Pergamon

Environmental Toxicology and Chemistry, Vol. 14, No. 8, pp. 1287-1298, 1995

Printed in the USA
0730-7268/95 $9.50 + .00

0730-7268(95)00068-2

COMPARATIVE PHOTODEGRADATION RATES OF ALACHLOR AND BENTAZONE IN NATURAL WATER AND DETERMINATION OF BREAKDOWN PRODUCTS

SERGE CHIRON,† JOAQUIN ABIAN,‡ MARTA FERRER,§ FRANCISCO SANCHEZ-BAEZA,§
ANGEL MESSEGUER§ and DAMIÀ BARCELÓ*†
Departments of †Environmental Chemistry, ‡Biomedical Analysis, and §Biological Organic Chemistry,
Jordi Girona, 18-26, 08034 Barcelona, Spain

(*Received* 20 *October* 1994; *Accepted* 27 *February* 1995)

Abstract—The photochemical degradation of the herbicides alachlor (2-chloro-2′,6′-diethyl-*N*-methoxymethylacetanilide) and bentazone (3-isopropyl-(1*H*)-2,1,3-benzothiadiazin-4(3*H*)-one-2,2-dioxide) in distilled water and in river water, under xenon arc lamp irradiation, was investigated. Analytical determinations were carried out by using a xenon arc photoreactor and on-line solid-phase extraction coupled to liquid-chromatography diode-array and liquid-chromatography mass-spectrometry detection systems. Photolysis experiments were performed at low concentration (20 μg/L), and the advantages of this methodology over conventional techniques are discussed. The photodegradation of alachlor and bentazone is a process depending on the water type, humic substances, and pH. When using a solution of 4 mg/L of humic matter, the estimated alachlor and bentazone half-lives were 84 and 150 min, respectively, using a total irradiance of 550 W/m^2 in the range of 300 to 800 nm. The degradation of alachlor and bentazone followed pseudo second- and first-order kinetics, respectively. The major photodegradation products of both herbicides were identified either by on-line solid-phase extraction (SPE)–liquid chromatography–thermospray mass spectrometry (LC/TSP-MS) or on-line-SPE-LC/TSP–tandem mass spectrometry (LC/TSP-MS/MS). In addition to that, a total of six transformation products of alachlor were synthesized in our laboratory and their MS spectra were compared with those of the breakdown products obtained. After photodegradation, a total of 14 photoproducts resulted from alachlor dechlorination with subsequent hydroxylation and cyclization processes. The two major photoproducts were identified as hydroxyalachlor and 8-ethyl-1-methoxymethyl-4-methyl-2-oxo-1,2,3,4-tetrahydroquinone. No significant breakdown products of bentazone could be identified.

Keywords—Alachlor Bentazone Photolysis Water Humic substances

INTRODUCTION

The herbicides bentazone (3-isopropyl-(1*H*)-2,1,3-benzothiadiazin-4(3*H*)-one-2,2-dioxide) and alachlor (2-chloro-2′,6′-diethyl-*N*-methoxymethylacetanilide) are commonly used for weed control in agricultural crops. Relatively high water solubilities and hydrolysis half-lives of alachlor and bentazone (500 mg/L; 15 d and 230 mg/L; >15 d, respectively) suggest that they may partition into ground waters and surface waters [1]. As a consequence of their widespread use (both over 500 tons a year in Europe) residue levels ranging from 0.1 μg/L to 10 μg/L have been detected in surface waters [2–4]. Information about the possible degradation pathways in aquatic natural systems is therefore important in order to know how long these compounds persist in the environment and to aid in identifying possible toxic transformation products.

Among the different transformation processes (biological and nonbiological), sunlight photoalteration of pesticide plays an important role in water [5,6]. Many discrepancies in pesticide photolysis data (i.e., half-lives data) come from the diversity of the aquatic systems (surface or distilled water) and irradiation sources (xenon arc lamps or mercury lamps) used. Although difficulties exist in modeling pesticide behavior under laboratory conditions, there is a need to conduct photodegradation studies useful for environmental purposes. Photolysis rates and photoproduct formation are actually dependent on the intensity and the wavelength distribution of the light used.

To accelerate photolysis testing, high-pressure Hg lamps with a wavelength output of approximately 254 nm were usually used in laboratory studies [6]. These studies pose difficulties for extrapolation to environmental conditions, as only the light <290 nm would be useful for fate measurements; moreover, it would be necessary to evaluate the quantum yield at a selected wavelength [7]. Under such practices with wavelengths out of the sunlight spectra, erroneous photodegradation mechanisms can be expected. The use of xenon arc lamps with an emission spectrum closely matching that of sunlight appears to be closer to environmental conditions [8–10], although xenon arc lamps do not account for daily and seasonal variation in sunlight. Photodegradation studies are usually carried out at high concentration levels (0.1 to 1 mg/L) in order to identify as many breakdown products as possible [6,11]. Nevertheless, photochemically induced formation of dimers may occur, and 2 to 5% organic solvents such as acetonitrile or methanol are needed to ensure pesticide water solubility, being responsible for solvent adduct formation [6].

---

*To whom correspondence may be addressed.

To avoid all these artifacts, our group is introducing a new method currently applied in our laboratory for monitoring purposes [3]. The pesticide photodegradation is investigated at low concentration by directly analyzing the irradiated solution using an on-line solid-phase extraction (SPE) followed by liquid chromatography-thermospray mass spectrometry (LC/TSP-MS). This approach can quantify the different pesticides and their transformation products (TPs) at levels between 0.02 to 10 μg/L [3,12]. With this methodology, photodegradation studies can be conducted at concentrations ranging from 10 to 50 μg/L of pesticide in the water samples. Experiments carried out in this way reflect more realistic environmental concentration levels.

The different studies on the photochemical transformation of pesticides generally involve an irradiation step, an isolation of the different photoproducts by solvent extraction followed by evaporation, and subsequent GC/MS determinations in order to identify the possible breakdown products formed [13,14]. However, most of the photodegradation reactions for pesticides in water occur through an oxidative pathway (hydroxy derivative formation for triazine; oxon analogs in the case of organophosphorus insecticides), leading generally to the formation of transformation products much more polar than the parent compounds. Consequently, they are not sufficiently volatile for GC analysis. In this respect, the use of LC/TSP-MS appears to be a good alternative to identify the more polar breakdown products, as our group did the last few years by irradiating relatively concentrated solutions of pesticides, e.g., 10 mg/L, and by directly analyzing the irradiated solution into the LC/TSP-MS [15].

One of the weaknesses in TSP ionization is its relatively little fragmentation together with a lack of structural information, which makes the use of the technique difficult for the identification of the tentative breakdown products formed. To enhance structural information, LC in combination with tandem MS has been successfully used in photodegradation studies [16].

Finally, structural confirmation needs to be carried out by analyzing authentic standards from synthetic origin—e.g., fenitrothion derivatives [6]. In this work our laboratory has undertaken the synthesis of six alachlor TPs not commercially available.

We must add that, until now, the combined use of on-line SPE-LC–diode array detection (DAD) and on-line SPE-LC/TSP-MS, although a powerful analytical technique for the trace determination of pesticides in waters, has not yet been employed in photodegradation studies of pesticides in water.

In view of the different approaches used for carrying out photodegradation studies of pesticides in water, the aims of our work were as follows: (a) the application of novel analytical techniques, involving on-line SPE-LC/DAD, LC/MS, and LC/MS-MS to the aquatic photodegradation studies of alachlor and bentazone at concentration levels of 10 to 50 μg/L by using a xenon arc lamp; (b) to study the degradation rate of alachlor and bentazone under various environmentally relevant conditions (using the authentic standards and commercially formulated compounds dissolved in distilled and/or river water containing or not containing humic substances). This should allow us to measure the enhancement of herbicide photodegradation in the presence of humic substances (HS) and to study the binding of alachlor and bentazone by dissolved HS; and (c) the identification of the main breakdown products by their synthesis and confirmation by on-line SPE-LC/DAD and on-line SPE-LC/TSP-MS. In addition, isolation of the breakdown products by off-line SPE and GC/MS determinations was also carried out was to identify the more volatile TPs and to correlate the data with the published literature.

EXPERIMENTAL SECTION

Chemicals

Analytical-grade alachlor and bentazone were purchased from Promochem (Wesel, Germany). Formulated alachlor used was Lasso EC (containing 480 g/L alachlor, Monsanto, St. Louis, MO). Formulated bentazone used was Basagran (containing 48% bentazone, BASF, Linsburgerhof, Germany). Rhône estuary humic materials were provided by Dr. P. Scribe (Univ. Pierre et Marie Curie, Paris, France). The LC-grade water, acetonitrile, and ammonium acetate were obtained from Merck (Darmstadt, Germany) and were passed through a 0.45-μm filter before use. Tripropylamine was obtained from Fluka Chemie (Buchs, Switzerland). Tripropylammonium formate was prepared by mixing amounts of tripropylamine (98%) and formic acid.

Chromatographic conditions

The eluent was delivered by two high-pressure pumps coupled to a model 715 automated gradient controller (Gilson, Villier le Bel, France) and a 1000S Applied Biosystems diode array detector (Foster City, CA). A Lichrocart cartridge column 60 RP-8 select B (25 cm × 4.6 mm i.d.) packed with 4-μm Supersphere from Merck (Darmstadt, Germany) was used. Gradient elution was performed in the case of alachlor from an eluent containing 5% of A (acetonitrile:water, 90:10) and 95% of B (water) to 100% of A in 35 min and back to the initial conditions in 5 min, at a flow rate of 0.9 ml/min. In the case of bentazone, gradient elution was performed from an eluent containing 5% of A (acetonitrile:water, 90:10) and 95% of B (water at pH = 3 with formic acid) to 45% A: 55% B in 35 min, and from 45% A:55% B up to 100% A in 3 min. Isocratic elution was performed during 10 min and back to the initial conditions in 4 min at a flow rate of 0.9 ml/min. In the on-line SPE method, a MUST column switching device from Spark Holland (AS Emmen, The Netherlands) was used. Sample preconcentration was carried out with an SSI model 300 LC pump from Scientific Systems Inc. (State College, PA). Precolumn consisted of a stainless steel membrane disk holder made in the workshop of the Free University of Amsterdam (The Netherlands) where 10 × 4.6 mm i.d. C_{18} Empore extraction disks were placed (J.T. Baker, Deventer, The Netherlands). The general analytical procedure was described elsewhere [3]. Disks were first conditioned by flushing 10 ml of methanol and then 10 ml of HPLC water at a flow rate of 1 ml/min. Later, 100 ml of water sample was preconcentrated at a flow rate of 2 ml/min. After switching the valve, the components were desorbed and separated by the gradient elution responsible for the analytical separation. Desorption of the pesticides from the disk holder was done in the backflush mode, thus avoiding dead volumes and chromatographic tailing.

Mass spectrometric analysis

GC/MS. A 30 m × 0.25 mm i.d. fused silica capillary column coated with chemically bonded phenyl-methyl DB 5 (J&W Scientific, Folsom, CA) was programmed from 60 to 280°C at 6°C/min and directly introduced into the ion source. The injection volume was 2 μl, and the injection mode was splitless. A Fisons MD 800 (Manchester, UK) was used for GC/MS in the electron impact (EI) mode. Helium was used as a carrier (30 cm/s). EI spectra were obtained at 70 eV.

Thermospray LC/MS

A Hewlett-Packard (Palo Alto, CA) model 5988A thermospray LC/MS quadrupole mass spectrometer and a Hewlett-Packard model 35741B instrument for data acquisition and processing were employed. In the case of bentazone analyses, thermospray temperature parameters were programmed from 100 to 90°C and from 180 to 170°C for the stem and tip, respectively. Ion source temperature was set at 250°C. Tripropylammonium formate (0.05 M, pH = 2.9) was used as mobile phase additive. In the case of alachlor analyses, thermospray temperature parameters were optimized from 110 to 100°C and from 200 to 190°C for the stem and tip, respectively. Ion source was set at 240°C in order to avoid thermal degradation of alachlor. Ammonium acetate (0.05 M) was used as mobile phase additive. The filament on setting was used in all experiments. Full-scan chromatograms were recorded, with conventional positive and negative ion chemical ionization, when analyzing spiked water.

Thermospray LC/MS-MS. A Finnigan (San Jose, CA) TSQ 700 triple quadrupole mass spectrometer equipped with a Finnigan thermospray source and interface was used. The interface and the interface block temperatures were set about 104 and 250°C, respectively. In these conditions the aerosol temperature was about 280°C. No temperature gradient programming of the interface was used for the run. Argon at 1 mTorr was used as collision gas. Collision energy was set at 25 eV.

Photolysis experiments

Irradiations were carried out using a Suntest apparatus from Heraeus (Hanau, Germany) equipped with a xenon arc lamp. Irradiance was set at 550 W/m^2. Additionally, 500 ml of distilled water, 500 ml of surface water (Ebro delta, Tarragona, Spain), and 500 ml of water samples containing 4 mg/L of humic matter were spiked with 20 μg/L of either formulated or analytical-grade alachlor/bentazone. Because the water solubility values of bentazone and alachlor are 500 μg/L and 230 μg/L, respectively, at 25°C, no methanolic solutions were required at this concentration level to ensure the pesticide solubility. Samples were kept in a quartz reaction reservoir, and temperature was set at 30°C using a cooling circuit. At different periods, water samples of 100 ml were removed from this reactor, preconcentrated after acidification at pH = 1 with sulfuric acid, and finally analyzed by on-line SPE-LC/DAD or on-line SPE-LC/TSP-MS. Experiments involving real river water or distilled water spiked with humic matter were undertaken so as to examine the enhancement of photodegradation by using this matrix.

Studies on the bonding ability of HS were carried out by adding 4 mg/L of HS to distilled water spiked with 20 μg/L of pure alachlor. The dissolved HS and herbicides remained in contact during 24 h at room temperature and in the dark so that adsorption equilibrium was reached. Solutions were irradiated during 15 h and either acidified at pH = 1 or not. The difference between pesticide concentrations obtained with or without sample acidification before analysis allowed us to estimate the pesticide-binding fraction in a similar way as reported elsewhere [17].

Synthesis of TPs of alachlor

The infrared (IR) spectra were recorded with a Bomen model MB120 apparatus (Montreal, Canada). The nuclear magnetic resonance (NMR) spectra (^{1}H, ^{13}C) were recorded on a Varian Gemini 200 or a Varian Unity 300 spectrometer (Palo Alto, CA). All NMR spectra were performed in neutralized $CDCl_3$ solution; chemical shifts are given in ppm using [^{1}H]tetramethylsilane and $^{13}CDCl_3$ as internal references. Other symbols are s: singlet; m: multiplet; t: triplet; d: doublet; ca: complex absorption; q: quadruplet; br: broad. The GC/MS-EI spectra (70 eV) were obtained using a Fisons model MD 800 mass spectrometer coupled to a Fisons GC 8000 apparatus equipped with a 25-m HP-5 capillary column. The elemental analyses were performed on a Carlo Erba 1108 instrument (Milan, Italy). All new compounds gave correct elemental analysis (±0.4%). Reagents were used as received from commercial sources. Unless otherwise stated, organic extracts coming from crude reaction mixtures were routinely washed with water and brine, and dried over $MgSO_4$. Compounds were purified by preparative TLC or flash chromatography on silica gel, using hexane:ethyl acetate or hexane: *tert*-butyl methyl ether as eluent mixtures. Unequivocal photoproduct structural confirmation needs to be carried out by comparison with authentic standards [5,6]. The syntheses of different putative phototransformation products derived from alachlor to be used as standards for identification purposes are shown in Figure 1 and are briefly described below.

2,6-Diethylacetanilide (1). 2,6-Diethylaniline (0.78 g, 5.2 mmol) was acetylated with acetic anhydride (6.3 mmol) and triethylamine (0.02 mmol) in CH_2Cl_2 (50 ml) to give compound **1** (0.85 g) in 85% yield. **1**: IR (film): 3,213, 1,650, 1,637, 1,537 cm^{-1}; ^{1}H NMR, δ: 7.3–7.0 (3 H, H Ar), 2.55 (m, 4 H, CH_2), 2.14 (s, 0.7 from 3 H, $COCH_3$), 1.71 (s, 0.3 from 3 H, $COCH_3$), 1.20 (t, 0.6 from CH_3, J = 9.5 Hz), 1.16 (t, 0.4 from 3 H, J = 10 Hz, CH_3); ^{13}C NMR, δ: 173.5 (CO), 169.4 (CO), 142.2 (*o*-Ar), 141.5 (*o*-Ar), 133.7 (C-1), 132.7 (C-1), 128.61 (*p*-Ar), 127.9 (*p*-Ar), 126.72 (*m*-Ar), 126.13 (*m*-Ar), 24.7 (CH_2), 23.0 ($CO\underline{C}H_3$), 19.9 ($CO\underline{C}H_3$), 14.4 (CH_3), 14.3 (CH_3); MS: 191 (M$^+$, 37), 176 (14), 148 (100), 134 (91).

N-*Acetyl*-N-*methoxymethyl-2,6-diethylaniline (2).* A 50% solution of NaOH (0.27 ml) was added dropwise to a solution of amide **1** (0.10 g, 0.52 mmol), methoxymethyl chloride (75 μl, 1 mmol) and phenyltriethylammonium chloride

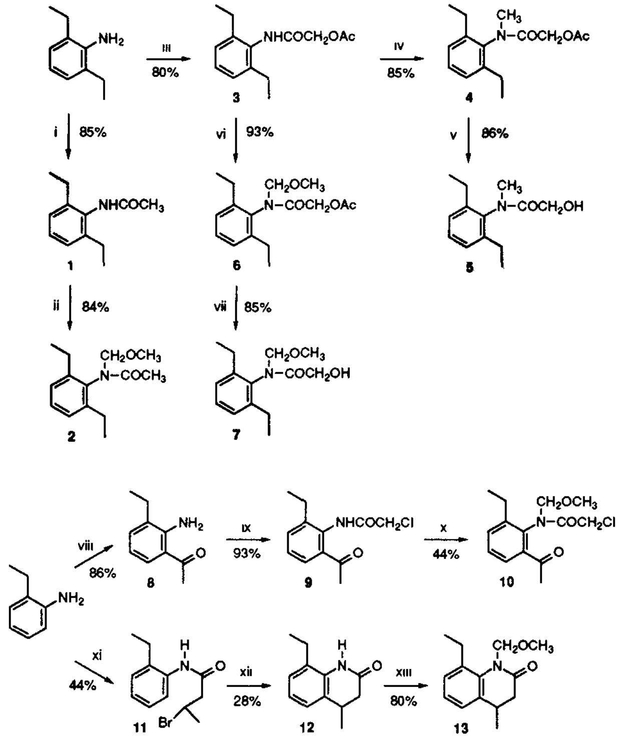

Fig. 1. Reaction conditions. (i) $Ac_2O/Et_3N/CH_2Cl_2$; (ii) $ClCH_2OCH_3$/50% $NaOH/CH_2Cl_2/Et_3PhNCl$; (iii) $ClCOCH_2OAc/Et_3N/CH_2Cl_2$; (iv) $NaH/CH_3I/THF$; (v) 50% NaOH/THF; (vi) $NaH/CH_3OCH_2Cl/THF$; (vii) 50% NaOH/THF; (viii) $CH_3CN/BCl_3/AlCl_3$/toluene, followed by 1 N H_2SO_4/reflux; (ix) $ClCOCH_2Cl/Et_3N/CH_2Cl_2$; (x) $ClCH_2OCH_3$/50% $NaOH/CH_2Cl_2/Et_3PhNCl$; (xi) 3-bromobutyric acid/Et_3N/ClCOOEt; (xii) $AlCl_3$/heptane; (xiii) $ClCH_2OCH_3$/50% $NaOH/CH_2Cl_2/Et_3PhNCl$.

(2.7 mg) in CH_2Cl_2 (150 μl), and the mixture was vigorously stirred for 1 h at 0 to 5°C. Purification of the crude reaction mixture gave 0.103 g of amide **2** (84% yield). **2**: IR (film): 1,674, 1,298 cm^{-1}; ^1H NMR, δ: 7.37–7.10 (3 H, ArH), 4.91 (s, 2 H, NCH_2O), 3.45 (s, 3 H, OCH_3), 2.58 (m, 4 H, CH_2), 1.75 (s, 3 H, $COCH_3$), 1.24 (t, 3 H, $J = 11.5$ Hz, CH_3); ^{13}C NMR, δ: 172.5 (CO), 141.5 (*o*-Ar), 139.3 (C-1), 128.5 (*p*-Ar), 128.0 (*p*-Ar), 126.6 (*m*-Ar), 126.3 (*m*-Ar), 79.6 (NCH_2O), 57.5 (OCH_3), 23.6 (CH_2), 21.9 ($CO\underline{C}H_3$), 14.4 (CH_3); GC-MS: 235 (M^+, 2), 161 (59), 146 (100).

N-*Acetoxyacetyl-2,6-diethylaniline (3).* Acetoxyacetyl chloride (0.47 ml, 4.3 mmol) was added to a solution of 2,6-diethylaniline (0.50 g, 3.3 mmol), triethylamine (1.4 ml, 10 mmol) in CH_2Cl_2 (50 ml), and the mixture was stirred at −20°C until reaction was completed (36 h). The workup of the crude reaction mixture led to a residue containing 0.68 g (80% yield) of compound **3**, which was used without further purification. **3**: ^1H NMR, δ: 7.45 (br. s, 1 H, NH), 7.30–7.07 (3 H, ArH), 4.72 (s, 2 H, $COCH_2O$), 2.56 (q, 4 H, $J = 7.5$ Hz, CH_2), 2.20 (s, 3 H, $OCOCH_3$), 1.18 (t, 6 H, $J = 7.5$ Hz, CH_3); ^{13}C NMR, δ: 169.5 (CO), 166.4 (CO), 141.2 (*o*-Ar), 131.5 (C-1, Ar), 127.8 (*p*-Ar), 125.9 (*m*-Ar), 62.4 ($CO\underline{C}H_2OAc$), 24.3 ($\underline{C}H_2CH_3$), 20.0 ($OCO\underline{C}H_3$), 14.0 (CH_3).

N-*Acetoxyacetyl*-N-*methyl-2,6-diethylaniline (4).* A solution of the amide **3** (0.64 g, 2.6 mmol) in tetrahydrofuran (THF) (10 ml) was added to a suspension of NaH (0.08 g, 3.2 mmol) in THF (3 ml), maintained at −20°C, and the mixture was stirred at this temperature for 30 min and for 30 min at 20°C. Then, a solution of methyl iodide (0.19 ml, 3.2 mmol) in THF (10 ml) was added to the reaction flask and the stirring was prolonged for 2 h. Treatment of the crude reaction mixture afforded 0.58 g of compound **4** (85% yield), which was used without further purification. **4**: ^1H NMR, δ: 7.40–7.08 (3 H, ArH), 4.16 (s, 2 H, $COCH_2O$), 3.19 (s, 3 H, NCH_3), 2.59 (q, 4 H, $J = 7.5$ Hz, CH_2), 2.13 (s, 3 H, $OCOCH_3$), 1.26 (t, 6 H, $J = 7.5$ Hz, CH_3).

N-*Hydroxymethylcarbonyl*-N-*methyl-2,6-diethylaniline (5).* A solution of amide **4** (0.52 g, 2.3 mmol) in THF (25 ml) was treated with 1% NaOH (50 ml) at 20°C until reaction was completed (15 min). Treatment and purification of the crude reaction mixture afforded alcohol **5** (0.43 g, 86% yield). **5**: IR (film): 3,425, 1,658 cm^{-1}; ^1H NMR, δ: 7.38–7.14 (3 H, ArH), 3.58 (d, 2 H, $J = 5$ Hz, $COCH_2OH$), 3.40 (t, 1 H, $J = 5$ Hz, OH), 3.24 (s, 3 H, NCH_3), 2.51 (q, 4 H, $J = 8$ Hz, CH_2), 1.24 (t, 6 H, $J = 8$ Hz, CH_3); ^{13}C NMR, δ: 172.3 (CO), 141.2 (*o*-Ar), 136.7 (C-1), 129.2 (*p*-Ar), 127.0 (*m*-Ar), 60.3 (NCH_2OH), 36.2 (NCH_3), 23.3 (CH_2), 14.5 (CH_3); GC-MS: 221 (M^+, 1.5), 190 (100).

N-*Acetoxyacetyl*-N-*methoxymethyl-2,6-diethylaniline (6).* Following the procedure described for the preparation of amide **4**, amide **3** (3.2 mmol) was treated with methoxymethyl chloride (0.3 ml, 3.8 mmol) to give a crude reaction mixture containing the amide **6** (0.87 g, 68% yield), which was used without further purification. **6**: ^1H NMR, δ: 7.37–7.10 (3 H, ArH), 4.94 (s, 2 H, $COCH_2O$), 4.16 (s, 2 H, NCH_2O), 3.48 (s, 3 H, OCH_3), 2.61 (q, 4 H, $J = 7.5$ Hz, CH_2), 2.13 (s, 3 H, $COCH_3$), 1.26 (t, 6 H, $J = 7.5$ Hz, CH_3); ^{13}C NMR, δ: 170.4 (CO), 168.6 (CO), 141.9 (*o*-Ar), 136.3 (C-1 Ar), 129.2 (*p*-Ar), 126.9 (*m*-Ar), 80.0 (NCH_2O), 61.7 ($\underline{C}H_2OAc$), 57.8 (OCH_3), 23.4 ($\underline{C}H_2CH_3$), 20.4 ($OCO\underline{C}H_3$), 14.3 (CH_3).

N-*Hydroxymethylcarbonyl*-N-*methoxymethyl-2,6-diethylaniline (7).* Following the procedure described above for the hydrolysis of amide **4**, compound **6** (0.85 g, 2.9 mmol) gave alcohol **7** (0.32 g, 89% yield). **7**: IR (film): 3,458, 3,450, 1,668, 1,458 cm^{-1}; ^1H NMR, δ: 7.4–7.16 (3 H, ArH), 4.89 (s, 2 H, NCH_2O), 3.53 (d, 2 H, $J = 4.5$ Hz, $COCH_2$), 3.41 (s, 3 H, OCH_3), 3.31 (t, 1 H, $J = 4.5$ Hz, OH), 2.54 (q, 4 H, $J = 7.5$ Hz, CH_2), 1.24 (t, 6 H, $J = 7.5$ Hz, CH_3); ^{13}C NMR, δ: 174.1 (CO), 141.7 (*o*-Ar), 135.6 (C-1), 129.3 (*p*-Ar), 129.0 (*p*-Ar), 126.9 (*m*-Ar), 80.3 (NCH_2O), 60.6 (CH_2OH), 57.7 (OCH_3), 23.5 (CH_2), 14.3 (CH_3); GC-MS: 251 (M^+, 1.5), 188 (100), 160 (83), 140 (64).

2-Acetyl-6-ethylaniline (8). Following the procedure reported by Mortimer (18), treatment of 2-ethylaniline (2.1 g, 17.4 mmol) with 20 ml of a 1 M solution of BCl_3 in heptane, followed by addition of acetonitrile (2.9 ml, 52 mmol) and $AlCl_3$ (2.3 g, 19 mmol), afforded compound **8** (0.86 g, 30% yield), which was used without further purification. **8** (19): ^1H NMR, δ: 7.62 (dd, 1 H, $J_1 = 1$, $J_2 = 8$ Hz, H-3, β-CO), 7.20 (da, 1 H, $J = 7.5$ Hz, H-5), 6.62 (t, 1 H, $J = 8$ Hz, H-4), 6.49 (br., 2 H, NH_2), 2.58 (s, 3 H, $COCH_3$), 2.49 (q, 2 H, $J = 7.5$ Hz, CH_2), 1.25 (t, 3 H, $J = 7$ Hz, CH_3).

*N-Chloromethylcarbonyl-2-acetyl-6-ethylaniline (**9**).* Chloroacetyl chloride (0.09 ml, 1.2 mmol) was added to a solution of amine **8** (0.15 g, 0.92 mmol) and triethylamine (0.4 ml, 2.7 mmol) in CH_2Cl_2 (15 ml), maintained at −20°C. The mixture was then stirred for 24 h at 20°C. The workup of the crude reaction mixture gave compound **9** (0.21 g, 93% yield), which was used without further purification. **9**: ^1H NMR, δ: 9.75 (br., 1 H, NH), 7.63 (dd, 1 H, $J_1 = 1$, $J_2 = 8$ Hz, H-3, β-CO), 7.49 (br. d, 1 H, $J = 7.5$ Hz, H-5), 7.32 (t, 1 H, $J = 8$ Hz, H-4), 4.18 (s, 2 H, CH_2Cl), 2.62 (q, 2 H, $J = 7.5$ Hz, CH_2), 2.61 (s, 3 H, $COCH_3$), 1.22 (t, 3 H, $J = 7.5$ Hz, CH_3).

*N-Chloromethylcarbonyl-N-methoxymethyl-2-acetylethylaniline (**10**).* This compound was obtained and isolated (0.10 g, 44% yield) from amide **9** (0.21 g, 0.86 mmol), by using the procedure described for the preparation of compound **2**. **10**: IR (film): 1,691, 1,454 cm^{-1}; ^1H NMR, δ: 7.70–7.30 (3 H, ArH), 5.45 (d, 1 H, $J = 10$ Hz, $COC\underline{H_a}H_bCl$), 4.32 (d, 1 H, $J = 10$ Hz, $COH_a\underline{H_b}Cl$), 4.14 (d, 1 H, $J = 14$ Hz, $NC\underline{H_a}H_bO$), 3.73 (d, 1 H, $J = 14$ Hz, $NCH_a\underline{H_b}O$), 3.35 (s, 3 H, OCH_3), 2.62 (m, 2 H, CH_2), 2.54 (s, $COCH_3$), 1.27 (t, 3 H, $J = 7.5$ Hz, CH_3); ^{13}C NMR, δ: 200.7 (CO), 168.5 (NCO), 143.1 (C-1), 139.3 (*o*-Ar), 135.1 (*o*-Ar), 132.2 (CH Ar), 128.9 (CH Ar), 125.9 (CH Ar), 79.6 (NCH_2O), 57.8 (OCH_3), 42.5 (CH_2Cl), 29.6 (CH_3CO), 23.1 (CH_2), 14.0 (CH_3); GC-MS: 283 (M^+, 0.5), 248 (23), 206 (25), 174 (100).

*9-Ethyl-4-methyl-2-oxo-1,2,3,4-tetrahydroquinoline (**12**).* A solution of 3-bromobutyric acid (0.300 g, containing 33% of butyric acid), in CH_2Cl_2 (20 ml), was treated with triethylamine (0.25 ml, 1.8 mmol), ethyl chloroformate (0.17 ml, 1.8 mmol) and after 1 min, with 2-ethylaniline (0.66 ml, 5.4 mmol). The mixture was stirred at 20°C until reaction was completed. After the workup and further purification, a 2:1 mixture of amide **11** and the corresponding nonbrominated amide derivative was isolated in 65% overall yield. **11**: ^1H NMR, δ: 7.48 (br., 1 H, NH), 7.63 (d, 1 H, H-2 Ar), 7.30–7.00 (3 H, ArH), 4.58 (hx, 1 H, $J = 7.0$ Hz, CHBr), 2.89 (d, 2 H, $J = 6.5$ Hz, $COCH_2$), 2.61 (q, 2 H, $J = 7.5$ Hz, CH_2), 1.79 (d, 3 H, $J = 6.5$ Hz, $CH(Br)\underline{CH_3}$), 1.21 (t, 3 H, $J = 7.5$ Hz, CH_3). Then, a solution containing this amide mixture (0.30 g) in heptane (7 ml) was stirred for 6 h at 65°C in the presence of $AlCl_3$ (0.52 g, 3.9 mmol). The workup and further purification of the crude reaction mixture gave amide **12** (0.040 g, 28% yield), which was used without further characterization. **12**: ^1H NMR, δ: 9.25 (br., 1 H, NH), 7.11 (t, 1 H, $J = 8$ Hz, ArH), 6.90 (d, 1 H, $J = 7.5$ Hz, ArH), 6.72 (d, 1 H, $J = 7.5$ Hz, ArH), 3.33 (m, 1 H, H-4), 2.80–2.45 (4 H, 2 H $C\underline{H_2}CH_3$, 2 H CH_2CO), 1.25 (t, 3 H, $J = 7.5$ Hz, CH_3), 1.20 (d, 3 H, $J = 7$ Hz, CH_3CH); ^{13}C NMR, δ: 171.2 (CO), 141.3 (Ar), 136.1 (Ar), 127.3 (Ar), 126.4 (Ar), 123.4 (Ar), 113.9 (Ar), 37.8 (CH_2CO), 27.4 ($CHCH_3$), 24.8 (CH_2), 19.4 ($CH\underline{CH_3}$), 15.3 (CH_3); GC-MS: 189 (M^+, 62), 174 (100), 146 (83), 128 (32), 91 (12), 77 (16).

*8-Ethyl-1-methoxymethyl-4-methyl-2-oxo-1,2,3,4-tetrahydroquinoline (**13**).* This compound was obtained and isolated (0.040 g, 80% yield) from amide **12** (0.040 g, 0.2 mmol), by using the procedure described for the preparation of compound **2**. **13**: IR: 1,687, 1,469 cm^{-1}; ^1H NMR, δ: 7.24–7.14 (br., 2 H, ArH), 6.96 (dd, 1 H, $J_1 = 3.5$, $J_2 = 5.5$ Hz, H-6), 5.73 (d, 1 H, $J = 10.5$ Hz, $C\underline{H_a}H_bOCH_3$), 4.94 (d, 1 H, $J = 10.5$ Hz, $CH_a\underline{H_b}CH_3$), 3.41 (s, 3 H, OCH_3), 3.29 (m, 1 H, $C\underline{H}CH_3$), 2.84–2.52 (4 H, 2 H CH_2, 2 H H-3), 1.26 (t, 3 H, $J = 7.5$ Hz, CH_3), 1.17 (d, 3 H, $J = 7$ Hz, CH_3CH); ^{13}C NMR, δ: 170.3 (CO), 140.8 (Ar), 138.6 (Ar), 128.5 (Ar), 127.3 (CH Ar), 124.0 (CH Ar), 114.2 (CH Ar), 73.9 (NCH_2O), 56.3 (OCH_3), 38.5 (CH_2CO), 26.8 ($CHCH_3$), 25.0 (CH_2), 18.8 ($\underline{CH_3}CH$), 15.4 (CH_3); GC-MS: 233 (M^+, 79), 218 (100).

RESULTS AND DISCUSSION

General considerations

The main advantages of using on-line SPE-LC/DAD and/or on-line SPE-LC/TSP-MS over an off-line solvent extraction or SPE followed by GC or LC determinations for pesticide analysis in water samples have been pointed out in various sources [12,19]. Application of this on-line methodology to the identification of photodegradation products after irradiation is new. It allows direct analysis of the water samples after irradiation, which is a guarantee of sample analytical integrity. When applying conventional techniques, the solvent must be evaporated generally to dryness and, consequently, losses of compounds can take place either during evaporation or by degradation during the time of exposure. With the method presented here this step is obviously avoided.

A second clear advantage when using on-line SPE-LC/TSP-MS is that it is not necessary to perform any derivatization of the sample, which is also a typical procedure in photolysis experiments for the identification of more polar TPs. Because the technique used is very sensitive (sample enrichment by a factor of 1,000 vs. conventional off-line LC technique) limits of detection (LODs) are closer to GC techniques, so it makes this approach particularly useful for the trace-level identification of the more polar TPs. Photolysis experiments have been carried out at concentrations as small as 20 μg/L. LC/TSP-MS has sometimes caused us problems in the identification of unknown TPs. The use of TSP–tandem MS/MS enhances the structural information obtained by the LC/MS combination, providing fragmentation patterns as important as those obtained in GC/MS using EI mode. In addition, use of off-line SPE and GC/MS will permit us to match the compounds that are identified by both techniques and to know to what extent the use of on-line SPE-LC/DAD and/or MS permits identification of a variety of more polar pesticides as compared to GC/MS approaches.

Degradation rates of alachlor and bentazone under a xenon arc lamp

Alachlor is fairly stable in river water and no losses via chemical hydrolysis processes were found after at least 1 month with solutions kept in the dark at pH 6.8 (ammonium acetate buffer). Alachlor absorbs radiation weakly between 290 nm, which is the lower wavelength limit for sunlight radiation, and 320 nm. The UV/visible spectrum and structure of alachlor are given in Figure 2. The direct photolysis of alachlor is therefore slow, and when irradiation of analytical-grade alachlor in distilled water was carried out, all of the alachlor was depleted within 10 h. The reaction did not appear to be first order. However, first-order kinetics were previously reported, but in the first case [13], a medium-

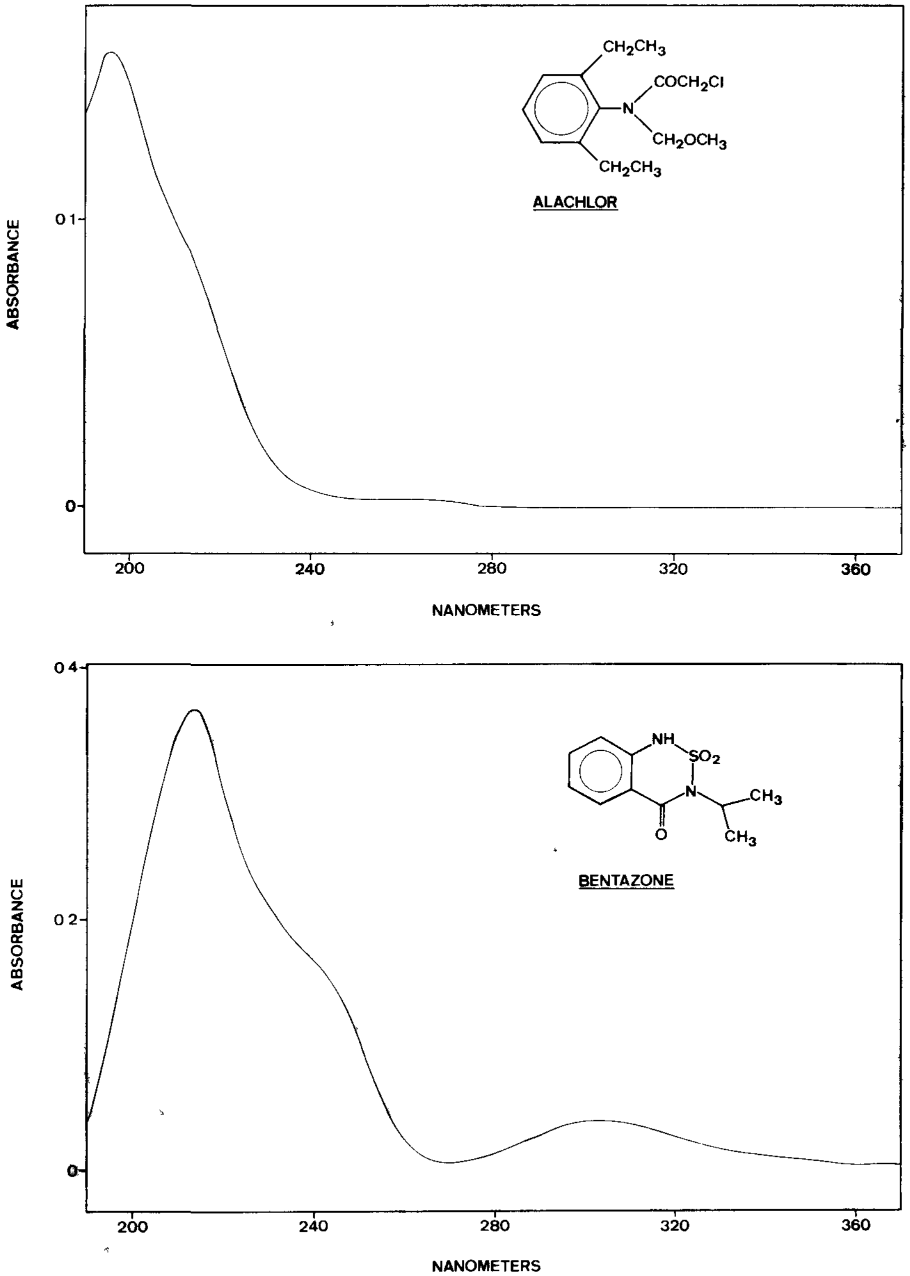

Fig. 2. Chemical structures and UV/visible spectra of alachlor and bentazone.

pressure mercury lamp was used, and in the second case [20], degradation was carried out in the presence of a catalyst, TiO_2. Both degradations may proceed by completely different mechanisms. From plots of $1/C - 1/C_0$ versus time, the reaction apparently fits a second-order kinetic, and the half-life ($t_{1/2}$) was estimated to be ~140 min (Table 1). The appearance of hydroxyalachlor followed a first-order kinetic, suggesting that a stable radical intermediate reacting with water is formed. The intermediate formation can be explained by the loss of chlorine, following the absorption of photons, and its structure is given in Figure 3. Such a radical is likely at the origin of complicated photolysis mechanisms. In addition, it has to be pointed out that alachlor undergoes conformational equilibrium in solution around the amide bond, which has no effect on alachlor loss rates in water.

No differences were observed between photolysis of analytical-grade alachlor and formulated alachlor. Humic substances (HS) are in natural water the largest fraction of dissolved organic matter (DOM; 40–60%), and the molecular interactions of DOM are dependent on temperature, pH, ionic strength, and type of ions in solution [21]. We have found that HS are partly bound to alachlor when added in

Table 1. Calculated rate constants and half-lives of alachlor in water under xenon arc lamp irradiation

Water type	Rate constant (ng ml^{-1} min^{-1})	Half-life (min)
Distilled water + pure alachlor	$1.79 \cdot 10^{-2}$	140
Distilled water + formulated alachlor	$1.94 \cdot 10^{-2}$	129
Ebro River water + pure alachlor	$2.98 \cdot 10^{-2}$	84

aqueous media at 4 mg/L. Previously reported results [22] have indicated that alachlor does not bind to water-soluble soil organic matter (WSSOM), although the experiments were not reported. This is somewhat strange since compounds with a similar K_{oc} value as alachlor (between 115 to 120 cc/g) such as simazine [22] and atrazine [23] were found to bind to WSSOM.

The binding of alachlor to HS was demonstrated by irradiating alachlor solutions during 15 h in order to ensure that the whole photodegradation of dissolved alachlor was achieved. However, changing the pH from 7 to 1 leads to the release of the pesticide. This alachlor fraction was bound to HS, and it was never available to photolysis action. The binding fraction was estimated around 5% of the initial alachlor concentration. Binding by physical sorption is reversible, and van der Waals forces are involved in these interactions rather than covalent binding to form stable aggregates. The masking behavior of humic matter was optimum within a contact time of 24 h before irradiation. The binding of anthracene and pyrene [21] by humic acids occurs very fast. It never took longer than 3 min because of the predominance of hydrophobic interactions. The higher time of contact required in the case of alachlor implies direct reaction with functional groups. At constant pH 6.8 an increase in ionic strength (0.05–0.1 M NaCl) induced a decrease in binding rate. The K_{oc} of alachlor depends, therefore, on its solubility in different waters.

Furthermore, different HS were extracted from Rhône River delta water samples with a water salinity rate of 0, 4.5, 6.2, and 33‰, respectively, and their binding behaviors were investigated. The binding fraction of alachlor increases when the water salinity rate increases, reaching 10% for HS extracted from water with 33‰ of salinity. The binding of pesticides by HS may not depend only on the chemical characteristics of the water but also on the structural characteristics of the HS. More studies are required to understand whether this fraction is irreversibly bound to HS under actual conditions or whether it is steadily released in water, becoming a constant source of contamination.

To study the influence of HS on photolysis rates, the alachlor binding fraction was omitted from kinetic equations by subtracting this constant amount from alachlor concentration values obtained during the kinetic experiments. By applying this correction, a decrease of 56 min in half-life was observed (see Table 1). In addition, chemical changes of aquatic humic material took place under xenon lamp irradiation. The broad peak recorded at retention time of 5 to 10 min (see Fig. 4) disappeared progressively, and new peaks at 17 and 18 min appeared in the chromatographic traces after 3 h of irradiation.

Complete photolysis of bentazone in its dissociated form (pK_a = 3.4, and water pH was set at 6.8 with a 0.1 M ammonium acetate buffer) took place within 16 h in distilled water. Bentazone is somewhat more stable than alachlor in hydrolysis (dark control) and photolysis in aqueous environment, although the UV/visible spectrum of bentazone (Fig. 1) shows stronger absorption at wavelengths above 300 nm than does alachlor. Reaction appeared pseudo-first-order with a $t_{1/2}$ about 2 h 30 min. The $t_{1/2}$ for formulated bentazone was 2 h 45 min. No quenching or photosensitizing effects can be attributed to the wetting agents in this case.

Bentazone was not observed to bind to any of the studied HS. These findings followed expectations since both bentazone and humics are substances with dominant anionic nature. However, when experiments were carried out with distilled water spiked with 4 mg/L of HS and bentazone in its dissociated form, photochemical degradation increased and the reaction was depleted within 8 h. If bentazone was in its

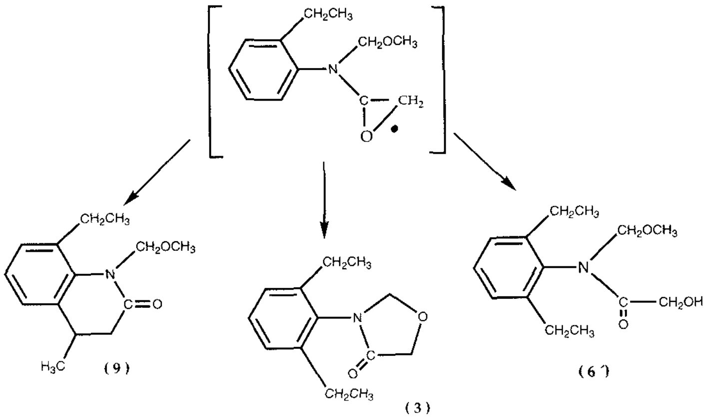

Fig. 3. Chemical structures of three photodegradation products of alachlor: (6′) hydroxyalachlor, (9) 8-ethyl-1-methoxymethyl-4-methyl-2-oxo-1,2,3,4-tetrahydroquinoline, and (3) [4-(2,6-diethylphenyl)-3-morpholinone].

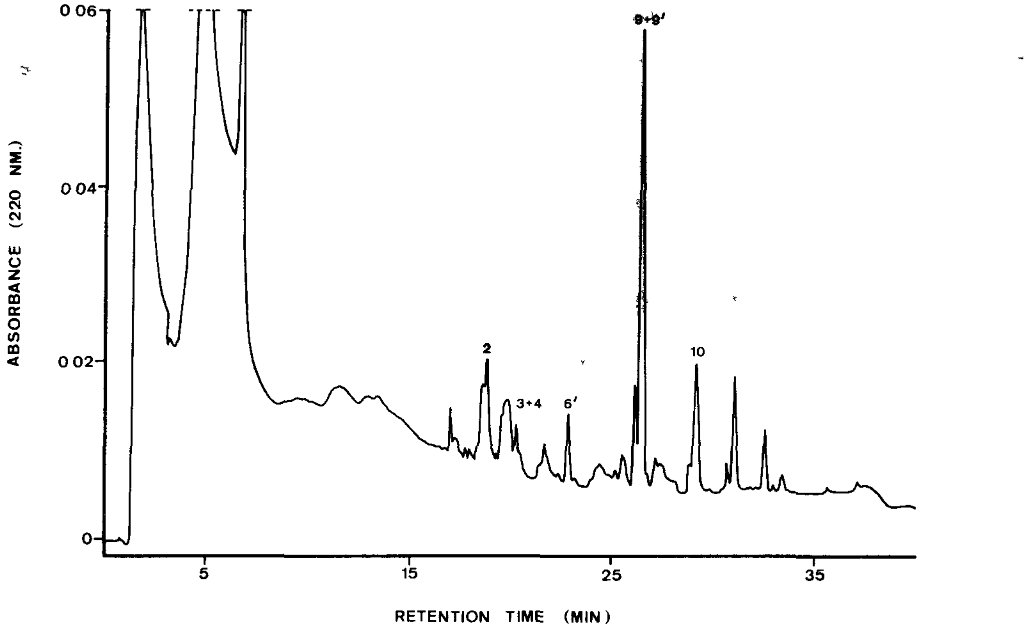

Fig. 4. On-line SPE-LC/UV chromatogram obtained at 220 nm after preconcentration on 10 × 4.6 mm i.d. C_{18} Empore extraction disks of 100 mL of Ebro River water sample spiked with 20 μg/L analytical-grade alachlor irradiated 5 h under a xenon arc lamp. Gradient elution program: from 5% of A and 95% B to 100% A in 35 min; back to initial conditions 5 min; flow rate 0.9 ml/min.

nondissociative form (reaction medium pH is set at 2 with sulfuric acid), degradation rate was slower, 11 h (see Fig. 5). As with alachlor, bentazone degradation kinetic is strongly influenced by the HS, which acted as photosensitizer and produced under xenon arc lamp reactive intermediate-like singlet oxygen ($^1O_2$), superoxide anions (O_2^-), and peroxy radical (ROO), which enhanced the degradation of the compound [24]. These findings suggest that oxidation or hydroxylation may be the main routes of photodegradation of alachlor and bentazone in water.

Identification of alachlor photoproducts

Fourteen photodegradation products have been detected using either UV or MS detection. Their compound molecular weights (mol. wts.) are given in Tables 2 and 3. Among them, four are isomers with a mol. wt. of 251: 2, 2', 6, and 6'. Two are isomers with a mol. wt. of 269: 1 and 1', and two others are isomers with a mol. wt. of 233: 8 and 8'. Ten breakdown products have a concentration evaluated above 0.5 μg/L: 1, 1', 2, 2', 3, 6', 8, 8', 9, and 9', and their occurrence in aqueous environment can be expected (see Table 2). At the end of the irradiation period, 10 h, the sum of their concentrations has been estimated at 40% of the alachlor originally present. Identical photoproducts have been found using distilled water and Ebro River water. The different compounds identified in full-scan traces, when using distilled water samples, were confirmed in Ebro River water experiments using the selected ion-monitoring mode. Each compound has been characterized by two diagnostic ions. Compound 9 (synthesis product 13, see Fig. 1) is the major degradation product in both experiments, but its concentration is higher in river water than in distilled water, showing that humic substances have a rather strong influence on the formation rate of TPs.

When photolysis experiments were carried out in distilled water plus 1% methanol, two additional compounds appeared in the LC/UV, and the LC/TSP-MS chromatograms (results not shown) exhibited a protonated molecular peak at $m/z = 266$. Two compounds of mol. wt. 251 have undergone a methylation in presence of a low concentration of methanol. Use of organic solvents in photolysis experiments can lead to a misinterpretation of what happens in an actual environment since adduct-solvent formation takes place [14]. The CH_3OCH_2 moiety was preserved under xenon arc lamp irradiation. Losses of 31 or 32 and 45 mass units corresponding to losses of OCH_3, $HOCH_3$, and CH_3OCH_2, respectively, is a common fragmentation pattern of all the compounds (see Tables 2 and 3).

In addition, compounds *N*-hydroxymethylcarbonyl-*N*-methyl-2,6-diethylaniline (**5**) and 2,6-diethylacetanilide (**1**), both from synthetic origin, were not found in our experiments. Compounds *N*-acetyl-*N*-methoxymethyl-2,6-diethylaniline (**2**) and *N*-chloromethylcarbonyl-*N*-methoxymethyl-2-acetyl-6-ethylaniline (**10**) were also not found in our photolysis experiments. After a dechlorination step, a first pathway involved a hydroxylation process. Figure 3 shows the chemical structure of three identified compounds. Hydroxyalachlor (6') is the second most important compound. Its identification was obtained by matching its GC/EI-MS and the LC/TSP-MS spectra with those of an available standard from synthetic origin.

For unequivocal characterization, tandem MS could be applied by using the $[M + H]^+$ ion as the precursor ion (Fig. 6).

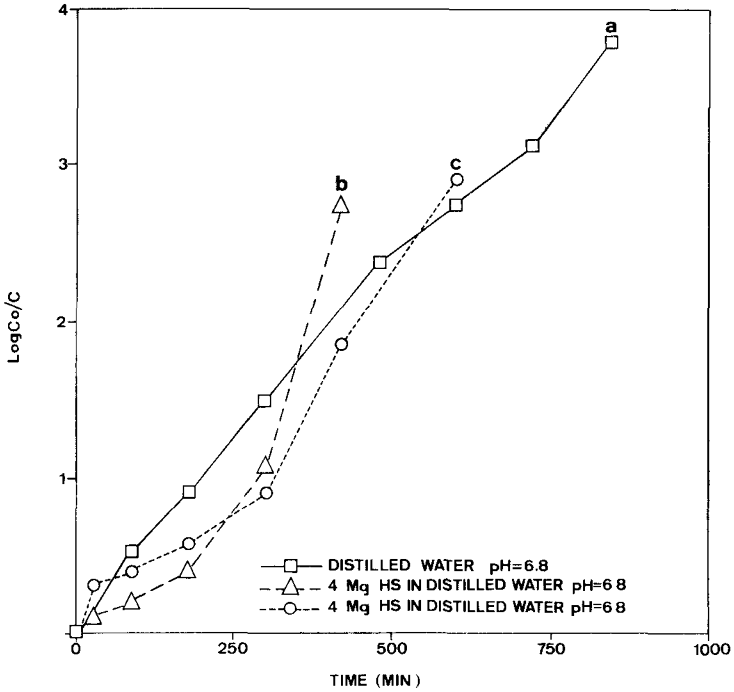

Fig. 5. Degradation rate of bentazone (**a**) in 500 ml distilled water; (**b**) in 500 ml distilled water spiked with 4 mg/L humic substances (HS) at pH 6.8; (**c**) in 500 ml distilled water spiked with 4 mg/L HS at pH 2. Irradiations were carried out using a Suntest apparatus from Heraeus (Hanau, Germany) equipped with a xenon arc lamp. Irradiance was set at 550 W/m^2. Samples were kept in a quartz reaction reservoir, and temperature was set at 30°C using a cooling circuit. At different periods, water samples of 100 ml were removed from this reactor, preconcentrated after acidification at pH = 1 with sulfuric acid, and finally analyzed by on-line SPE-LC/DAD or on-line SPE-LC/TSP-MS.

Table 2. Photodegradation products' molecular weight (mol. wt.), retention time (RT), and main ions and their relative abundance in LC/TSP-MS-positive ionization mode using 0.05 M ammonium acetate as additive in the mobile phase

Compounds	RT	Mol. wt.	Main ions—PI mode and (relative abundance)
1	16.9	269	270 $[M+H]^+$ (100), 287 $[M+NH_4]^+$ (1), 328 $[M+CH_3COONH_4\text{-}H_2O]^+$ (16), 252 (20), 238 (6), 220 (10), 208 (6)
1′	16.9	269	Isomer of 1; same ions as 1
2	17.2	251	252 $[M+H]^+$ (100), 269 $[M+NH_4]^+$ (25), 310 $[M+CH_3COONH_4\text{-}H_2O]^+$ (4), 234 (80), 220 (20), 202 (4)
2′	18.1	251	Isomer of 2; same ions as 2
3	19.6	219	220 $[M+H]^+$ (100), 261 $[M+H+CH_3CN]^+$ (50)
4	19.6	251	252 $[M+H]^+$ (100), 269 $[M+NH_4]^+$ (80), 220 (85), 190 (4)
5	21.1	191	192 $[M+H]^+$ (65), 209 $[M+NH_4]^+$ (100)
6	21.1	251	Isomer of 6′; same ions as 6′; see below
7	22.8	221	222 $[M+H]^+$ (35), 239 $[M+NH_4]^+$ (100), 280 $[M+CH_3COONH_4\text{-}H_2O]^+$ (85)
6′	23.3	251	252 $[M+H]^+$ (100), 269 $[M+NH_4]^+$ (18), 237 $[M+H\text{-}CH_3]^+$ (5), 220 $[M+H\text{-}OHCH_3]^+$ (58), 208 $[M+H+H\text{-}CH_2OCH_3]^+$ (100)
8	24.3	233	234 $[M+H]^+$ (100), 251 $[M+NH_4]^+$ (8), 275 $[M+H+CH_3CN]^+$ (10), 292 $[M+59]^+$ (15), 219 (10), 202 (12)
8′	24.9	233	Isomer of 8; same ions as 8
9	25.9	233	234 $[M+H]^+$ (100), 251 $[M+NH_4]^+$ (18), 275 $[M+H+CH_3CN]^+$ (8), 292 $[M+59]^+$ (12), 219 $[M+H\text{-}CH_3]^+$ (16), 202 $[M+H\text{-}HOCH_3]^+$ (90), 190 $[M+H+H\text{-}CH_2OCH_3]^+$ (15)
9′	25.9	233	Isomer of 9; same ions as 9
10	29.1	269	270 $[M+H]^+$ (80), 287 $[M+NH_4]$ (100), 238 $[M+H\text{-}HOCH_3]^+$ (10), 226 $[M+H+H\text{-}CH_2OCH_3]^+$ (4)

For analytical separation, see Figure 4.

Table 3. Photodegradation products' molecular weight (mol. wt.), retention time (RT), and main ions and their relative abundance in GC/MS using EI mode

Compounds	RT	Mol. wt.	Main ions—EI mode and (relative abundance)
5	5.9	191	191 (100), 173 (25), 163 (52), 147 (61), 133 (80)
7	14.4	221	221 (8), 206 (10), 189 (70), 190 (35), 174 (20), 164 (60), 146 (100), 132 (40)
9	16.20	233	233 $[M]^+$ (10), 201 $[M\text{-}HOCH_3]^+$ (60), 202 $[M\text{-}OCH_3]^+$ (30), 188 $[M\text{-}CH_2OCH_3]^+$ (52), 186 $[M\text{-}CH_2OCH_3\text{-}H_2]^+$ (100), 146 $[M\text{-}OCNCH_2OCH_3]^+$ (55)
6	16.82	251	251 $[M]^+$ (1), 219 $[M\text{-}HOCH_3]^+$ (20), 220 $[M\text{-}OCH_3]^+$ (8), 188 $[M\text{-}HOCH_3\text{-}CH_2OH]^+$ (100), 160 $[M\text{-}HOCH_3\text{-}COCH_2OCH_3]^+$ (55)
8	17.38	233	233 $[M]^+$ (5), 218 (100), 188 (40), 173 (5), 160 (12)
10	17.52	269	269 $[M]^+$ (6), 237 $[M\text{-}HOCH_3]^+$ (12), 224 $[M\text{-}CH_2OCH_3]^+$ (10), 188 $[M\text{-}HOCH_3\text{-}CH_2Cl]^+$ (80), 160 $[M\text{-}HOCH_3\text{-}COCH_2Cl]^+$ (100)

The MS-MS spectrum of hydroxyalachlor shows losses of 32 mass units due to losses of CH_3OH from the methoxymethyl lateral chain. Additionally, a product ion is observed at *m/z* 162 that corresponds to the loss of both CH_3OH and the $COCH_2OH$ chain. This product ion is characteristic of the unmodified *n*-methyl diethyl anilide moiety and could be used with advantage in the search for other degradation products bearing this common structural feature by using the parent acquisition mode.

A second photodegradation pathway involved the cyclisation of the compound after losing a chlorine atom. Compound 9 was identified as 8-ethyl-1-methoxymethyl-4-methyl-2-oxo-1,2,3,4-tetrahydroquinone. The unequivocal identification of compound 9 was provided by matching its LC/TSP-MS and GC/EI-MS spectra with those of a synthetic standard (Compound 13, Fig. 1). Also, its electron impact GC/MS spectrum was similar to that reported in reference [13]. Compound 9′ is likely a stereoisomer of compound 9. Compound 3 with a mol. wt. of 219 was tentatively identified as 4-(2,6-diethylphenyl)-3-morpholinone. No significant fragmentation patterns were given by LC/TSP-MS spectrum, but the support for the structure identification was provided by the fact that the fragmentation $[M - 32]^+$ was not observed; the CH_3OCH_2 moiety must be involved in a ring structure.

In a similar way, the sunlight photodegradation of metolachlor in water leads to the identification of a morpholinone compound [5]. After 2 h of irradiation further photodegradation occurred and compounds 1, 1′, 2, 2′, 8, and 8′ were

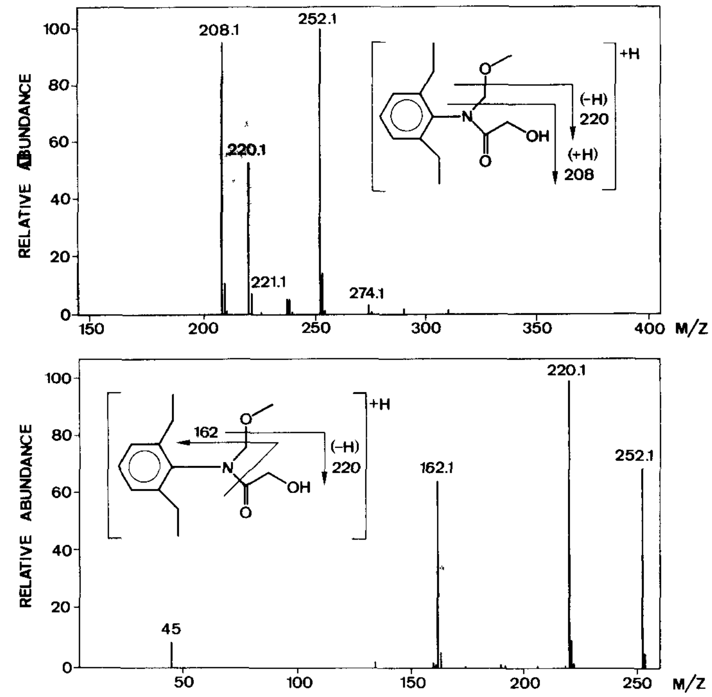

Fig. 6. LC/TSP-MS and LC/TSP-MS/MS spectra of hydroxyalachlor under positive ionization mode. For other conditions, see Experimental Section.

detected (see Tables 2 and 3). Identification of the very polar photoproducts have proven to be complicated. Adducts obtained with ammonium acetate $[M + H]^+$, $[M + NH_4]^+$, $[M + H + CH_3CN]^+$ and with methylamine $[M + CH_3NH_3]^+$ used as additive in the mobile phase avoided a wrong estimation of the molecular weights. No structure could surely be attributed to these compounds. Their full identification is still under investigation and may require other analytical methods such as NMR after an isolation step. To understand the origin of these further photodegradation products and to better clarify the photodegradation pathway of alachlor, both hydroxyalachlor (6′) and compound 9 were irradiated under identical conditions with those used for alachlor. Both products are stable in distilled water, and no breakdown product could be identified after 5 h of irradiation.

Identification of bentazone photoproducts

No significant photodegradation products have been detected in UV after 12 h of irradiation in distilled water sample spiked at 20 μg/L with analytical-grade bentazone, although aromatic ring breakdown is not an easy photochemical process (results not shown). Nevertheless, use of TiO_2 allows the cleavage of aromatic ring, leading to complete mineralization of the herbicide [25]. An exhaustive investigation on the photochemistry of bentazone at concentrations ranging from 5 mg/L to 500 mg/L in pure water has already been published and postulated that bentazone underwent, as a main pathway, an oxidative photodimerization involving the N-1 nitrogen of one bentazone moiety and the benzene of another one [11]. Dimerization did not occur at low concentration, and none of the two dimers previously described have been detected either in UV or in MS detection. Photodimerization process can be regarded in this case as an artifact of the experimental conditions. The pH of the reaction mixture had fallen to 2 after 5 h of irradiation, and only one photoproduct was detected in LC/TSP-MS (figure not shown) using negative ionization (NI) mode corresponding to a deprotonated molecular $[M - H]^-$ ion at $m/z = 135$ tentatively identified as amino-2-benzoic acid. Concentration-level estimation was difficult because these compounds have such low breakthrough volume on C_{18} sorbent that it could not be quantitatively recovered.

CONCLUSIONS

This study points out, in line with earlier findings, the role of dissolved organic matter in photodegradation kinetic experiments. Humic substances affect the photodegradation of both common herbicides, alachlor and bentazone, showing the importance of carrying out photolysis experiments in natural waters. It was also confirmed that alachlor and bentazone are moderately persistent as regards to photolysis, and the same TPs have been found in distilled water as in natural water. The present work has expanded the list of alachlor photolysis products available up till now. This is due, mainly, to the use of aqueous solutions and the use of on-line SPE-LC/TSP-MS for detection purposes.

Hyphenated LC/MS techniques appear to be a powerful tool to detect and identify the more polar photodegradation products, and they exceeded the possibilities of GC/EI-MS in this area. The LC/tandem MS/MS overcomes the lack of information in the spectra obtained under thermospray LC/MS conditions but failed in the identification of isomers. In these conditions, only a preliminary pathway could be formulated for alachlor involving a dechlorination step followed either by a hydroxylation process or a cyclisation process. Both processes may occur simultaneously, but more experiments are required to identify unequivocally the new transformation products formed. The bentazone photodegradation pathway is still not well elucidated because of the likely formation of highly polar breakdown products, and their extraction from water remains a difficult topic.

Finally, on-line SPE-LC/DAD or LC/MS techniques were successfully used for photolysis experiments in water. Studies could be carried out at concentrations as little as 20 μg/L, close to environmental level, avoiding solvent adducts when using 1% methanolic solution for alachlor and photochemically induced dimers for bentazone. It is our plan in a future work to achieve the determination of a great number of highly polar alachlor TPs and to develop analytical methods so as to include these new alachlor TPs in our current monitoring survey. This will be the only way to be sure of their formation in aquatic systems and to avoid artifacts from laboratory experiments.

Acknowledgement—S. Chiron acknowledges financial support from the Human Capital and Mobility Program from the Commission of the European Communities (Envirotrace). This work was supported by the Environment Research and Development Program 1991–1994 from the Commission of the European Communities (Contract EV5V-CT92-0061) and by CICYT (Contract AMB93-1427-CE).

REFERENCES

1. **Fielding, M., D. Barceló, A. Helweg, S. Galassi, L. Torstensson, P. Van Zoonen, R. Wolter** and **G. Angeletti.** 1992. Pesticides in ground and drinking water. Water Pollution Research Report 27. Commission of the European Communities, Brussels, Belgium, pp. 1–136.
2. **Durand, G., V. Bouvot** and **D. Barceló.** 1992. Determination of trace levels of herbicides in estuarine waters by gas and liquid chromatographic techniques. *J. Chromatogr.* **607**:319–327.
3. **Chiron, S., A. Fernandez-Alba** and **D. Barceló.** 1993. Comparison of on-line solid-phase disk extraction to liquid-liquid extraction for monitoring selected pesticides in environmental waters. *Environ. Sci. Technol.* **27**:2352–2359.
4. **Pereira, W.E.** and **F.D. Hostettler.** 1993. Nonpoint source contamination of the Mississippi River and its tributaries by herbicides. *Environ. Sci. Technol.* **27**:1542–1552.
5. **Kochany, J.** and **R.J. Maguire.** 1994. Sunlight photodegradation of metolachlor in water. *J. Agric. Food Chem.* **42**:406–412.
6. **Durand, G., J.L. Abad, F. Sanchez-Baeza, A. Messeguer** and **D. Barceló.** 1994. Unequivocal identification of compounds formed in the photodegradation of fenitrothion in water/methanol and proposal of selected transformation pathways. *J. Agric. Food Chem.* **42**:814–821.
7. **Wan, H.B., M.K. Wong** and **Ch.Y. Mok.** 1994. Comparative study on the quantum yields of direct photolysis of organophosphorus pesticides in aqueous solution. *J. Agric. Food Chem.* **42**:2625–2630.
8. **Miller, G.C.** and **R.G. Zeep.** 1983. Extrapolating photolysis rates from the laboratory to the environment. *Residue Rev.* **85**: 89–107.
9. **Yager, J.E.** and **C.D. Yue.** 1988. Evaluation of the xenon arc lamp as a light source for aquatic photodegradation studies:

Comparison with natural sunlight. *Environ. Toxicol. Chem.* **7**:1003–1011.

10. **De Bertrand, N.** and **D. Barceló.** 1991. Photodegradation of carbamate pesticides aldicarb, carbaryl and carbofuran in water. *Anal. Chim. Acta* **254**:235–244.
11. **Nilles, G.P.** and **M.J. Zabik.** 1975. Photochemistry of bioactive compounds: Multiphase photodegradation and mass spectral analysis of Basagran. *J. Agric. Food Chem.* **23**:410–415.
12. **Chiron, S., S. Dupas, P. Scribe** and **D. Barceló.** 1994. Application of on-line solid-phase extraction followed by liquid chromatography–thermospray mass spectrometry to the determination of pesticides in environmental waters. *J. Chromatogr.* **665**:295–305.
13. **Somich, C.J., P.C. Kearney, M.T. Muldoon** and **S. Elsasser.** 1988. Enhanced soil degradation of alachlor by treatment with ultraviolet light and ozone. *J. Agric. Food Chem.* **36**:1322–1326.
14. **Durand, G., M. Mansour** and **D. Barceló.** 1991. Identification and determination of fenitrothion photolysis products in water–methanol by gas chromatography–mass spectrometry. *Anal. Chim. Acta* **243**:259–271.
15. **Durand, G., N. De Bertrand** and **D. Barceló.** 1991. Application of thermospray liquid chromatography–mass spectrometry in photochemical studies of pesticides in water. *J. Chromatogr.* **554**:233–250.
16. **Abián, J., G. Durand** and **D. Barceló.** 1993. Analysis of chloroatrazines and their degradation products in environmental samples by selected various operating modes in thermospray HPLC/MS/MS. *J. Agric. Food Chem.* **41**:1264–1273.
17. **Pörschmann, J.** and **U. Stottmeister.** 1993. Methodical investigation of interactions between organic pollutants and humic material in coal wastewaters. *Chromatographia* **36**:207–211.
18. **Mortimer, R.D.** 1993. A convenient synthesis of 2-ethyl-6-(1)-hydroxyethyl)aniline – An alachlor metabolite synthon. *J. Agric. Food Chem.* **41**:976–977.
19. **Barceló, D.** 1991. Occurrence, handling and chromatographic determination of pesticides in the aquatic environment. *Analyst* **116**:681–689.
20. **Moza, P.N., K. Hustert, S. Pal** and **P. Sukul.** 1992. Photocatalytic decomposition of pendimethalin and alachlor. *Chemosphere* **25**:1675–1682.
21. **Schlautman, M.A.** and **J.J. Morgan.** 1993. Effects of aqueous chemistry on the binding of polycyclic aromatic hydrocarbons by dissolved humic materials. *Environ. Sci. Technol.* **27**:961–969.
22. **Pennington, K.L., S.S. Harper** and **W.C. Koskinen.** 1991. Interactions of herbicides with water-soluble soil organic matter. *Weed Sci.* **39**:667–672.
23. **Madhun, Y.A., J.L. Young** and **V.H. Freed.** 1986. Binding of herbicides by water-soluble organic materials from soil. *J. Environ. Qual.* **15**:64–68.
24. **Mopper, K., X. Zhou, R.J. Kieber, D.J. Kieber, R.J. Sikorski** and **R.D. Jones.** 1991. Photochemical degradation of dissolved organic carbon and its impact on the oceanic carbon cycle. *Nature* **353**:60–62.
25. **Pelizzeti, E., V. Maurino, C. Minero, O. Zerbinati** and **E. Borgarello.** 1989. Photocatalytic degradation of bentazone by TiO_2 particles. *Chemosphere* **18**:1437–1445.