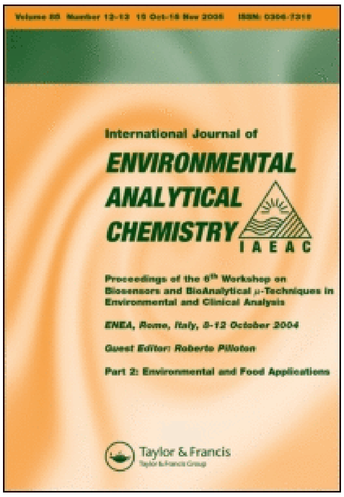

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PHOTODEGRADATION OF MUSK XYLENE

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Upon UV irradiation (λ>280 nm) in model organic solvents, musk xylene (**1**) underwent intramolecular cyclization in the presence of cyclohexane and methanol while photoreduction dominated in the cyclohexene medium. On layers of cellulose stearate the photodegradation of **1** took place readily both under sun simulator and in natural sunlight and several photoproducts identified in solvent experiments were detected. Dissolved in water **1** was also readily photodegraded, mainly yielding a photocyclization product. Photoproducts were isolated by preparative HPLC and analysed by means of HPLC, GC, GC-MS, MS, ^{1}H- and ^{13}C-NMR.

Keywords: Musk xylene; photodegradation; model organic solvents; layer of cellulose stearate; water

INTRODUCTION

A synthetic nitro musk, musk xylene (1-*tert*-butyl-3,5-dimethyl-2,4,6-trinitrobenzene, **1**), has widely been used as fragrance in the manufacture of cosmetics and detergents. Its total worldwide consumption in detergents in 1992 was estimated to be about 1000 t.[1] This substance is produced on a large scale especially in India and China. In an investigation by Sommer[2] it was found that 17 of 41 detergents and 7 of 60 cosmetics contained musk xylene, which demonstrates its main presence in detergents.

Since its first evidence in freshwater fish in 1981[3] musk xylene was further detected in a variety of samples from surface water, waste water[4], fish[5] and human tissues[6] as potential environmental pollutant. This persistent, lipophilic substance has a bioconcentration factor as high as 4.1 × 10^3 in fish, comparable to those of polychlorinated biphenyls (PCBs) and other organochlorine compounds. Unlike the main contamination route of humans by organohalogenated

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compounds through food consumption, it is assumed that the dermal sorption of musk xylene from cosmetics and detergents (in clothing) is probably the more important contamination route.[6]

In relation to the above mentioned facts, questions should be raised about the environmental degradation pathways of musk xylene. It is already known from two reports that photochemical degradation plays an indispensable part in it. More than 20 years ago Döpp et al.[7] reported the photochemistry of crystal musk xylene in which 1-hydroxy-2-indolinone was identified as the main photoproduct and several minor metabolites were also found during UV irradiation. They further showed the mechanism of the photochemical process of musk xylene. Recently, Schmidt et al.[8] studied the photodegradation of this substance in water and also identified two photoproducts reported by Döpp et al.[7]

Therefore, in this work the research was focused on the photodegradation of musk xylene, with the main emphasis put on the photochemical behavior in different reaction media including model organic solvents with different functionalities (cyclohexane, methanol, and cyclohexene), layers of cellulose stearate and water.

EXPERIMENTAL

Materials

Musk xylene (purity > 99 % by HPLC) was kindly provided by the Th. Geyer GmbH & Co. KG (Renningen, Germany). The solvents (cyclohexane, methanol, and cyclohexene) were distilled before use. Milli-Q (Millipore S. A., Molsheim, France) purified water was employed as reaction medium. The layer of cellulose stearate (derivatized cellulose filter paper, substitution degree 12.8 %) was prepared after the method developed by Ambruster.[9]

Photolysis

For kinetic degradation investigations, 20 mg musk xylene were dissolved in 40 mL cyclohexane, methanol, and cyclohexene, respectively. For preparative isolation of photoproducts 40 mg musk xylene were dissolved in 40 mL cyclohexane, methanol, and cyclohexene and irradiated for 12 h, 8 h, and 2 h, respectively. Due to the extremely low solubility of musk xylene in water, the concentration prepared was correspondingly as low as about 400 µg/L. The irradiation both in organic solvents and in water was carried out in round quartz cuvettes (volume: 40 mL) at room temperature using a metal halogen lamp (Sol 500) equipped with glass filter WG 295 (Schott, cut-off λ>280 nm).

The photolysis on layers of cellulose stearate was undertaken both under a sun simulator (2 parallel UV-B lamp tubes, TL 100/12, Philips) at the distance of 20 cm and in natural sunlight (July 31st, 1997 in Hohenheim, Stuttgart). Before the irradiation the layer of cellulose stearate was immersed in a solution of 100 mg musk xylene in 25 mL methanol for 10 seconds, air dried in the dark and then cut in pieces of 0.5 cm^2 size. By this procedure the initial musk xylene contents were about 5.5 – 6.0 mg/g.

Product isolation

The photolysis mixtures were evaporated to dryness, dissolved in methanol and chromatographed on preparative HPLC equipped with Kronlab Nucleosil C18 column (250 mm x 20 mm, Sinsheim, Germany), Knauer pump 64, Knauer variable wavelength monitor (Berlin, Germany) and Shimadzu Chromatopac C-R3A integrator (Kyoto, Japan) using the following eluent at a flow rate of 10 mL/min.: isocratic methanol/water = 80/20 for cyclohexane and methanol photolysis mixtures and methanol/water = 85/15 for the cyclohexene.

Synthesis of photoproducts for identification

4-tert-Butyl-2, 6-dimethyl-3, 5-dinitroaniline (5) and 6-tert-butyl-2, 4-dimethyl-3,5-dinitroaniline (6)

A mixture of 1.0 g musk xylene, 2 mL c-HCl, 500 mg Fe powder and 50 mL methanol was heated to boiling under reflux for 2 h. After evaporation of the solvents in vacuo, water (150 mL) and diethyl ether (50 mL) were added. The aqueous layer was neutralized with 1 N Na_2CO_3 solution and the mixture was extracted with diethyl ether (3 x 50 mL). The organic layers were combined and dried (Na_2SO_4), and solvents were removed in vacuo. The residue was dissolved in methanol and the isolation was achieved on TLC (silica gel 60 F_{254}, 2 mm thickness, Merck, Germany) with petroleum ether/diethyl ether (10/90). Altogether 310 mg of **5** and **6** were obtained, but it was difficult to isolate them from each other completely because the zones of them overlapped. Therefore, only the upper and lower parts were scraped off in order to get pure **5** and **6**.

High performance liquid chromatography (HPLC)

The degradation analyses of musk xylene were carried out on a Hewlett Packard 1050 HPLC system (Waldbronn, Germany) equipped with an autosampler, a photodiode array detector and an Eurospher 100-C18 (250 × 4 mm) column (Knauer, Berlin). During irradiation 1 mL samples were taken from the reaction mixtures of organic solvents. In the case of cyclohexane and cyclohexene, the solvents were evaporated by a stream of nitrogen and the residues were redissolved in 1 mL of methanol. 20 µL of the methanolic solutions were injected onto the LC column. Cellulose stearate samples were each extracted with 5 mL methanol; the injection volume was also 20 µL. At a flow rate of 0.8 mL/min and with the detections at 230 nm and 254 nm, cyclohexane and methanol photolysis mixtures as well as the extracts of cellulose stearate layers were analysed with gradient methanol/water (50/50 [0–5 min]→95/5 [20–30 min]→50/50 [35–45 min]) as eluent while isocratic methanol/water (85/15) eluent was employed for cyclohexene mixture.

Gas chromatography (GC)

During irradiation of musk xylene in water, 2 mL samples were taken every 10 minutes and extracted twice with 10 mL diethyl ether. After removal of diethyl ether, the residues were dissolved in 1 mL of methanol and analysed by a Perkin Elmer Autosystem XL GC equipped with DB-5 capillary column (30 m × 0.25 mm × 0.25 µm), autosampler and ECD. Hydrogen was used as carrier gas and splitless injection method was chosen. The injection volume was 1 µL.

Nuclear magnetic resonance (NMR) spectroscopy

^{1}H-NMR and ^{13}C-NMR as well as ^{1}H, ^{13}C-COSY and DEPT measurements were performed on a Bruker AC-250, ARX-300 and ARX-500 (Karlsruhe, Germany) respectively. Spectra are reported in parts per million (ppm) downfield from tetramethylsilane as internal standard.

Mass spectrometry (MS)

MS was obtained with Finnigan MAT 95 instrument (Bremen, Germany).

GC/MS-coupling

The spectra were achieved with a Perkin Elmer gas chromatograph GC 8420 (Ueberlingen, Germany) with PVMS 54 column, 25 m × 0.25 mm × 0.3 µm, coupled with Finnigan MAT Ion Trap 800 detector (Bremen, Germany).

UV/VIS spectrometry

The measurements were carried out on Perkin Elmer UV/VIS spectrometer Lambda 2 (Ueberlingen, Germany).

Melting point

Melting points were taken on a digital melting point apparatus (Electrothermal Eng. Ltd.) and are uncorrected.

Photoproducts

1-hydroxy-3,3,5,7-tetramethyl-4,6-dinitro-2-indolinone (2)

Yellow powder, yield: 7.1 mg (for irradiation in cyclohexane) and 5.4 mg (for irradiation in methanol). M.p.: 241–242°C (decomp.); UV (methanol): λ_{max} (log ε) = 257 nm (4.10); ^{1}H-NMR (500 MHz, CD_3COCD_3): $\delta = 1.39$ (6H, $>C(CH_3)_2$), 2.14 (3H, $-CH_3$), 2.52 (3H, $-CH_3$), 10.37 (1H, >N-OH); ^{13}C-NMR: (125 MHz, CD_3COCD_3): $\delta = 12.14$ ($-CH_3$), 12.18 ($-CH_3$), 22.85 ($>C(\underline{C}H_3)_2$), 43.27 ($>\underline{C}(CH_3)_2$), 114.72, 115.45, 125.26, 140.60, 146.73, 153.76 (6 arom. C), 176.10 (-CO-N<); MS (70 eV): *m/z* 295 (100 %, M^+), 278 (19 %), 261 (8 %), 43 (21 %).

3,3,5,7-tetramethyl-4,6-dinitro-2-indolinone (3)

Yellow powder, yield: 1.2 mg. M.p.: 223–224°C (decomp.); UV (methanol): λ_{max} (log ε) = 206 nm (4.38), 243 nm (3.98), 328 nm (2.95); ^{1}H-NMR (500 MHz, $CDCl_3$): $\delta = 1.47$ (6H, $>C(CH_3)_2$), 2.19 (3H, $-CH_3$), 2.29 (3H, $-CH_3$), 9.32 (1H, >NH); ^{13}C-NMR (125 MHz, $CDCl_3$): $\delta = 12.37$ ($-CH_3$), 12.59 ($-CH_3$), 22.62 ($>C(\underline{C}H_3)_2$), 46.47 ($>\underline{C}(CH_3)_2$), 114.05, 115.87, 127.38, 129.44, 146.22, 151.66 (6 arom. C), 182.56 (-CO-N<); MS (70 ev): *m/z* 279 (90 %, M^+), 262 (100 %), 245 (8 %), 230 (15 %), 172 (26 %), 91 (47 %).

6-methoxy-5,5,8,10-tetramethyl-7,9-dinitro-3-oxa-2-azabicyclo[4.4.0]deca-1,7,9-triene (4)

White powder, yield: 3.8 mg. M.p.: 106–107°C; UV (methanol): λ_{max} (log ε) = 248 nm (4.07), 292 nm (3.77); ^{1}H-NMR (500 MHz, $CDCl_3$): $\delta = 1.23$ (3H, $>C(CH_3)_2$), 1.28 (3H, $>C(CH_3)_2$), 1.66 (3H, $-N{=}C-C(CH_3){=}C-NO_2$), 2.07 (3H, $-C(CH_3){=}C-NO_2$), 3.33 (3H, $-OCH_3$), 3.77 (1H, doublet, $>CH_2-O-$), 3.83 (1H, doublet, $>CH_2-O-$); ^{13}C-NMR (125 MHz, $CDCl_3$): $\delta = 12.57$ ($-C(\underline{C}H_3){=}C-NO_2$), 21.20 ($>C(\underline{C}H_3)_2$), 22.17 ($>C(\underline{C}H_3)_2$), 24.46($-N{=}C-C(\underline{C}H_3){=}C-NO_2$), 32.08 ($>\underline{C}(CH_3)_2$), 52.75 ($-OCH_3$), 73.30 ($\underline{C}-OCH_3$), 76.66 ($>CH_2-O-$), 124.72, 128.49, 144.82, 146.51, 148.50 ($5 \times Csp^2$); MS (70 eV): *m/z* 311 (5 %, M^+), 296 (67 %), 280 (99 %), 265 (100%), 264 (3 %), 219 (3 %), 189 (4 %), 115 (8 %), 91 (8 %), 77 (9 %).

4-tert-butyl-2,6-dimethyl-3,5-dinitroaniline (5)

Yellow crystals, yield: 7.9 mg. M.p.: 186–188°C ; UV (methanol): λ_{max} (log ε) = 245 nm (4.18), 291 nm (3.42); ^{1}H-NMR (250 MHz, $CDCl_3$): δ= 1.39 (9H, $-C(CH_3)_3$), 1.99 (6H, $-CH_3$), 4.01 (2H, $-NH_2$): ^{13}C-NMR (63 MHz, $CDCl_3$): δ = 13.04 ($-CH_3$), 30.62 ($-C(\underline{C}H_3)_3$), 36.41 ($-\underline{C}(CH_3)_3$), 114.97, 119.72, 142.81, 150.40 (arom. C); GC-MS (70 eV): *m/z* 267 (29%, M$^+$), 252 (100 %), 235 (13 %), 218 (24 %), 188 (15 %), 144 (20 %), 91 (30 %), 57 (46 %).

6-tert-butyl-2,4-dimethyl-3,5-dinitroaniline (6)

Yellow crystals. M.p.: 122–123°C; UV (methanol): λ_{max} (log ε) = 207 nm (4.56), 247 nm (4.12), 293 nm (3.43); ^{1}H-NMR (300 MHz, $CDCl_3$): δ = 1.53 (9H, $-C(CH_3)_3$), 1.99 (3H, $-CH_3$), 2.07 (3H, $-CH_3$), 4.29 (2H, $-NH_2$); ^{13}C-NMR (75 MHz, $CDCl_3$): δ = 12.20 ($-CH_3$), 13.06 ($-CH_3$), 28.92 ($-C(\underline{C}H_3)_3$), 35.82 ($-\underline{C}(CH_3)_3$), 109.64, 115.08, 124.04, 143.80, 150.30, 150.86 (6 arom. C); GC-MS (70 eV): *m/z* 267 (20 %, M$^+$), 252 (11%), 218 (46 %), 160 (11 %), 57 (100%).

RESULTS AND DISCUSSION

Upon UV irradiation (λ>280 nm) musk xylene was photodegraded in the presence of cyclohexane, methanol and cyclohexene, as shown in Figure 1. Photolysis was much more efficient in cyclohexene than in methanol as well as in cyclohexane. The half-lives were estimated to be 51 min, 247 min, and 495 min, respectively.

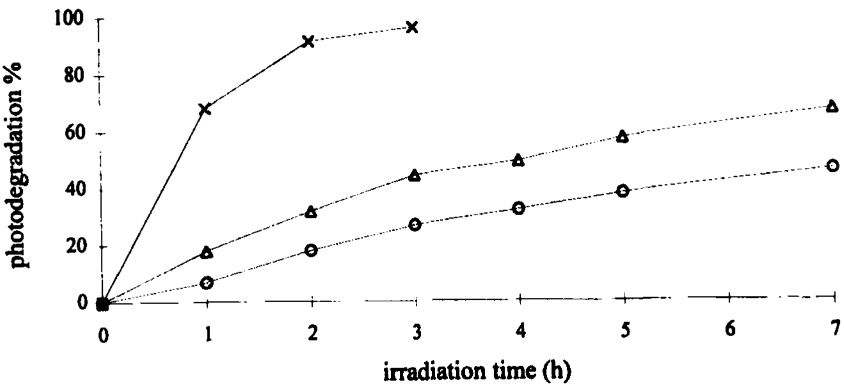

FIGURE 1 Photodegradation (λ>280 nm) of musk xylene in different organic solvents (20 mg/40 mL); O, cyclohexane; Δ, methanol; X, cyclohexene

In methanol and cyclohexane as reaction media the photocyclization products predominate exclusively throughout the irradiation period, which originate from intramolecular attack of an electronically excited nitro group on the neighbouring *tert*-butyl moiety. Comparable to the results obtained by Döpp et al.[7], the main photoproduct identified in our study was proved to be 1-hydroxy-3,3,5,7-tetramethyl-4,6-dinitro-2-indolinone (**2**) that was formed through several reaction steps (Figure 2). The N-hydroxy group of this product showed instability during GC-MS analysis while direct inlet MS measurement delivered the molecular mass of 295 convincingly. Furthermore, the blue color formed with iron(III)-chloride in methanol is characteristic for 1-hydroxy-2-indolinones.

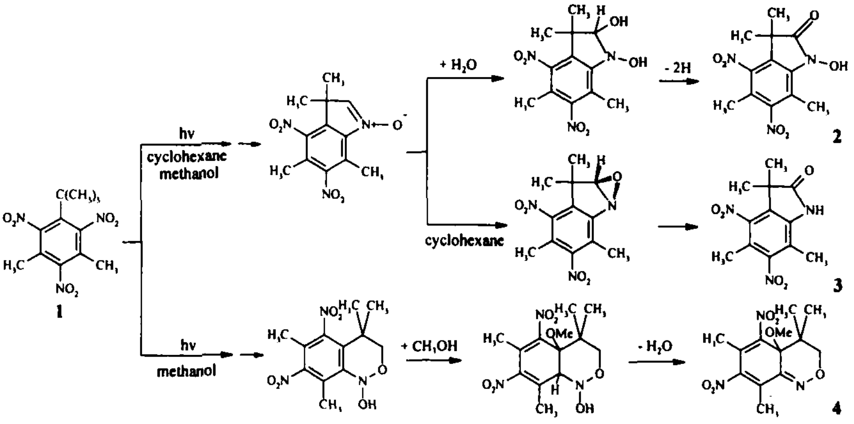

FIGURE 2 Photodegradation scheme of musk xylene (1) in methanol and cyclohexane

Depending on the solvents used, some different minor photoproducts were obtained. The cyclohexane irradiation mixture delivered 3,3,5,7-tetramethyl-4,6-dinitro-2-indolinone (**3**). The GC-MS analysis of **3** yielded the same fragment distribution as that of **2** and its formation could be postulated to experience an oxaziridine step. In the presence of methanol the participation of solvent molecules in the photoreaction process led to the formation of 6-methoxy-5,5,8,10-tetramethyl-7,9-dinitro-3-oxa-2-azabicyclo[4.4.0]deca-1,7,9-triene (**4**) (Figure 2). Since formation of compound **4** was unexpected, we want to present the spectroscopic arguments by which we have arrived at this structure; ^{1}H, ^{13}C chemical shifts and coupling constants are given in Figure 3. The ^{13}C-NMR data of **4** only show 5 sp^{2}-carbons and thus definitely rule out a benzenoid structure. The molecular ion at *m/z* 311 in the EI-MS spectrum of **4** formally corresponds to the addition of the solvent CH_3OH to the starting compound **1** under elimination of water. This hypothesis is strengthened by the NMR findings. The singlet of a methyl group at 3.33 ppm in the ^{1}H-NMR spectrum and the resonance at 52.8 ppm for the respective carbon as well as the ^{1}J(H,C) coupling constant of 145.5 Hz unequivocally prove the existence of a methoxy group. There are only two further ^{13}C signals (73.3 and 76.7 ppm) in the range expected for *O*-substituted sp^3-carbons. Based on ^{13}C-DEPT measurements the resonance at 76.7 ppm can be clearly assigned to a CH_2-function. The diastereotopic protons of this group (3.83 and 3.77 ppm) show coupling constants ^{1}J(H,C) 146.5 Hz and ^{2}J(H,H) (-)11.5 Hz which likewise prove linkage to an oxygen. Since the CH_2-O partial structure is incorporated in a ring, the methoxy group must be bound to the quaternary carbon at 73.3 ppm.

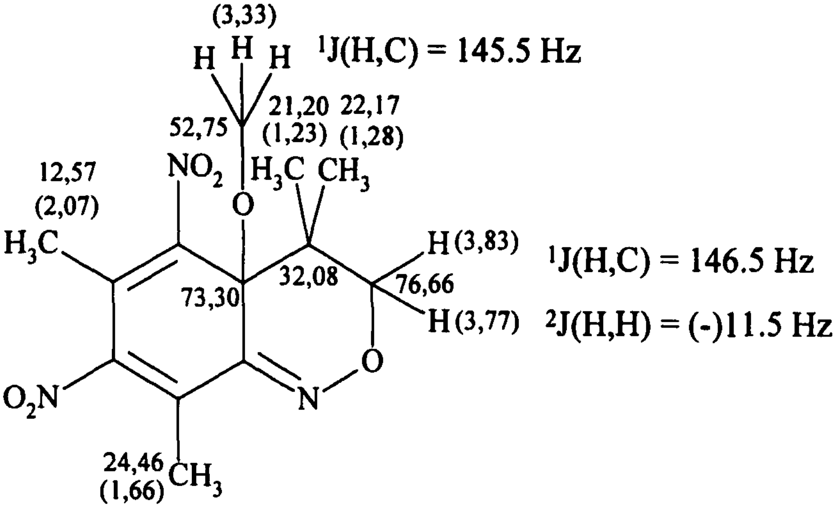

FIGURE 3 Hydrogen/carbon assignment of photoproduct **4** is validated by ^{1}H, ^{13}C-COSY measurements

In contrast, cyclohexene with H-donor properties[10] influenced the photodegradation process of musk xylene differently. The photoproducts identified in cyclohexane as well as in methanol photolysis mixtures were not found. Instead, photoreduction products **5** and **6** were representative in the cyclohexene medium, which must have been formed through nitroso and hydroxylamino intermediates[11] (Figure 4). Though the mass spectra of both gave the same molecular ion (M^+) at *m/z* 267, the ^{13}C-NMR analysis clearly indicated the symmetric structure of **5**. Due to electronic and steric reasons **5** was also preferentially formed than **6**. When air oxygen existed in cyclohexene, the formation of **6** could be effectively inhibited. Under the O_2 free condition the ratio of yield of **5** and **6** amounted to

24:1. As **5** and **6** could not be well separated from each other on HPLC and the amount of **6** was also too small for identification, a chemical method in which Fe/HCl was employed to reduce musk xylene was chosen to synthesize **6** so that the characterization of this compound could be guaranteed.

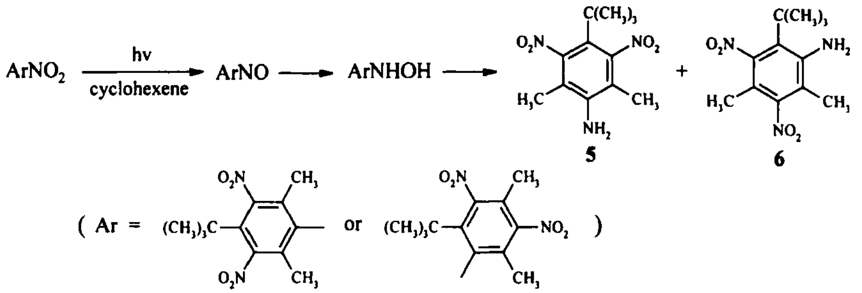

FIGURE 4 Photoreduction scheme of musk xylene in cyclohexene

Based on the fundamental research carried out in model organic solvents the study was further focused on the photostability of musk xylene on the layer of cellulose stearate both under sun simulator and in natural sunlight (Figure 5). The aim was to simulate clothes which may have residues of musk xylene after washing with flavored detergents and hence act as reaction medium if exposed in the sun. On both conditions musk xylene underwent the same photochemical process that resulted in the formation of several products already identified and characterized in model organic solvents (**2**, **3** and **5**). Both intramolecular cyclization and photoreduction occurred in this reaction medium.

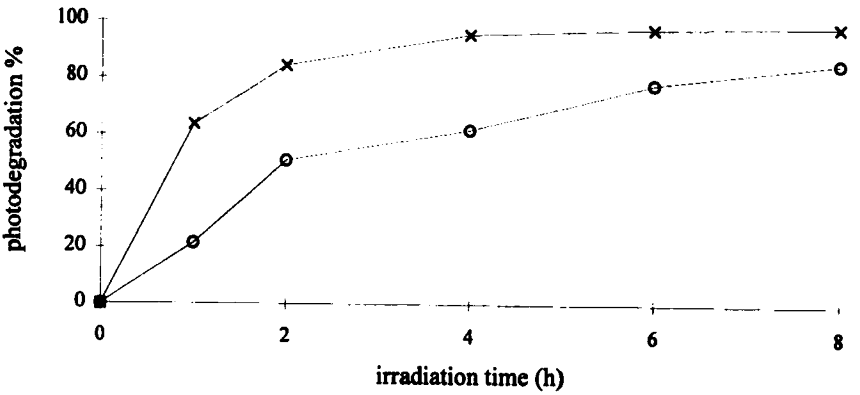

FIGURE 5 Photodegradation of musk xylene on layers of cellulose stearate. The initial contents: 5.8 mg/g (x, in sunlight) and 5.4 mg/g (O, under sun simulator), respectively

Furthermore, following the fact that musk xylene was often detected in surface water, waste water[4] and fishes[5] in the past few years, we also carried out the research about the photodecomposition of musk xylene in water. After 60 minutes irradiation (λ>280 nm) about 95 % of the initial content was converted (Figure 6). In comparison with the research about photochemical degradation of musk xylene in water performed by Schmidt et al.[8] who identified **3** and 3,3,5,7-tetramethyl-4,6-dinitro-3H-indole as photoproducts with GC-MS, we only found the formation of photoproduct **2** which was determined with GC-MS and HPLC.

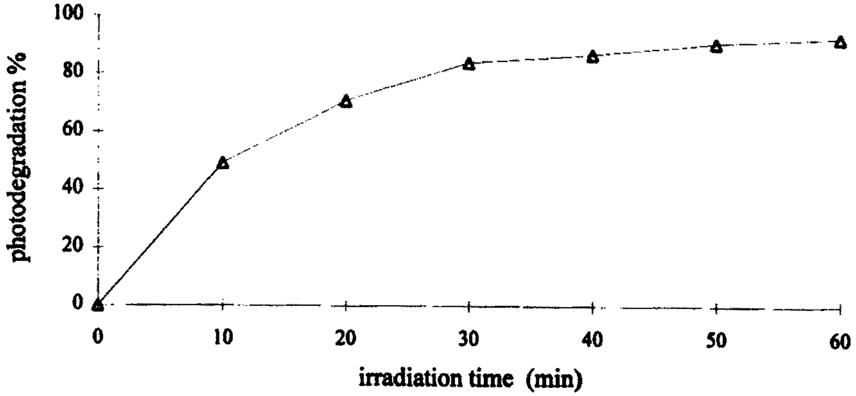

FIGURE 6 Photodegradation of musk xylene in water (16 μg/40 mL)

CONCLUSION

From the results obtained it can be concluded that reaction environments have significant impact on the photoreactivity and reaction process of musk xylene which showed a considerable tendency to be photochemically degraded.

It was reported that according to the OECD regulation 301C and measured as BOD, the biological degradation of musk xylene amounted to 0–6 % in 4 weeks. Hence, it must be classified as undegradable or very difficult to degrade under the given condition.[1] As a persistent and lipophilic compound, little is known so far about its chemical and biological transformation in the environment. In contrast, the present study indicates that photochemical degradation may be a reasonable pathway for musk xylene to be eliminated effectively. Unfortunately, it is not clear whether or not the photoproducts formed could be much greater dangers to the environment than the parent compound.

Maekawa et al.[12] reported that musk xylene was carcinogenic in B6C3F1 mice at the dose levels of 0.075 and 0.15 % in the diet for 80 weeks. Moreover, in the study of distribution, metabolism, and excretion of musk xylene in rats Minegishi et al.[13] identified amino derivatives as main metabolites, but found no evidence of carcinogenicity by them. As for the other photoproducts characterized in our study, it still lacks the findings about their toxicological behaviour. Therefore, photoproducts should be included in environmental and biological analyses and monitorings. It is also necessary to further investigate the properties of different photometabolites, influences on the environment and persistence.

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