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BY-PRODUCTS FORMATION DURING DEGRADATION OF ISOPROTURON IN AQUEOUS SOLUTION. I: OZONATION

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Abstract—The degradation of the herbicide isoproturon during its ozonation in aqueous solution has been investigated with the aim of identifying intermediate as well as final by-products formed. At ambient temperature, phosphate-buffered (pH = 7) isoproturon aqueous solutions (10, 10^{-1} and 10^{-3} mg/l) were ozonated in a semi-batch reactor, under a continuous flow of ozonated air whose ozone concentration was 9 and 0.9 mg O_3/l_{air} for the highest and the two lower herbicide concentrations respectively. Measured steady-state ozone concentrations during the two sets of experiments (i.e. the highest and the lower isoproturon concentration) were 1.9 and 0.7 mg O_3/l. Under all of the above conditions, isoproturon was always completely removed in a period ranging between 5 and 15 min, essentially by reacting with molecular ozone. High-performance liquid chromatography-mass spectrometry (HPLC-MS) analyses indicate that primary degradation by-products are formed either by introducing OH groups in the aromatic ring and/or in the side-chain substituents, or by breaking down the isopropyl alkyl chain. The results also show that these primary intermediates are successively degraded yielding low molecular weight compounds such as aldehydes, simple organic acids and α-oxo-acids, which have been identified by gas chromatography-electron capture detection (GC-ECD), ion chromatography (IC) and GC-MS, respectively. On the basis of the analytical results, a pathway for the degradation of isoproturon by ozone has been proposed. © 2001 Elsevier Science Ltd. All rights reserved

Key words—by-products, isoproturon, herbicide, water, ozonation, HPLC, mass-spectrometry

INTRODUCTION

The application of herbicides on agricultural soils is a well established and effective practice to control weed growth. Herbicides are applied to soils repeatedly and because of their rather slow degradation (Khadrani *et al.*, 1999; Madhun and Freed, 1987; Pieuchot *et al.*, 1996), their occurrence in groundwaters as well as surface waters has become more and more frequent. There is, therefore, concern regarding herbicide contamination of water resources (Fielding *et al.*, 1992; Lacorte *et al.*, 1996) and also an interest in reliable technologies for removing herbicides from water. Even though this latter goal is commonly achieved by appropriate physico-chemical methods such as adsorption onto activated carbon, membrane filtration and advanced oxidation processes (Di Giano, 1987; O'Brien *et al.*, 1987; Camel and Bermond, 1998; Ollis *et al.*, 1991), the possibility that herbicides can be removed and/or degraded even during the disinfection step routinely carried out at drinking water treatment plants should not be discounted. From a chemical standpoint the commonly used chemical disinfectants (i.e., Cl_2/HOCl, ClO_2, BrCl/HOBr, O_3, etc.) are all oxidant species potentially able to react with herbicides causing their removal and/or degradation (EPA, 1986). Therefore, the degradation by-products should be expected, and their identification is an important issue as little is known about their nature, and their toxicity could be even higher than that of the parent compounds.

With this aim, long-term investigations have been carried out to assess whether herbicides react with disinfectants, and if so, to identify the resulting by-products (Mascolo *et al.*, 1994, 1995). The present paper is the first part of an investigation to study the degradation of the herbicide isoproturon during its reactions with two disinfectants: ozone (part I) and chlorine (part II).

Currently, ozone applications for water treatment are numerous and widespread all over the world in spite of its two major technological drawbacks (Rice and Netzer, 1984), *viz.* firstly, under conditions common to water and wastewater treatment, ozone is an unstable gas and it must, therefore, be generated and applied at its point of use; secondly, as ozone is only partially soluble in water, its introduction into water or wastewater requires a very efficient

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*Author to whom all correspondence should be addressed. Tel.: +39-080-5820519; fax: +39-080-5313365; e-mail: mascolo@area.ba.cnr.it gas–liquid contact to maximise the mass transfer of ozone from the gas phase to the liquid phase. From the chemical point of view, in aqueous solution molecular ozone can remain as O_3, or it can decompose to form very reactive species such as the hydroxyl radical ($OH^{\bullet}$) which is a stronger oxidising agent ($E_0 = 2.80\,V$) than molecular ozone ($E_0 = 2.07\,V$). Factors affecting ozone decomposition include: pH, presence of specific water constituents (i.e., initiators, promoters, and inhibitors of free radical reactions), ionic strength and temperature (Langlais *et al.*, 1991). The chemical effects of ozone in water are then a result of the following: its direct reaction with dissolved compounds; its decomposition into secondary oxidants (e.g. $OH^{\bullet}$); the subsequent reactions of these secondary oxidants with solutes (Hoigné and Bader, 1976).

Molecular ozone, being a weaker oxidising agent than $OH^{\bullet}$, is a rather selective oxidant and because of this, most water and wastewater treatment processes are carried out with relatively low ozone dosages (Rice, 1997). Larger amounts of more reactive oxidants such as $OH^{\bullet}$ would be required due to their consumption in side oxidation reactions. From the kinetic point of view, as $OH^{\bullet}$ is a much stronger and less-selective oxidant than ozone, its reaction rate constants are about nine orders of magnitude greater than those of ozone for the same organic compound (Hoigné and Bader, 1983).

Referring to reaction mechanisms, molecular ozone can react with organic substrates either through cyclo-addition to unsaturated bonds or electrophilic and nucleophilic reactions (Langlais *et al.*, 1991). Electrophilic reactions take place on molecular sites with a high electron density such as those found in aromatic compounds substituted with electron donor groups (–OH, NH_2, etc.). Conversely, nucleophilic reactions occur on molecular sites showing an electron deficit and, more frequently, on carbons carrying electron-withdrawing groups (–NO_2, –Cl, etc.). As for the hydroxyl radical, it reacts with organics by three major mechanisms: hydroxy addition, hydrogen abstraction, and electron transfer (Huang *et al.*, 1993; Legrini *et al.*, 1993).

In practice, even though pesticides removal by ozonation is often achieved successfully, the complete destruction of these contaminants (i.e. the total mineralization of organic carbon to carbon dioxide) is seldom obtained. Therefore, the formation of several ozonation by-products is usually to be expected (Reynolds *et al.*, 1989) as found for other pollutants (Brambilla *et al.*, 1993; Ku *et al.*, 1998; Prados and Ciba, 1997; Yao *et al.*, 1998).

Isoproturon is a herbicide belonging to the class of phenyl-urea derivatives, widely used in agriculture to control broadleaf and grassy weeds in cereals and other crops (Tomlin, 1997). Because in Europe the annual consumption of isoproturon is estimated to be over 1000 tons, it has been included in the European "black list" of pesticides (Fielding *et al.*, 1992). It can be biodegraded, like other phenyl-urea herbicides (Cullington and Walker, 1999; Esposito *et al.*, 1998; Vroumsia *et al.*, 1996). It is also broken down by advanced oxidation processes (De Laat *et al.*, 1996; Meijers *et al.*, 1995; Prados *et al.*, 1995; Roche and Prados, 1995) and several degradation by-products have been identified (Allemane *et al.*, 1995; Aguer and Richard, 1996; Pramauro *et al.*, 1993; Richard and Bengana, 1996). However, isoproturon degradation by-products have been identified only for the system O_3/H_2O_2 (Allemane *et al.*, 1995), i.e., when the predominant oxidant species should be the $OH^{\cdot}$ radical (Glaze, 1987; Glaze *et al.*, 1987). Furthermore, in this study (Allemane *et al.*, 1995) highly polar by-products would not have been identified, as only GC-MS analyses without any derivatization (which would only detect relatively volatile non-polar compounds) were carried out. Therefore, the specific aim of the work reported in this paper was to identify the main by-products, including polar compounds, formed during the reaction between isoproturon and ozone alone at neutral pH, i.e., when the predominant oxidant species should be molecular ozone (O_3) (Glaze, 1987; Glaze *et al.*, 1987).

By-products identification was first performed under laboratory conditions which were analytically convenient (isoproturon initial conccentration = 10 mg/l; transferred ozone dose ozone = 45.9 mg/l) even though these conentrations were far more extreme than those which would actually be encountered at real water treatment plants. Follow-up work involved searching for the by-products identified during this preliminary step when experimental conditions closer to reality were used (i.e., isoproturon initial concentration = 100 and 1 μg/l; transferred ozone dose ≈2 mg/l).

EXPERIMENTAL SECTION

Chemicals

Isoproturon [3-(4-isopropylphenyl)-1,1-dimethyl-urea] 99% purity, (Polyscience, Niles, L, USA) was used without further purification. Water and methanol used for liquid chromatography were HPLC-grade (Rathburn, Walkerburn Scotland). Methanol and methylene chloride used for solid–liquid extractions were pesticide-grade from Rathburn. Diazomethane was produced from *N*-methyl-*N*-nitroso-*p*-toluenesulphonamide (Aldrich, Milwaukee, WI, USA) according to a published procedure (Aldrich, 1993). All other chemicals were analytical grade from Aldrich. Double-distilled water was used for preparing all aqueous solutions.

OZONATION EXPERIMENTS

A 10 mg/l isoproturon aqueous solution was prepared by introducing appropriate amount of isoproturon into a 500 ml flask and dissolving it (with stirring) in phosphate buffer solution (10 mM) at pH = 7. The solution was then placed into a Normag reactor (Normag, Hofheim am Taunus, Germany) for ozonation. Ozonated air (9 mg O_3/l) was produced by a Fisher 502 ozonator (Meckenheim, Germany) and bubbled into the Normag reactor at a flow rate of 60 l/h. Experiments at lower isoproturon concentration (100 and 1 ppb) were carried out with a flow rate of 6 l/h of ozonated air (9 mg O_3/l). Ozone output was monitored before each experiment by determining (by titration with sodium thiosulphate) the amount of free iodine liberated from a potassium iodide solution. Ozone transferred during the reaction was determined by the following equation:

$$\begin{aligned} O_3 \text{ transferred} &= (O_3\text{consumed} + O_3 \text{ residual in water}) \\ &= (O_3 \text{ in the (in)gas} - O_3 \text{ in the (off) gas}) \end{aligned}$$

Samples were withdrawn from the reaction mixtures at scheduled times, quenched with sodium thiosulfate, and analysed by HPLC. Residual ozone concentrations in the aqueous solutions were determined by the Indigo method (Standard Methods, 1999).

ANALYTICAL DETERMINATIONS

The concentration of unreacted isoproturon and the production of isoproturon by-products were monitored by HPLC-UV with a 1050 chromatographic system (Agilent Technologies, Palo Alto, CA, USA) equipped with a Spherisorb C-8, 5 μm (250 × 4.6 mm i.d.) column from Thames Chromatography (Windsor, England) and a 1050 series multiple wavelength detector, set at 240 nm. Samples, injected *via* a 10 μl Rheodyne loop, were eluted with a methanol/50 mM ammonium acetate 40/60 v/v solution at a flow rate of 1 ml/min. HPLC-MS analyses were carried out by ionspray-mass spectrometry with an API 165 mass spectrometer (Applied Biosystems/MSD Sciex, Foster City, CA, USA) equipped with an ionspray interface. The ionspray conditions (positive ions) were: nebulizer gas (air) = 1.2 l/min, curtain gas (nitrogen) = 1 l/min, needle voltage = 5500 V, orifice voltage = 20 V and ring voltage = 200 V. The column and operating conditions were the same as used for the HPLC-UV analyses. The flow from the HPLC-UV was split to allow just 50 μl/min to enter the ionspray interface. Quenched samples (isoproturon starting concentration = 10 mg/l) to be analysed by HPLC-MS for by-products identification were concentrated 150 times using the following solid-phase extraction (SPE) procedure: after conditioning a 500 mg Bondelut C-18 solid phase cartridge (Varian, Harbor City, CA, USA) by loading sequentially methylene chloride/methanol (1/1 v/v solution), methanol and water, 150 ml of quenched sample was passed through the cartridge. After a 5 min drying step, the cartridge was eluted using methanol. The volume of the extract was reduced to 1 ml using a gentle stream of purified air. The formation of by-products was followed by HPLC-MS, operating in selected ion monitoring (SIM), by monitoring the $[M+H]^+$ ions of previously identified by-products. In these determinations, quenched samples with an initial concentration of 10 mg/l isoproturon were analysed without any pre-concentration step, while those with an initial isoproturon concentration of 0.1 and 0.001 mg/l were concentrated (100 and 10,000 times, respectively) by SPE using Carbograph-4 cartridges (0.5 and 1 g, respectively, obtained from Lara s.r.l., Italy). The SPE procedure used was the same as that for the C-18 cartridges.

The quantitative determination of formic, acetic and oxalic acids was carried out by ion chromatography using a Dionex 4000i (Dionex, Sunnyvale, CA, USA) LC system working in suppressed ion mode, equipped with a conductivity detector. Samples, injected via a 50 μl loop, were eluted at a flow rate of 1 ml/min through an OmniPac PAX-500 analytical column (250 × 4 mm i.d.) with the following gradient (solvent A = 0.75 mM NaOH with 1.25% methanol; solvent B = 50 mM NaOH with 1.25% methanol): isocratic 100% A for 5 min, then programmed from 100% A to 100% B over 25 min, then isocratic until time = 35 min. For the determination of other organic acids, 20 ml of water sample were acidified to pH = 2 with sulphuric acid and liquid–liquid extracted with diethyl ether (2 × 3 ml). The extracts were dried with sodium sulphate, methylated with diazomethane and their volume reduced to 1 ml for gas-chromatography/mass-spectrometry (GC-MS) analyses. GC-MS analyses were carried out with a VG (Manchester, UK) TS-250 trisector magnetic mass spectrometer equipped with an electron impact (EI) source and a PDP-11 microcomputer (Digital, Maynard, MA, USA) for data acquisition. The mass spectrometer was interfaced to a 5890 gas-chromatograph (Agilent Technologies, Palo Alto, CA, USA). The column used was a SPB-5 (30 m length, 0.25 mm i.d. and 0.25 μm film thickness) from Supelco (Bellefonte, PA, USA) directly interfaced to the ion source through a transfer line heated to 280°C. One microlitre of the sample was injected into a splitless injector at 200°C. The operating conditions were: carrier gas: helium, flow rate: 1 ml/min, initial temperature: 40°C (1 min), programming rate: 15°C/min up to 280°C, then isothermal for 5 min. EI mass spectra were obtained at 70 eV electron energy with the ion source at 180°C. The magnetic analyser was scanned from 40 to 400 a.m.u. over a period of 1 s, with a resolution of 500 (10% valley). The background-subtracted mass spectra were matched against those in the NIST mass spectra library and interpreted on the basis of the observed fragmentation.

Determination of aldehydes was carried out by derivatization with pentafluorobenzylhydroxylamine hydrochloride (PFBHA, Aldrich) according to a published procedure (Le Lacheur *et al.*, 1993). The derivatives were analysed by gas-chromatography/electron capture detection (GC-ECD) with a HP 5890 GC (Agilent Technologies, Palo Alto, CA, USA). One microlitre of the of sample was injected into a 30 m × 0.25 mm DB-1 column (J&W, Folsom, CA, USA). The GC oven temperature programme was as follows: isothermal at 30°C for 3 min; 30 to 300°C at 8°C/min; isothermal for 10 min. The injector and detector were kept at 250 and 320°C, respectively.

RESULTS AND DISCUSSION

Figure 1 shows the isoproturon decay curves recorded during ozonation carried out in aqueous solutions at pH = 7, and at three different initial isoproturon concentrations (10, 10^{-1} and 10^{-3} mg/l). These decay curves demonstrate that, regardless of the conditions, ozone is very effective in removing isoproturon, and it is only the time (5–15 min) required to do so that is affected by the conditions. In Fig. 1, as reported in the experimental section, the applied ozone dose in water per minute was 18 and 1.8 mg/l for the highest and for the two lowest herbicide concentrations, respectively. As for the transferred ozone [O_3 transferred = (O_3 consumed + O_3 residual in water) = (O_3 in the (in)gas−O_3 in the (off)gas)], in the case of the highest herbicide concentration (10 mg/l) consumption was measurable only during the first 15 min, as afterwards the difference (O_3 in the (in)gas−O_3 in the (off)gas) became lower than the analytical experimental error. During this 15 min period, the measured amount of transferred ozone was 45.9 mg/l (44 mg/l as consumed and 1.9 mg/l as residual), i.e., the ozone transfer efficiency was 17% and the ratio (mg/l of

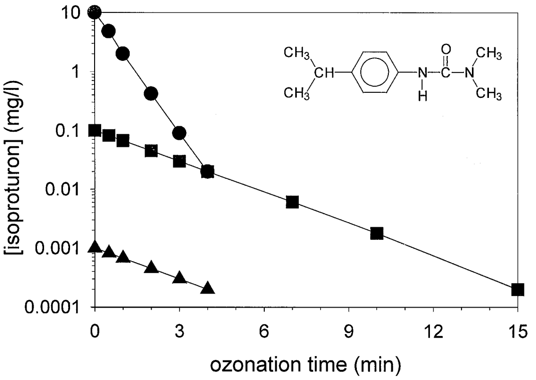

Fig. 1. Isoproturon structure and ozonation decays at the pH = 7. Experimental conditions: ●: $[\text{ozone}]_{\text{air}} = 9$ mg/l at 1 l/min, ozone consumed = 44 mg/l, residual ozone concentration are in water = 1.9 mg/l; ■: $[\text{ozone}]_{\text{air}} = 9$ mg/l at 0.1 l/min, ozone consumed < 1 mg/l, residual ozone concentration in water = 0.7 mg/l; ▲: $[\text{ozone}]_{\text{air}} = 9$ mg/l at 0.1 l/min, ozone consumed < 1 mg/l, residual ozone concentration in water = 0.7 mg/l.

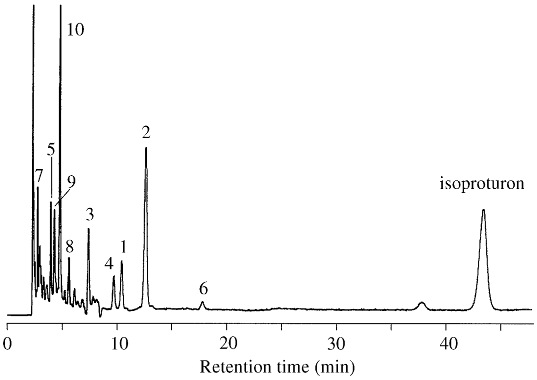

Fig. 2. HPLC-UV chromatogram of isoproturon by-products after 3 min of ozonation at pH = 7. Initial herbicide concentration = 10 mg/l. By-products identification numbers as reported for chemical structures in Fig. 5. Other experimental conditions are in the text.

ozone consumed)/(mg/l of herbicide removed) was 44/10 = 4.4.

The experimental error associated with the measurement of the consumed ozone when the lower herbicide concentrations were used, renders such a measurement meaningless. In these cases, however, some reasonable assumptions can be applied. Assuming that the stoichiometry of the reaction between ozone and isoproturon is the same, even for the lowest herbicide concentrations (10^{-1} and 10^{-3} mg/l), the ozone consumed in these cases [4.4 × mg/l of isoproturon)] should be less than 1 mg/l. Considering that the residual ozone measured after 15 min in these cases was 0.7 mg/l, the amount of transferred ozone falls into a range lower than 2 mg/l, which represents that actually measured at real water treatment plants (Bonnelye and Richard, 1997). When the highest herbicide concentration was used (i.e., 10 mg/l ≅ 50 μM), given that the kinetic constants of ozone with isoproturon and OH^- ions are 141 $M^{-1}s^{-1}$ (De Laat *et al.*, 1996) and 70 $M^{-1}s^{-1}$ (Staehelin and Hoignè, 1985), respectively, a rapid calculation indicates that ozone (after its transfer into water) will react more rapidly with isoproturon (k [isoproturon] $\cong 7 \times 10^{-3}\,s^{-1}$) than with hydroxide ion to form $OH^{\bullet}$ ($k_{OH^-}\,[OH^-] \cong 7 \times 10^{-7}\,s^{-1}$ at pH = 6 and $7 \times 10^{-4}\,s^{-1}$ at pH = 9). It follows that molecular ozone is the main species responsible for isoproturon degradation because of the high initial concentration used. Further evidence of this is that the pH has a negligible influence on isoproturon decay because, as long as isoproturon is present in the solution, $OH^{\bullet}$ formation by reaction of ozone with hydroxide ion is very low, whatever the pH. As isoproturon ozonations carried out in the pH range 6–9 gave rise to similar decay curves, they have not been reported.

To monitor for degradation by-products, the approach used was to initially apply the identification techniques to the samples in which the highest isoproturon concentration was used (i.e., 10 mg/l), and then to search for the by-products so identified in the samples where lower herbicide concentrations (i.e., 10^{-1} and 10^{-3} mg/l) were present.

As is evident in the HPLC-UV chromatogram shown in Fig. 2, which refers to the highest herbicide concentration, after a reaction time for isoproturon with ozone of 3 min several by-products whose retention times are all shorter than that of isoproturon are formed. This means that all the detected by-products are more polar than isoproturon, and that they still maintain the aromatic structure as all are UV detectable. In order to identify the by-products detected by HPLC-UV, HPLC-MS analyses of quenched samples were carried out. Table 1 reports the molecular weight (MW) of each detected by-product as well as the major ions in their ionspray mass spectra. Examining Table 1 it is possible to note that by-products 1–3 have the same MW (222 a.m.u.) which corresponds to the chemical structure of hydroxylated isoproturon. The formation of such hydroxylated by-products is not novel as they have also been identified during oxidation reaction of other phenyl-urea herbicides (Aguer and Richard, 1996; Richard and Bengana, 1996). As shown in Fig. 3a, the presence in the mass spectrum of by-product 3 of an ion of $m/z = 165$ indicates that the hydroxyl group (OH–) in this by-product is located on the benzylic carbon of isoproturon structure. In fact, 165 a.m.u. is the molecular weight of a hydroxylated isoproturon after the loss of the hydroxypropyl group [$(CH_3)_2C$–OH]. The lack of the ion ($m/z = 165$) in the spectra of by-products 1 and 2 reported in Fig. 3b, suggests they are isomers with the hydroxyl group located on the isoproturon aromatic ring. As for by-product 5, it has a molecular weight (180 a.m.u.) which is lower than that of isoproturon

Table 1. Mass spectral data for isoproturon ozonation by-products analysed by HPLC-MS. By-products identification numbers as reported for chemical structures in Fig. 5

Derivative	MW	$[M+Na]^+$, $[M+H]^+$ and main fragment ion masses (relative intensity)
Isoproturon	206	229(20), 207(100), 165(18), 72(37)
1	222	245(13), 223(100), 181(39), 72(5), 46(9)
2	222	245(13), 223(100), 181(32),72(5), 46(8)
3	222	245(10), 223(100), 205(7), 165(15), 134(4)
4	208	231(30), 209(100), 167(50)
5	180	203(18), 181(100), 72(50)
6	196	219(100), 197(58), 72(8)
7	198	219(100), 197(65), 72(15)
8	212	235(30), 213(100), 143(36), 72(9)
9	228	251(50), 229(100), 159(40), 89(32), 72(11)
10	228	251(65), 229(100), 89(21), 72(15)

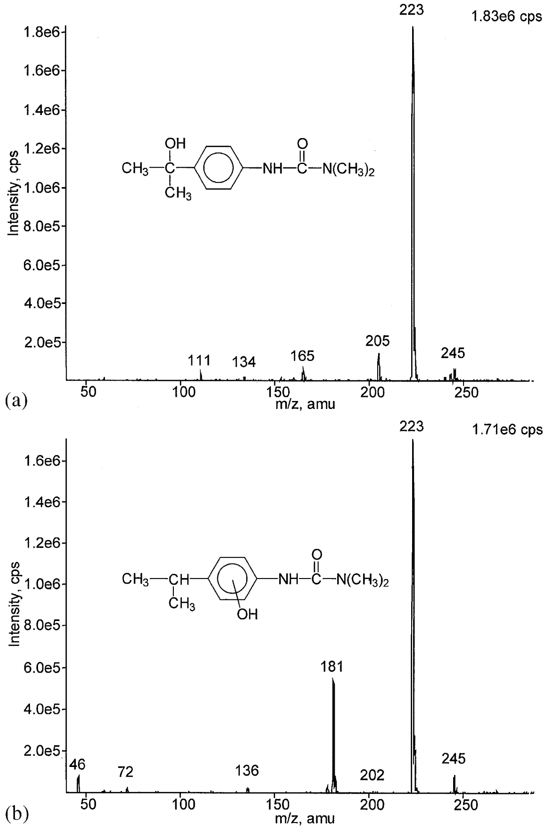

Fig. 3. Ionspray mass spectra of by-product 3 (a) and 1 (b). Instrumental conditions in the text.

(206 a.m.u.) suggesting that it arises from some structural breakdown of the parent molecule. In fact, its molecular weight corresponds to that of an isoproturon derivative where the isopropyl side chain, hydroxylated (as in the by-product 3) or not (as in the isoproturon itself), is replaced by an hydroxyl group. By-products 6 and 8–10 (two isomers) have molecular weights that increase progressively by 16 a.m.u. from that of by-product 5 (180 a.m.u.). This suggests that they are formed by oxidation of by-product 5 by successive introduction of hydroxyl groups into the aromatic ring and/or on the nitrogen bonded to the ring. This hypothesis is substantiated by the fact that all these by-products are UV-detectable. By-products 9 and 10 are probably isomers because they have the same MW (228 a.m.u.). Isomer formation during ozonation of aryl compounds is very likely because of the lack of selectivity of hydroxyl radical reactions. However, for these by-products another structure of the same MW is also possible in which the aromatic ring has been opened giving rise to a structure derived from muconic acid. Considering by-product 4, its MW (208 a.m.u.) is compatible with a derivative whose structure is that of isoproturon where a methyl group (CH_3–) has been replaced by a hydroxyl (OH–). Whether the methyl group has been replaced from the isopropyl [$(CH_3)_2CH$–] or from *N*-dimethyl [$(CH_3)_2N$–] group of the isoproturon molecule, can be determined by checking the mass spectrum of this by-product reported in Table 1. The presence of the ion $m/z = 167$, which corresponds to a fragment where the isopropyl group [$(CH_3)_2CH$–] has been lost, clearly points to the second alternative. For by-product 7 (MW = 198 a.m.u.), its structure is similar to by-product 4 where the isopropyl group [$(CH_3)_2CH$–] has been lost and two more hydroxyl groups have been gained.

In order to assess whether the above by-products were formed simultaneously or sequentially, their formation and/or decomposition with respect to time was followed by HPLC-MS. Furthermore, to check whether such by-products can be formed under conditions typically used at water treatment plants, their evolution was monitored using lower isoproturon initial concentrations (i.e., 0.1 and 0.001 mg/l) as well as lower (10 times) applied ozone dosage. As shown in Fig. 4, the results show that for all the investigated isoproturon concentrations, the primary by-products numbered as 1–3 are those which are quantitatively predominant, and that an initial rather fast isoproturon oxidation is followed by a slower primary by-products (1–3) degradation. Figure 4 also demonstrates, as expected, that by lowering the initial isoproturon concentration the concentration of each primary by-product maximizes at longer reaction times. Looking at Fig. 4 it is also possible to

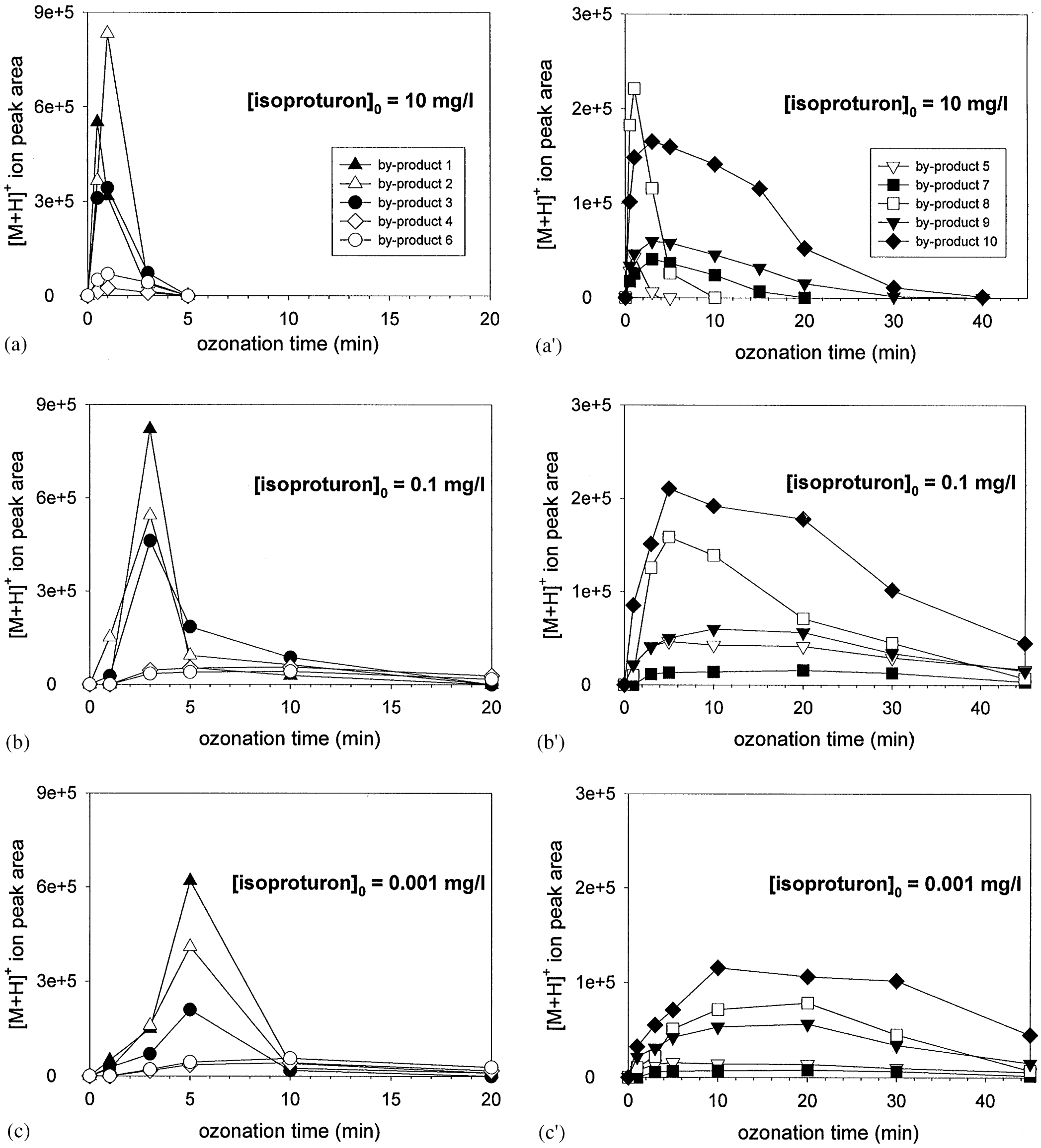

Fig. 4. Evolution of isoproturon by-products at pH = 7. The curve numbers identify the detected compounds whose identification numbers are those reported for chemical structures in Fig. 5. Experimental conditions (a) and (a'): $[\text{isoproturon}]_0 = 10\,\text{mg/l}$, $[\text{ozone}]_{\text{air}} = 9\,\text{mg/l}$ at 1 l/min, ozone consumed = 44 mg/l, residual ozone concentration in water = 1.9 mg/l; (b) and (b'): $[\text{isoproturon}]_0 = 0.1\,\text{mg/l}$, $[\text{ozone}]_{\text{air}} = 9\,\text{mg/l}$ at 0.1l/min, ozone consumed < 1 mg/l, residual ozone concentration in water = 0.7 mg/l, quenched samples concentrated 100 times; (c) and (c'): $[\text{isoproturon}]_0 = 0.001\,\text{mg/l}$, $[\text{ozone}]_{\text{air}} = 9\,\text{mg/l}$ at 0.1 l/min, ozone consumed < 1 mg/l, residual ozone concentration in water = 0.7 mg/l, quenched samples concentrated 10,000 times.

conclude that during real drinking water treatment, the most likely by-products to be formed are those numbered from 4 to 10.

On the basis of the above results, the isoproturon ozonation pathway shown in Fig. 5 has been proposed. As previously explained, in the present investigation the mechanism of isoproturon degradation is mainly based on the reaction with molecular ozone. The pathway depicted in Fig. 5 indicates that the initial attack of molecular ozone occurs either on the aromatic ring or on an alkyl group (isopropyl and methyl). This explains why the by-products identified in the present investigation are different from those found in previous studies, in which the main degradation pathway was due to reaction of OH$^\bullet$ radical with either a C–N or a C–H bond (Allemane *et al.*, 1995; Prados *et al.*, 1995). Moreover, it is interesting to note that the chemical structures of the primary by-products identified during previous investigations (e.g., quinone-, aniline-, benzaldehyde-, nitrobenzene-, isopropylbenzene- and phenol-like structures) are structurally simpler than those found

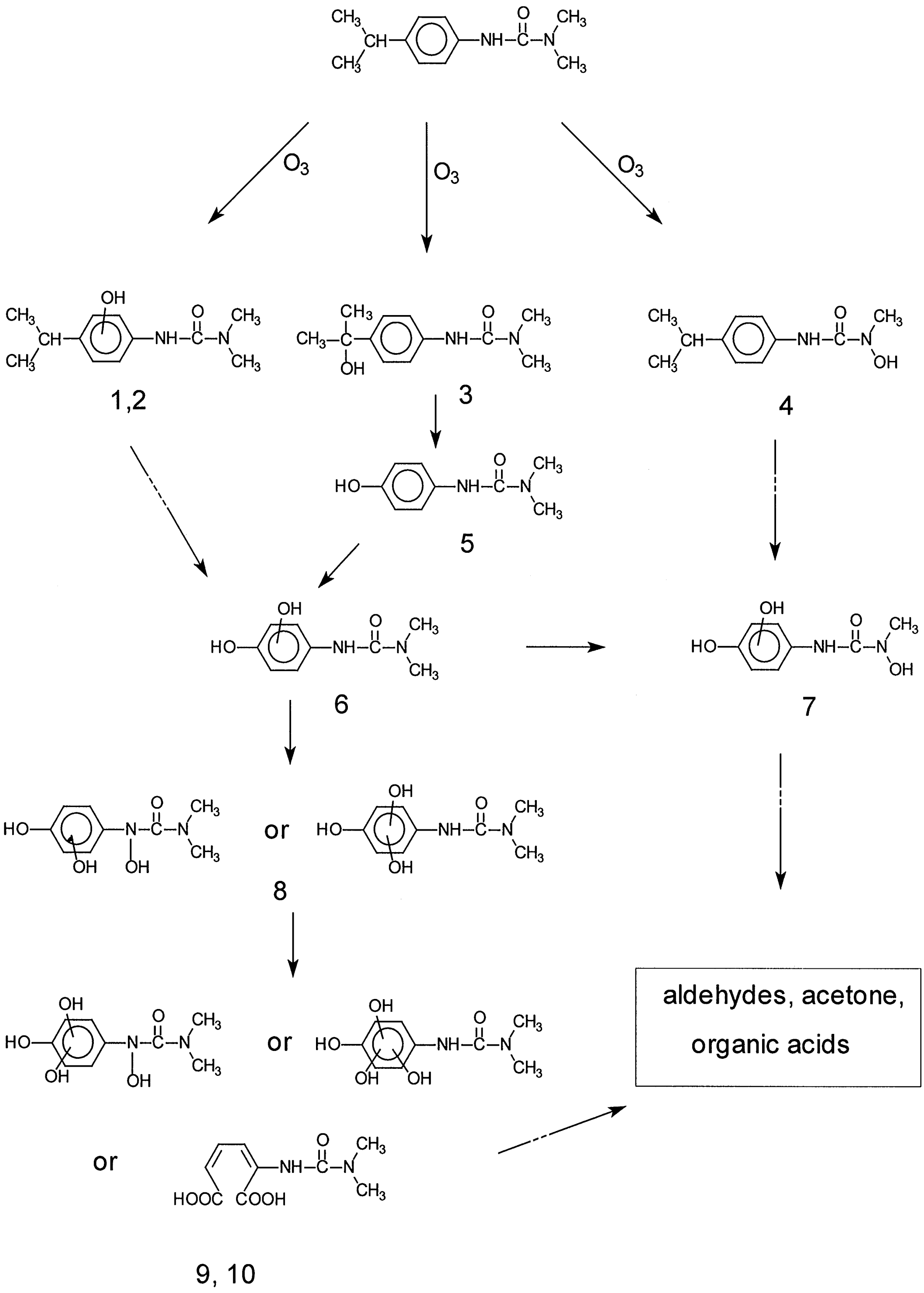

Fig. 5. Proposed pathway for isoproturon oxidation by ozone.

during the present study (i.e., 1–4 in Fig. 5). This is not unexpected as $OH^\bullet$ is a stronger oxidant and more reactive than O_3. Also, it is important to point out that during the present investigation because HPLC-MS was used, it has been possible to identify polar by-products not detectable (if formed) in the previous studies (Allemane *et al.*, 1995; Prados *et al.*, 1995) which only utilised GC-MS.

As shown in Fig. 4, by-products 7, 9 and 10 undergo further degradation that lead to their disappearance. As with longer reaction times no by-products were detected using HPLC-UV, this is probably be due to aromatic ring cleavage by reaction with ozone and $OH^•$. It is to be expected that at longer reaction times, when the isoproturon has already disappeared, dissolved ozone can react with $OH^•$ ion to give rise to $OH^•$ radical. Ozone chemistry, supports such an assumption, as during extensive ozonation of aromatic structures, low MW compounds such as aldehydes, ketones and acids are formed (Corless *et al.*, 1990). Accordingly, such compounds were looked for and found as described in the experimental section. The quantitative plots of detected carbonyl compounds, reported in Fig. 6, show that formaldehyde, acetone and glyoxal are the main compounds formed, with their concentrations being far greater than that of the others. As for acetone, its formation is probably due to the carbon–carbon cleavage of the isopropyl group of by-product 3. Furthermore, Fig. 6 shows that aldehydes, once formed, are further degraded as proved by the fact that their concentration regularly decreases after the first 5 min. From a chemical viewpoint, the most likely products of aldehyde oxidation are carboxylic acids. Consequently, as reported in the experimental section, ozonated samples were analysed also for organic acids and the results are reported in Fig. 7. This figure shows that, during the ozonation, the formation of the identified acids (i.e., formic, acetic, and oxalic) is practically continuous, indicating that such products are the real end by-products of the overall ozonation reaction. This conclusion was expected considering the very low values available in literature of the kinetic constants of the reactions between ozone and aliphatic carboxylic acids (Hoigné, 1987). The measured concentrations of formic acid, much higher than those expected from the formaldehyde concentrations reported in Fig. 6, prove that this acid is the end by-product of several previously formed intermediates.

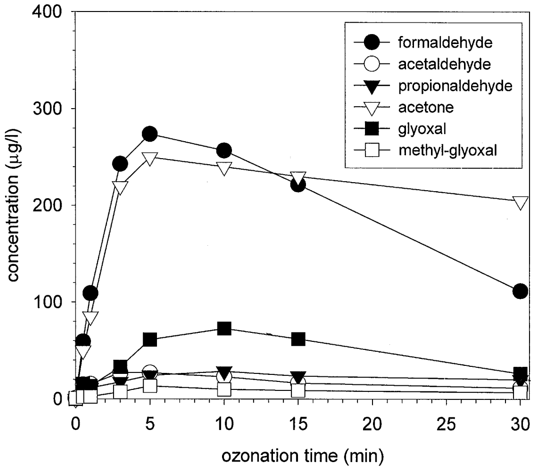

Fig. 6. Aldehydes and acetone formation during isoproturon ozonation at pH = 7. Experimental conditions: $[\text{isoproturon}]_0 = 10\,\text{mg/l}$, $[\text{ozone}]_{\text{air}} = 9\,\text{mg/l}$ at 1 l/min, ozone consumed = 44 mg/l, residual ozone concentration in water = 1.9 mg/l.

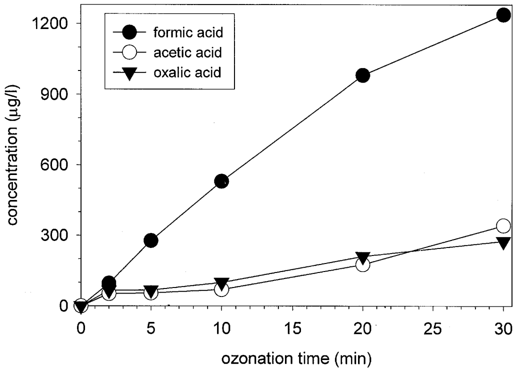

Fig. 7. Formation of organic acids during isoproturon ozonation at pH = 7. Experimental conditions: $[\text{isoproturon}]_0 = 10\,\text{mg/l}$, $[\text{ozone}]_{\text{air}} = 9\,\text{mg/l}$ at 1 l/min, ozone consumed = 44 mg/l, residual ozone concentration in water = 1.9 mg/l.

Finally, ozone chemistry also suggests that during the investigated reaction the formation of α-oxo-acids can occur. This was confirmed by GC-MS analyses of methylated samples. As reported in Table 2, α-oxo-acids with five or six carbon atoms were detected. As no authentic standards were available, they could not be quantified. However, the peak areas found for these compounds were very low, suggesting that they were only present at low concentrations. Presumably, such compounds are formed by breakdown of the aromatic structures of some isoproturon primary degradation by-products, and can give rise (by decarboxylation) to lower molecular weight derivatives.

CONCLUSIONS

A laboratory-scale investigation has been carried out to identify the degradation by-products of a

Table 2. Low molecular weight organic acids identified by GC-MS analyses after derivatization with diazomethane

Chemical structure	IUPAC name
$CH_3(CH_3)C{=}C(OCH_3)COOCH_3$	2-butenoic acid, 2-methoxy-3-methyl, methyl ester
$CH_3CH(CH_3)CH{=}C(OCH_3)COOCH_3$	2-pentenoic acid, 2-methoxy-4-methyl, methyl ester
$CH_3CH_2CH_2COCH_2COOCH_3$	hexanoic acid, 3-oxo, methyl ester
$CH_3CH{=}C(OCH_3)COOCH_3$	2-butenoic acid, 2-methoxy, methyl ester

herbicide during ozonation. Isoproturon, a phenylurea derivative, was chosen as a model compound because of its widespread use.

On the basis of the results the following conclusions have been drawn:

- Under the fixed experimental conditions, some of which are similar to those encountered at real water treatment plants, isoproturon is completely removed by ozone;
- the main reaction of isoproturon is with molecular ozone;
- during ozonation, several intermediates that maintain the aromatic ring of the isoproturon are formed as a result of primary isoproturon degradation;
- successive degradations of such intermediates, by reaction with ozone and $OH^{\bullet}$, lead to the breakdown of their aromatic structures and to the formation of low molecular weight compounds such as aldehydes, simple organic acids and α-oxo-acids.

From the drinking water quality point of view, an issue worthy to be assessed is whether the toxicity of the identified intermediates is lower or greater than that of isoproturon.

REFERENCES

Aguer J. P. and Richard C. (1996) Transformation of fenuron induced by photochemical excitation of humic acids. *Pesticide Sci.* **46**, 151–155.

Aldrich (1993) Diazald®, MNNG and diazomethane generators, technical information bulletin, No. AL-180.

Allemane H., Prados-Ramirez M., Croué J. P. and Legube B. (1995) Identification of the initial isoproturon oxidation by-products resulting from the ozone/hydrogen peroxide system. *Rev. Sci. l'Eau* **8**, 315–331.

Bonnelye V. and Richard Y. (1997) Changes in ozone demand of water during the treatment process. *Ozone Sci. Engng* **19**, 339–350.

Brambilla A. M., Calvosa L., Monteverdi A., Polesello S. and Rindone B. (1993) Ozone oxidation of polyethoxylated alcohols. *Water Res.* **27**(8), 1313–1322.

Camel V. and Bermond A. (1998) The use of ozone and associated oxidation processes in drinking water treatment. *Water Res.* **32**(11), 3208–3222.

Corless C. E., Reynolds G. L., Graham N. J. D. and Perry R. (1990) Ozonation of pyrene in aqueous solution. *Water Res.* **24**(9), 1119–1123.

Cullington J. E. and Walker A. (1999) Rapid biodegradation of diuron and other phenylurea herbicides by a soil bacterium. *Soil Biol. Biochem.* **31**(5), 677–686.

De Laat J., Maouala-Makata P. and Dorè M. (1996) Rate constants for reactions of ozone and hydroxyl radicals with several phenyl-ureas and acetamides. *Environ. Technol.* **17**, 707–716.

Di Giano F. A. (1987) In *Control of Organic Substances in Water and Wastewater*, ed. Berger, B. B., Noyes data Corporation, Park Ridge, NJ, USA Chapter 3.

EPA (1986) *Municipal Wastewater Disinfection: Design Manual.* EPA/625/1-86/021, Cincinnati, OH, USA.

Esposito E., Paulillo S. M. and Manfio G. P. (1998) Biodegradation of the herbicide diuron in soil by indigenous actinomycetes. *Chemosphere* **37**(3), 541–548.

Fielding, M., Barcelo, D., Helweg, A., Galassi, S., Torstensson, L., Van Zoonen, P., Wolter, R., Angeletti G. (1992) Pesticides in ground and drinking water. *Water Pollution Research Report 27, Commission of the European Communities, Directorate-General for Science, Research and Development,* Brussels, Belgium.

Glaze W. H. (1987) Drinking water treatment with ozone. *Environ. Sci. Technol.* **21**, 224–229.

Glaze W. H., Kang J. W. and Chapin D. H. (1987) The chemistry of water treatment involving ozone, hydrogen peroxide and ultraviolet radiation. *Ozone Sci. Engng* **9**(4), 335–342.

Hoigné J. (1987) In Stucki S., *Process Technologies for Water Treatment* pp. 121–143. Plenum Press, New York.

Hoigné J. and Bader H. (1976) The role of hydroxyl radical reactions in ozonation processes in aqueous solutions. *Water Res.* **10**, 377–386.

Hoigné J. and Bader H. (1983) Rate constants of reactions of ozone with organic and inorganic compounds in water—I. Non-dissociating organic compounds. *Water Res.* **17**, 173–183.

Huang C. P., Dong C. and Tang Z. (1993) Advanced chemical oxidation: its present role and potential future in hazardous waste treatment. *Waste Management* **13**, 361–377.

Khadrani A., Seigle-Murandi F., Steiman R. and Vroumsia T. (1999) Degradation of three phenylurea herbicides (chlortoluron, isoproturon and diuron) by micromycetes isolated from soil. *Chemosphere* **38**(13), 3041–3050.

Ku Y., Chang J. L., Shen Y. S. and Lin S. Y. (1998) Decomposition of diazinon in aqueous solution by ozonation. *Water Res.* **32**(6), 1957–1963.

Lacorte S., Ehresmann N. and Barceló D. (1996) Persistence of temephos and its transformation products in rice crop field waters. *Environ. Sci. Technol.* **30**(3), 917–923.

Langlais B., Reckhow D.A. and Brink D.R., Ozone in Water Treatment, Application and Engineering (pp. 12–13). Lewis Publishers, Chelsea, MI, USA.

Legrini O., Oliveros E. and Braun A. M. (1993) Photochemical processes for water treatment. *Chem. Rev.* **93**, 671–698.

Le Lacheur R. M., Sonnenberg L. B., Singer P. C., Christman R. F. and Charles M. J. (1993) Identification of carbonyl compounds in environmental samples. *Environ. Sci. Technol.* **27**, 2745–2753.

Madhun Y. A. and Freed V. H. (1987) Degradation of the herbicides bromacil, diuron and chlortoluron in soil. *Chemosphere* **16**(5), 1003–1011.

Mascolo G., Lopez A., Földenyi R., Passino R. and Tiravanti G. (1995) Prometryne oxidation by sodium hypochlorite in aqueous solutions: kinetics and mechanism. *Environ. Sci. Technol.* **29**(12), 2987–2991.

Mascolo G., Lopez A., Passino R., Ricco G. and Tiravanti G. (1994) Degradation of sulphur containing s-triazines during water chlorination. *Water Res.* **28**(11), 2499–2506.

Meijers R. T., Oderwald-Muller E. J., Nuhn P. A. N. and Kruithof J. C. (1995) Degradation of pesticides by ozonation and advances oxidation. *Ozone Sci. Engng* **17**, 673–686.

O'Brien R. P., Rizzo J. L., Schuliger and W. G. (1987) In *Control of Organic Substances in Water and Wastewater*, ed. Berger B. B., Noyes data Corporation, Park Ridge, NJ, USA (Chapter 11).

Ollis D. F., Pelizzetti E. and Serpone N. (1991) Destruction of water contaminants. *Environ. Sci. Technol.* **25**(9), 1523–1529.

Pieuchot M., Perrin-Ganier C., Portal J-M. and Schiavon M. (1996) Study of the mineralization and degradation of isoproturon in three soils. *Chemosphere* **33**(3), 467–478.

Prados M., Roche P. and Allemane H. (1995) State of the art in the pesticides oxidation field. In *Proceedings of the*

12th Ozone World Congress, *Lille, France*, 15–18 May, Vol. 1, pp. 99–113.

Prados M. and Ciba N. (1997) Atrazine by-products formation in xidation processes. In *Proceedings of the 13th Ozone World Congress*, Kyoto, Japan, 26–31 October, Vol. 2, pp. 651–656.

Pramauro E., Vincenti M., Augugliano V. and Palmisano L. (1993) Photocatalytic degradation of monouron in aqueous TiO_2 dispersions. *Environ. Sci. Technol.* **27**(9), 1790–1795.

Reynolds G., Graham N., Perry R. and Rice R. G. (1989) Aqueous ozonation of pesticides: a review. *Ozone Sci. Engng* **11**, 339–383.

Rice R. G. (1997) Application of ozone for industrial wastewater treatment—a review. *Ozone Sci. Engng* **18**, 477–515.

Rice R. G. and Netzer A. (1984) *Handbook of Ozone Technology and Applications*, Vol. 2, Ann Arbor Science Publisher Inc., Ann Arbor, MI, USA.

Richard C. and Bengana S. (1996) pH effect in the photocatalytic transformation of a phenyl-urea herbicide. *Chemosphere* **33**(4), 635–641.

Roche P. and Prados M. (1995) Removal of pesticides by use of ozone or hydrogen peroxide/ozone. *Ozone Sci. Engng* **17**, 657–672.

Staehelin J. and Hoignè J. (1985) Decomposition of ozone in water in the presence of organic solutes acting as promoters and inhibitors of radical chain reactions. *Environ. Sci. Technol.* **19**(12), 1206–1213.

Standard Methods for the Examination of Water and Wastewater, 20th ed. (1999) American Public Health Association (APHA), American Water Works Association (AWWA) and Water Environment Federation (WEF), Washington, DC, USA.

Tomlin, C. D. S. Ed. (1997) *The Pesticide Manual. 11th edition*, British Crop Protection Council, Farnham, UK.

Vroumsia T., Steiman R., Seigle-Murandi J., Bennoit-Guyod L. and Khadrani A. (1996) Biodegradation of three substituted phenylurea herbicides (chlortoluron, diuron and isoproturon) by soil fungi. *A comparative study*. *Chemosphere* **33**(10), 2045–2056.

Yao J. J., Huang Z. H. and Masten S. J. (1998) The ozonation of pyrene: pathway and product identification. *Water Res.* **32**(10), 3001–3012.