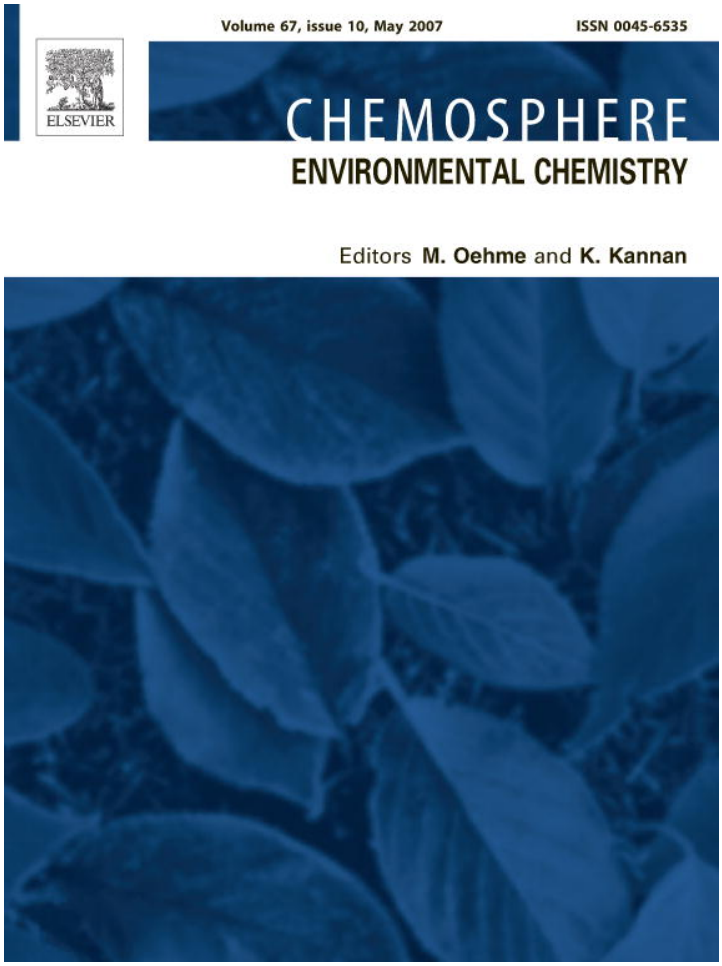

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Phototransformation products of tamoxifen by sunlight in water. Toxicity of the drug and its derivatives on aquatic organisms

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Abstract

Transformation of tamoxifen has been observed in water by prolonged sunlight irradiation. The main photoproducts, isolated by chromatographic techniques, have been identified by spectroscopic means. Photoisomerization, photocyclization and, to a lesser extent, photooxygenation appear to be involved in the degradation of the drug. The acute and chronic toxicity of the parent drug and its photoproducts were tested on non-target aquatic organisms (*Brachionus calyciflorus*, *Thamnocephalus platyurus*, *Daphnia magna* and *Ceriodaphnia dubia*). Exposure to all the compounds induced mainly chronic effects without significant differences among the parental and derivative compounds.

Keywords: Tamoxifen; Drug phototransformation; Photoisomerization; Photocyclization; Photooxygenation; Acute and chronic toxicity

1. Introduction

Pharmaceuticals are among the emerging issues in environmental chemistry (Heberer, 2002; Kümmerer, 2004). They are produced and administrated for human and animal care and can reach the environment to variable extents. Some authors reported information on the environmental occurrence of drugs, pointing out that significant amounts of several pharmaceuticals may be discharged in the environment and not be completely removed by waste water treatment plants; they have been detected in surface waters in the ng l^{-1} up to the μg l^{-1} range and occasionally in ground waters (Ternes, 1998; Heberer et al., 2002; Zuccato et al., 2006). Different studies suggest that therapeutic substances at concentrations found in the environment may have effects on aquatic organisms (Daughton and Ternes, 1999; Ferrari et al., 2003; Isidori et al., 2005). Therefore, a knowledge of physical, biological, and chemical processes in the aquatic system, such as adsorption, degradation, photolysis and hydrolysis, is of great importance. Only very limited information on these processes is available to date (Kümmerer, 2004). Hence, the presence and the possible effects of the transformation products on the environment should also be investigated.

The aim of the present work was to isolate the main products derived from photolysis of the drug tamoxifen (**1**) in water by sunlight exposure and to evaluate the acute and chronic toxicity of the drug and its photochemical derivatives on aquatic life utilizing the rotifer *Brachionus calyciflorus* and the crustaceans, *Thamnocephalus platyurus*, *Daphnia magna* and *Ceriodaphnia dubia*. The bioassays included acute and chronic tests with more than one test species in order to address biological effects of chemicals on different organizational structures and sensitivities of non-target organisms.

Tamoxifen is a non-steroidal antiestrogen that has been used to successfully treat breast cancer for many years. More recently, tamoxifen has been used as a prophylactic

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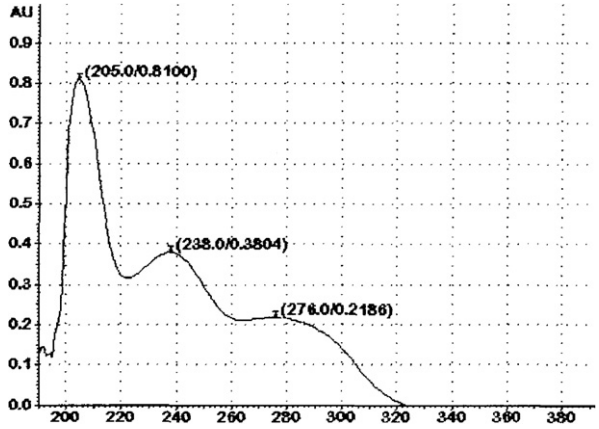


Fig. 1. UV spectrum of tamoxifen.

agent in women considered at significant risk of developing the disease (Fisher et al., 1998). This drug has been detected in surface water samples collected in UK from some estuaries at concentrations up to 71 ng l^{-1} (Thomas and Hilton, 2004) and in sewage treatment plants at concentrations of 20–40 ng l^{-1} (Ashton et al., 2004). Irradiation of the drug has been described under UV light of 254 nm using methanol as solvent (Wilson and Ruenitz, 1993). Several analytical methods for the estimation of the drug and its metabolites are based on the photochemically assisted conversion of the drug to fluorescent products (Nieder and Jaeger, 1987; Salamoun et al., 1990). On the other hand the strict instruction that tamoxifen solution should be stored in the dark suggests its photosensitivity even to daylight (Nieder and Jaeger, 1987; Wilson and Ruenitz, 1993). This agrees with the observation that tamoxifen exhibits a strong UV band at λ 277 nm ($\varepsilon = 14\,792$ l mol^{-1} cm^{-1}) with a tail at $\lambda > 310$ nm (Fig. 1). In this study we examine the irradiation in water by sunlight under as close to natural conditions as possible. The effect of pH and of natural photosensitizers, such as humic acid and nitrate, on the rate of degradation is also discussed. Humic acid and nitrate are likely to be encountered in aquatic environments and are reported to be capable of oxidizing a range of organic compounds (Zepp et al., 1985, 1987). To accelerate the degradation and/or to isolate the photodegradation products a solar simulator and UV lamp (Pyrex filter) were also used.

2. Material and methods

2.1. Chemicals

Tamoxifen, analytical standard grade (90%), KNO_3, humic acid, NaN_3, phenyl ethyl ketone were supplied by Aldrich and used without further purification.

2.2. Equipment and methods

NMR spectra were recorded on a Varian Inova-500 instrument operating at 499.6 and 125.62 MHz for ^1H and ^{13}C, respectively, and referenced with deuterated solvents ($CDCl_3$). Mass spectra (EI-MS) were obtained with a GCMS-QP5050A (Shimadzu) equipped with a 70 eV EI detector. The column used was Zebron ZB-5 30 m × 0.25 mm with a film thickness of 0.25 μm. The temperature of the injector as well as of the detector was 230 °C and the acquisition mode was full scan. The following oven temperatures were used; start temperature 60 °C followed by an increase to 160 °C at a rate of 20 °C/min where it was held for 8 min. Then, temperature was increased to 280 °C at a rate of 20 °C/min and held for 8 min. UV/Vis spectra were recorded in methanol on a Perkin–Elmer Lambda 7 spectrophotometer. IR spectra were recorded on a Jasco FT/IR-430 instrument. Irradiations were performed by exposure to sunlight (May-2005) or using a 150-W solar simulator equipped with a Xenon lamp and a filter to simulate irradiation at the earth surface (Oriel Instruments). A photoreactor (Helios Italquartz) equipped with a 500 W high-pressure mercury lamp (through a Pyrex glass filter, $\lambda > 300$ nm) was also used for UV irradiation. Analytical and preparative TLC were made on Kieselgel 60 F_{254} plates with 0.2 mm and 0.5 or 1 mm layer thickness, respectively (Merck).

2.3. Experimental procedures

2.3.1. Irradiation of tamoxifen by solar simulator

Drug (40 mg) was dispersed in MilliQ water (500 ml) and irradiated in open flask by a solar simulator. After 80 h the dispersion was evaporated and analyzed by ^1H NMR and TLC. The residue (38 mg) was chromatographed on preparative TLC [eluent: benzene/triethylamine/petroleum ether (9/1/10)] to give, at decreasing Rfs, tamoxifen (90%), its isomer **2** (traces), phenanthrenes **3** and **4** (ca. 2%).

Similar experiments were carried out in the presence of nitrate (10 ppm), with humic acid (5 ppm), at pH 4.0 and 9.0 by adjusting the pH with HCl 2 M and KOH 2 M, respectively. After 80 h each dispersion was evaporated in vacuum and the residue analysed by NMR and TLC. Analyses of the residues obtained by irradiation in the presence of nitrate, of humic acid and at pH 9 showed that tamoxifen was unchanged. Analysis of the residue at pH 4.0 (37 mg) showed, in addition to drug **1**, a mixture of products. Chromatography on preparative TLC gave tamoxifen (70%), isomer **2** (4%), a mixture containing phenanthrenes **3** and **4**, with an overall yield of about 8%.

2.3.2. Sunlight irradiation of tamoxifen

Dispersions of the drug (40 mg) in MilliQ water (500 ml) and at pH 4.0 were exposed in open Pyrex flasks to solar light (May, 2005, Naples-Italy) for 1 month. MilliQ water was constantly added to maintain the initial concentrations. After evaporation of water, each residue was chromatographed on preparative TLC. The residue in milliQ water (38 mg) gave tamoxifen (50%), isomer **2** (4%), two phenanthrenes **3** and **4** (traces), ketone **5** (trace) and a

complex polar fraction (10%). The latter was treated with HCl 2 N and extracted with ethyl acetate. The organic layer was analyzed by ^{1}H NMR spectrum showing the presence of benzoic acid (**6**), identified by comparison with an authentic sample. Mixture obtained at pH 4.0 (30 mg) was purified by preparative TLC chromatography to give tamoxifen (40%), isomer **2** (4%), mixture of phenanthrenes **3** and **4**, and ketone **5** with an overall yield of 10%, the polar fraction (20%) contained benzoic acid.

2.3.3. Irradiation of tamoxifen by UV lamp (Pyrex filter)

Two solutions of tamoxifen (40 mg) were prepared in 50 ml of a mixture of water at pH 2.0 and methanol (1:1 v/v). The solutions were saturated with argon or oxygen and irradiated for 7 h by UV lamp. After extraction with $CHCl_3$, the residues were analyzed by ^{1}H NMR. The residue obtained by irradiation under oxygen atmosphere afforded tamoxifen (23%), the isomer **2** (21%), phenanthrene **3** (9%), phenanthrene **4** (14%), ketone **5** (9%).

Irradiation under argon atmosphere led to tamoxifen (47%) and the isomer **2** (36%) with trace amounts of phenanthrenes **3** and **4**.

Two solutions of tamoxifen (4 mg) in CH_3OH/MilliQ water (1:1, 4 ml) and CD_3OD/D_2O (1:1, 4 ml), respectively, were irradiated for 10 h. The ^{1}H NMR analysis of both mixtures showed the drug (ca. 90%), compounds **2**–**4** (<10%) and traces of compound **5**. Similar results were obtained when a solution of tamoxifen (8 mg) in CH_3OH/MilliQ water (1:1, 10 ml) was irradiated in the presence of NaN_3 (5 equiv) for 10 h.

2.3.4. Methylene blue-sensitized photooxygenation of tamoxifen

Irradiation of tamoxifen (20 mg) in MilliQ water (200 ml) was carried out in the presence of methylene blue (1:10 with **1**) by the solar simulator under oxygen-saturated conditions. After 57 h, TLC gave tamoxifen (85%), ketone **5** (6%) and minor unidentified products.

2.3.5. Irradiation of ethyl phenyl ketone (7)

Compound **7** (40 mg) was dissolved in 150 ml of MilliQ water and irradiated in the presence of methylene blue (1:10 with **7**) under oxygen atmosphere by the solar simulator for 57 h. Then, after extraction with $CHCl_3$, the residue was analyzed by ^{1}H NMR showing the starting material (94%), benzoic acid (ca. 2%). Similar results were obtained when a dispersion of ethyl phenyl ketone in MilliQ water was exposed to sunlight for one month (July-2005 – Naples-Italy).

2.4. Photoproducts characterization

Isomer **2**: ^{1}H NMR: δ ($CDCl_3$) 7.20–6.80 (m, 14H, Ar–H), 4.21 (m, 2H, CH_2O), 3.02 (m, 2H, CH_2N), 2.59 (s, 6H, $(CH_3)_2N$); 2.50 (q, $J = 7.6$ Hz, 2H, CH_2CH_3), 0.94 (t, $J = 7.6$ Hz, 3H, CH_2CH_3). ^{13}C NMR: δ ($CDCl_3$) 155.3, 143.1, 142.2, 138.0, 137.1, 132.0, 130.8, 129.6, 127.9, 127.8, 126.6, 126.1, 114.1, 63.5, 57.0, 44.2, 29.0, 14.3. EIMS *m/z* 371 $[M]^+$, 300 $[M-C_4H_9N]^+$, 72 $[C_4H_{10}N]^+$, 58 $[(CH_3)_2N{=}CH_2]^+$.

Phenanthrene **3**: ^{1}H NMR: δ ($CDCl_3$) 8.70 (m, 1H, H-5), 8.13 (m, 2H, H-4 and H-8), 7.64 (m, 2H, H-6 and H-7), 7.50 (t, $J = 7.0$ Hz, 2H, H-10 and H-12), 7.45 (t, $J = 7.5$ Hz, 1H, H-11), 7.28 (d, $J = 8.0$, 2H, H-9 and H-13), 7.20 (d, $J = 9.0$ Hz, 1H, H-1), 7.02 (d, $J = 9.5$ Hz, 1H, H-2), 4.50 (m, 2H, CH_2O), 3.22 (m, 2H, CH_2N), 2.86 (q, $J = 7.5$ Hz, 2H, CH_2CH_3), 2.72 (s, 6H, $(CH_3)_2N$), 1.18 (t, $J = 7.4$ Hz, 3H, CH_2CH_3). EIMS *m/z* 369 $[M]^+$, 298 $[M-C_4H_9N]^+$, 72 $[C_4H_{10}N]^+$, 58 $[(CH_3)_2N{=}CH_2]^+$.

Phenanthrene **4**: ^{1}H NMR: δ ($CDCl_3$) 8.78 (m, 1H, H-5), 8.72 (d, $J = 8.0$ Hz, 1H, H-4), 8.17 (m, 1H, H-8), 7.67 (m, 2H, H-6 and H-7), 7.57 (m, 1H, H-3), 7.41 (m, 1H, H-2), 7.33 (d, $J = 8.0$ Hz, 1H, H-1), 7.24 (d, $J = 8.5$ Hz, 2H, H-9 and H-13), 7.07 (d, $J = 8.0$ Hz, 2H, H-10 and H-12), 4.37 (m, 2H, CH_2O), 3.14 (m, 2H, CH_2N), 2.91 (q, $J = 8.0$ Hz, 2H, CH_2CH_3), 2.66 (s, 6H, $(CH_3)_2N$), 1.19 (t, $J = 7.0$ Hz, 3H, CH_2CH_3). EIMS *m/z* 369 $[M]^+$, 298 $[M-C_4H_9N]^+$, 72 $[C_4H_{10}N]^+$, 58 $[(CH_3)_2N{=}CH_2]^+$.

Ketone **5**: IR ($CHCl_3$): ν_{max} 1654 (C=O) cm^{-1}. ^{1}H NMR: δ ($CDCl_3$) 7.84 (d, $J = 8.5$ Hz, 2H, H-7 and H-7′), 7.75 (d, $J = 7.5$ Hz, 2H, H-3 and H-3′), 7.58 (t, $J = 8.4$ Hz, 1H, H-9), 7.49 (t, $J = 8.4$ Hz, 2H, H-8 and H-8′), 7.00 (d, 2H, $J = 8.5$ Hz, H-2 and H-2′), 4.62 (m, 2H, CH_2O), 2.70 (m, 2H, CH_2N), 2.98 (s, 6H, $(CH_3)_2N$). ^{13}C NMR: δ ($CDCl_3$) 195.2 (CO), 160.4 (C-1), 137.7 (C-6), 132.5 (C-3), 132.1 (C-9), 131.3 (C-4), 129.7 (C-7), 128.2 (C-8), 114.1 (C-2), 63.2 (CH_2O), 56.8 (CH_2N), 44.0 ($(CH_3)_2N$). EIMS *m/z* 269 $[M]^+$, 198 $[M-C_4H_{10}N]^+$, 105 $[C_7H_5O]^+$, 58 $[(CH_3)_2N{=}CH_2]^+$.

2.5. Toxicity testing

Tamoxifen, its photoproducts and the sunlight-irradiation mixture were initially dissolved in dimethylsulphoxide (DMSO) and then diluted further in double-deionized water to make the final stock solutions. The DMSO concentration in the exposure solutions, including controls, was 0.01% (v/v) which is a non-effective dose as estimated in preliminary tests. Compound concentrations used in the definitive tests were based on results from range-finding tests. The test solutions were prepared by mixing the appropriate volumes of the stock solutions to be tested and the considered growth medium.

Acute bioassays were performed using resting eggs of invertebrate species provided by MicroBioTest (Nazareth, Belgium) from which test organisms could be hatched. Organisms, age 0–2 h, were exposed to different concentrations of drug and its derivatives in synthetic waters following the respective standard guides (ASTM, 1991; ISO, 1996) and for the crustacean *T. platyurus* the manufacturer procedure. The mortality of the *B. calyciflorus*, *T. platyurus* and the immobilization of the *D. magna* were evaluated after 24 h exposure to chemicals.

Chronic bioassays were carried out on the rotifer *B. calyciflorus* and the crustacean *C. dubia*. The test on *B. calyciflorus* was based on the population growth inhibition in 48 h of exposure. It was conducted according to the standard ISO/CD 20666 (ISO, 2001a). One test organism, born from cysts after 18 h of incubation, was distributed into each well of 24-well microplates, four replicates per concentration and a negative control, introducing 0.9 ml of the test sample and 100 μl of a fresh suspension of 10^7 cells/ml of a green alga as food. The microplates were incubated at 25 ± 1 °C in the dark for 48 h. At the end of the test the number of female rotifer was determined and, by comparing it with the control, the population growth inhibition percentage was determined for each concentration. The test on *C. dubia* was similarly based on a population growth inhibition but performed in 7 days on organisms, less than 24 h old, obtained by acyclical parthenogenesis of individual adult females (ISO, 2001b). Single organisms were individually exposed in beakers with 20 ml of test solution to seven concentrations (two-fold dilutions) in synthetic reconstituted aerated hard ISO medium. Ten replicates were incubated at 25 °C with a 16:8 h light:dark cycle (500 lux). Daphnids were fed at each daily renewal of the test medium with 100 μl algae, food fish (5 g l^{-1}) and yeast (5 g l^{-1}). Organisms were monitored for survival, and released neonates were counted each day prior to renewals and then discharged. From the comparison between the number of offspring at the end of the test in the sample batch and the control, it was possible to calculate the concentration which gave rise to 50% population growth inhibition, indicated as EC50. All results were analyzed using Toxicity Data Analysis and Database Software (Toxcalc™) (1996) to determine the respective L(E)C50s.

3. Results and discussion

3.1. Phototransformation studies

Dispersion of tamoxifen exposed to prolonged sunlight (1 month) in water caused 50% conversion. Purification of the irradiation mixture, by preparative TLC, gave *cis*-isomer **2**, phenanthrenes **3** and **4**, ketone **5** and benzoic acid (Fig. 2). Isomeric phenanthrene derivatives were identified by comparison of spectral data with those previously reported (Wilson and Ruenitz, 1993). Compound **2** was characterized by NMR and MS spectra as *cis*-isomer of the drug. It was previously detected by analytical methods which employ UV irradiation (Salamoun et al., 1990). Structure **5** was assigned on the basis of spectroscopic means (^{1}H NMR, ^{13}C NMR, MS, IR). In particular, the IR spectrum shows the presence of a strong band at 1654 cm^{-1} due to the conjugated carbonyl function as confirmed by ^{13}C NMR signal at δ 195.2. Moreover, MS spectrum exhibits the peak of molecular ion at m/z 269 with a characteristic peak at m/z 105 ($C_6H_5CO^+$). Compound **5** is a fragmentation product attributable to an oxidative cleavage of the C=C double bond. This cleavage is reported to occur starting from hydroperoxides, via Hock cleavage, (Porter, 1992) or from dioxetanes, via characteristic C–C and O–O bonds cleavages (Foote and Clennan, 1995). To get an appreciable amount of **5** and confirm the suggested hypothesis, we tried preparing the peroxidic intermediate by carrying out a methylene blue-photooxygenation of **1**. Methylene blue is known to be an efficient sensitizer for singlet oxygen which adds to double bond leading to dioxetanes and/or, in the presence of allylic hydrogens, gives an ene-type reaction leading to allylic hydroperoxides (Foote and Clennan, 1995). The methylene blue-sensitized photooxygenation reaction of tamoxifen was very slow and after 57 h a mixture of products were obtained among which ketone **5** was found in addition to the unreacted drug. No trace of the expected complementary ethyl phenyl ketone **7** was detected either by NMR or by TLC. However, control experiments showed that compound **7** was unstable under photoxidative conditions (both under methylene blue-sensitized irradiation and by one-month solar exposure), and benzoic acid was detected in the reaction mixtures.

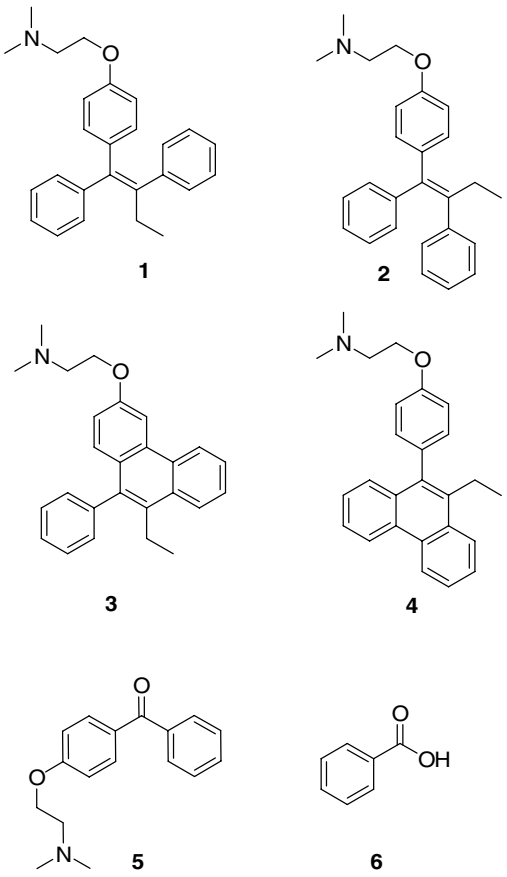
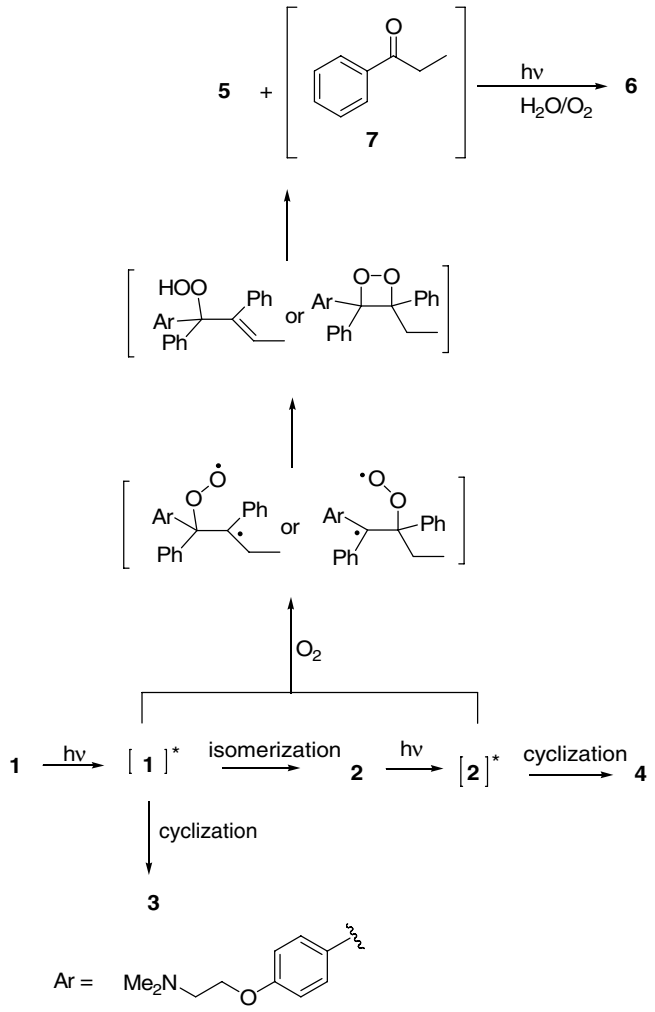


Fig. 2. Tamoxifen (**1**) and its photoproducts under sunlight irradiation in water.

Experiments showed that the drug was recovered unchanged by keeping it in the dark in aqueous solution (at different pHs or in the presence of humic acid or nitrate)



Scheme 1. Suggested photodegradation pathways of tamoxifen in water.

even after 30 days. Hence, all the products are formed by photo-induced reactions (Scheme 1). *cis–trans* Isomerization is a typical alkene photochemical reaction while formation of products such as **3** or **4** is observed in the photochemistry of stilbene-type products (Gilbert, 2004). In the irradiation of tamoxifen, due to the presence of geminal phenyl rings, both isomers are capable of dehydrogenation leading to two possible phenanthrene products **3** and **4**. Actually, cyclization occurs via a dihydrophenanthrene intermediate which may be undetected especially under oxidative conditions (Gilbert, 2004). Photooxygenation leading to ketone **5** is a minor reaction and appears not to involve singlet oxygen. Indeed, control experiments showed that the irradiation of tamoxifen was not accelerated in deuterated solvents, e.g. D_2O/CD_3OD, and was not affected by the presence of NaN_3, a typical singlet oxygen quencher (Foote and Clennan, 1995). More probably, the excited molecule **1** (and **2**) should be trapped by diradical ground-state molecular oxygen. Abstraction of β-hydrogen and shift of the double bond should lead to a hydroperoxide intermediate which, probably via a Hock-cleavage, should give ketone **5** and ethyl phenyl ketone (**7**). The slight increase of ketone formation under acidic conditions appears to support this assumption (Porter, 1992). However, it cannot be excluded that, partly, ring closure of the diradical intermediate to a dioxetane takes place and **5** and **7** are formed through the well-known O–O and C–C bonds cleavage. The undetected ketone **7** probably undergoes an aerobic Norrish Type I cleavage to benzoic acid, as found in the photooxygenation of other ketones (Kopecký, 1992).

The increasing degradation rate at acidic pH could be due to greater solubility of the protonated drug in water than the neutral form [pKa 9.24, measured according to Flexser et al. (1935)]. The stability of tamoxifen in the presence of nitrate and humic acid could be ascribed to filter action of these additives, which exhibit strong absorption bands in region 300–310 nm (Zepp et al., 1985, 1987).

3.2. Toxicity studies

The results of acute toxicity for tamoxifen and its derivatives are summarized in Table 1 and expressed as L(E)C50s. The confidence range for L(E)C50s was also computed using the maximum likelihood method. The interval estimation is expressed in a lower and upper bound that enclose the true value of L(E)C50 with a probability of 95%. Both the active pharmaceutical principle and photoproducts **2**–**5** affected the exposed organisms, especially the rotifer *B. calyciflorus* and the crustacean *T. platyurus* with LC50 values ranging from 0.40 to 1.59 mg l^{-1}. The LC50 values of tamoxifen and compound **2** were significantly different ($p < 0.05$) for rotifers and *T. platyurus* showing no overlapping confidence intervals. A less toxic

Table 1
L(E)C50 in mg/l for acute toxicity tests with 95% confidence range

Compounds	*B. calyciflorus*	*T. platyurus*	*D. magna*
Tamoxifen	0.97 (0.82–1.14)	0.40 (0.32–0.50)	1.53 (1.26–1.85)
2	1.07 (0.97–1.17)	0.47 (0.36–0.61)	1.74 (1.53–1.99)
3	0.95 (0.73–1.23)	0.94 (0.86–1.04)	2.82 (2.30–3.45)
4	1.06 (0.91–1.24)	1.59 (1.21–2.09)	NE[a] 5
5	1.31 (1.11–1.54)	1.28 (1.02–1.60)	3.27 (2.54–4.20)
Irradiation[b] mixture	17.79 (16.34–19.37)	15.97 (13.15–19.39)	NE[a] 10

[a] NE = No effect at.
[b] By sunlight.

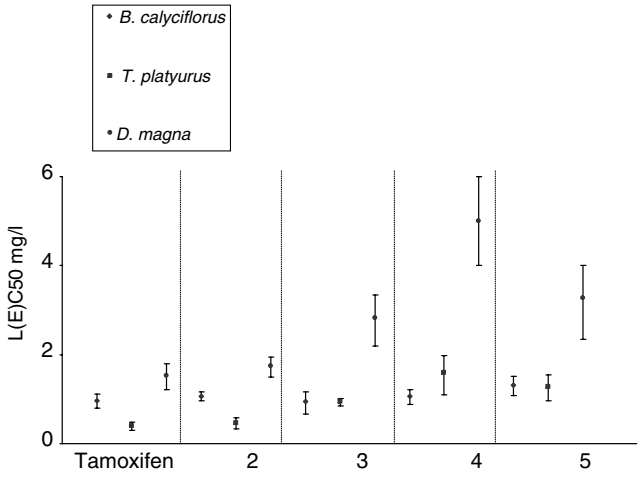


Fig. 3. Differences in acute toxicity for the organisms tested of tamoxifen and its derivatives.

potential was found for *D. magna* that showed EC50s enclosed in confidence ranges statistically different from the other organisms as shown in Fig. 3. No acute effect was found for compound **4** up to 5 mg l^{-1}. The irradiation mixture showed the lowest L(E)C50 values for *B. calyciflorus* and *T. platyurus* while no effect was found at the maximum concentration tested (10 mg l^{-1}) for *D. magna*. Generally, acute toxicity tests are performed at high concentrations of toxicants to obtain the respective LC50 or EC50 values even if the amounts tested are far from the environmental occurrence of pharmaceuticals. In fact, drugs are found in surface waters or effluents at very low concentrations, below the amounts utilized to obtain acute toxicity responses on non-target organisms. Also tamoxifen and its derivatives showed toxicity values below the environmental detection (ppt range) but adverse effects were found at concentrations quite low when compared to other classes of pharmaceuticals (Ferrari et al., 2003; Isidori et al., 2005).

As expected, in chronic bioassays tamoxifen and its photoproducts appeared toxic for the organisms tested. As reported in Table 2, the compounds investigated revealed the greatest effects on *C. dubia* with a toxic potential three orders of magnitude higher than acute tests. The most toxic of compounds was **2** showing an EC50 equal to 4.1×10^{-4} mg l^{-1}. These concentrations may not represent an ecological risk because they are still far from occurrence data of tamoxifen and particularly of its derivatives, for which no detection data are available. However, the drugs may act as persistent compounds simply because of their continual infusion into aquatic media via STP effluents, which prolong a multigenerational exposure for the resident organisms (Daughton and Ternes, 1999; Ferrari et al., 2003). On the other hand, even though concentrations of drugs may be low, they are commonly found in complex mixtures that could induce additive, synergistic or antagonist effects (Cleuvers, 2003). This is the case of the irradiation mixture, made up of tamoxifen and all the derivatives and reported in Tables 1 and 2, for which the lowest effects were found both in acute and chronic toxicity tests.

Table 2
EC50 in mg/l for chronic toxicity tests with 95% confidence range

Compounds	*B. calyciflorus* (48 h)	*C. dubia* (7 d)
Tamoxifen	0.25 (0.10–0.38)	8.1×10^{-4} (2.2×10^{-4}–1.4×10^{-3})
2	0.26 (0.19–0.32)	4.1×10^{-4} (1.1×10^{-4}–9.4×10^{-3})
3	0.156 (0.05–0.25)	2.8×10^{-3} (1.0×10^{-3}–8.7×10^{-3})
4	0.123 (0.08–0.16)	7.7×10^{-4} (1.2×10^{-4}–3.1×10^{-3})
5	0.125 (0.09–0.15)	8.9×10^{-4} (5.9×10^{-4}–3.4×10^{-3})
Irradiation mixture[a]	15.58 (14.09–17.01)	9.6×10^{-3} (2.1×10^{-3}–1.6×10^{-2})

[a] By sunlight.

4. Conclusions

Isomerization and cyclization are the main photoinduced reactions of tamoxifen even under natural conditions. Photooxygenation can also occur and this corresponds to the recent studies on antioxidant properties of the drug which have shown its capability of reacting with OH radicals (Leguene et al., 2002).

Our data demonstrate that exposure of non-target organisms to tamoxifen and its derivatives raises questions about the potential hazard posed by these compounds for the aquatic fauna, especially for chronic exposure.

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