL and the contrasting results of toxicity tests before and after irradiation evidence that further studies should be addressed to the monitoring and biological activity of the drug as well as its photoproducts. The latter are easily formed in aquatic environments and can cause possible additive, synergistic or antagonistic effects.

Our experiments evidenced moreover that MTL was ingested and incompletely depurated by *D. magna* and an increase in lipid droplets was observed. Taken together, these responses indicate that MTL exposure restricted energy acquisition, which might be the mechanisms underlying the observed acute toxicity of MTL to daphnia. Nevertheless, the relative significance of these response needs deeper investigations.

These are the first ecotoxicological data for MTL as well as its transformation products and suggest the opportunity to acquire further information and, also, extend to other bioindicators.

Credit author statement

A.Siciliano: Conceptualization, Supervision, Writing, Reviewing and Editing - M. Guida: Funding acquisition, Supervision, Writing, Reviewing and Editing- M.R. Iesce: Supervision, Writing original draft, Reviewing and Editing - G. Libralato: Supervision _F. Temussi: Conceptualization, Supervision, Writing, Reviewing- E. Galdiero: Supervision- F. Carraturo: Supervision and Reviewing - F. Cermola:

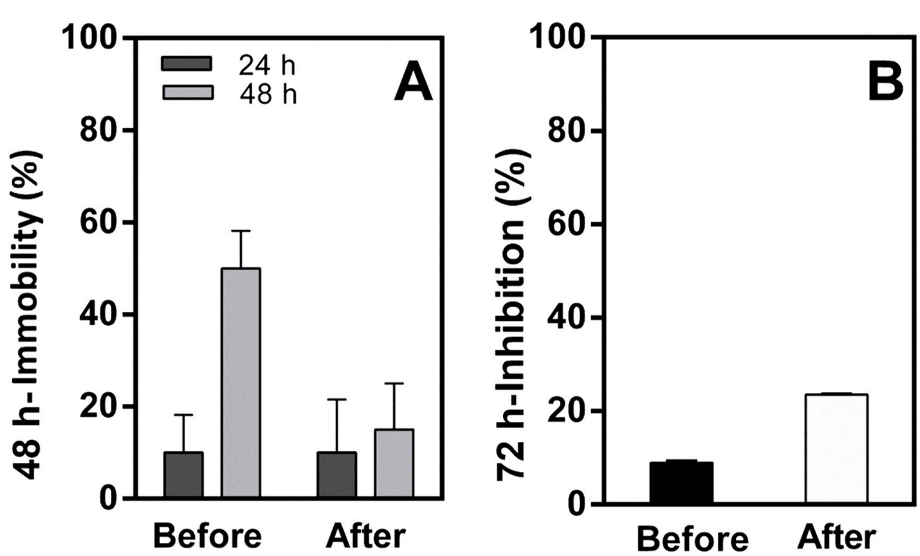


Fig. 6. Toxicity on D. magna (A) and on R. subcapitata (B) before and after photodegradation of Montelukast (MTL) using 13 mg/L of the drug.

Supervision and Reviewing- M DellaGreca: Project administration, Writing, Reviewing and Editing.

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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