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Using mass spectrometry to highlight structures of degradation compounds obtained by photolysis of chloroacetamides: Case of acetochlor

Yasmine Souissi[a], Sophie Bourcier[a,*], Sélim Ait-Aissa[b], Emmanuelle Maillot-Maréchal[b], Stéphane Bouchonnet[a], Christophe Genty[a], Michel Sablier[a]

[a] Ecole Polytechnique, Laboratoire des Mécanismes Réactionnels, CNRS, Route de Saclay, 91128 Palaiseau Cedex, France
[b] INERIS, Unité Ecotoxicologie in vitro et in vivo, Parc ALATA, BP2, 60550 Verneuil en Halatte, France

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ABSTRACT

The photooxidation of acetochlor (a pesticide belonging to the acetamides group) using a polychromatic UV irradiation in ultrapure water was studied. This study reports the efficiency of mass spectrometry for the characterization of photodegradation products of acetochlor. Decompositions of protonated ions MH^+ are proposed in electrospray (ESI) mode for LC–MS, while electron ionization (EI) and chemical ionization modes (CI) are used for GC–MS. The knowledge of fragmentation and the use of a combination of experiments (MS/MS, high resolution) allow the characterization of photoproducts. Structural elucidation is assisted by the use of photolysed deuterated compounds. Fifteen major degradation products have been characterized, five by LC-QTOF, six photoproducts by GC-ITMS, and four are observed by both techniques. In vitro bioassays based on the quantification of receptor-mediated activity demonstrated that acetochlor photolysis engenders a moderate but significant estrogenic activity. Moreover, a quantitative structure–activity relationship (QSAR) approach was used to assess the potential toxicity effect of acetochlor and its by-products. The predictions were analyzed showing a variety of toxicity profiles of acetochlor photoproducts depending on the toxicological investigated endpoint.

1. Introduction

Due to their extensive use worldwide, an increasing number of biologically active compounds belonging to a large variety of chemicals appear in different aquatic environments under the form of the parent molecules and their degradation products. The contamination of soils, ground and/or surface water is a growing environmental concern all over the world [1]. As a consequence, a large group of organic compounds have been labeled as water emerging contaminants by the Environmental Protection Agency [2] and the European Union [3]. The resulting directives which prioritize some of these compounds due to their high toxicity, high environmental persistence, and bioaccumulation potential include pesticides, among which are the chlorinated herbicides. These are of particular environmental concern due to their solubility and bioavailability. This represents a threatening issue for aquatic environment and human health. Herbicides contaminated lands and farming areas may then require remediation to reduce water resources contamination. In the present study, we focused on acetochlor [2-chloro-N-(ethoxymethyl)-N-(2-ethyl-6-methylphenyl)acetamide], a member of the chloroacetanilide class of broad leaf herbicides, which is currently authorized and widely used as a pre- and post-emergence treatment. It is mainly absorbed by the roots and leaves, inhibiting photosynthetic electron transport of the host [4].

The chloroacetanilide herbicides are frequently detected in water bodies located nearby agriculture areas after soil leaching and urban runoff [5,6]. While need to be monitored, their persistence in soil as well as the emergence of potential breakdown products is a non desirable effect that may lead to ecological and human health alterations [7,8] In fact, chloroacetanilide degradation products were reported to be detected with even greater frequency than the parent compound [9].

Recently, chloroacetanilide herbicides and their degradation products have been demonstrated to be potential endocrine disruptors. Acetochlor was also previously reported to have interactions with uterine estrogen receptors and to cause the alteration of thyroid hormone dependent gene expression [10,11].

Other studies pointed out that neutral chloroacetanilide herbicide degradates require closer attention as they became considered

* Corresponding author at: Ecole Polytechnique, Laboratoire des Mécanismes Réactionnels, CNRS, Route de Saclay, 91128 Palaiseau Cedex, France.
Tel.: +33 1 69 33 48 06.
E-mail address: sophie.bourcier@dcmr.polytechnique.fr (S. Bourcier).

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as micropolluants for regulation in drinking water [2,12]. Those degradates have been reported to possess at least a modicum persistency and they may affect organisms while they yield toxic or other deleterious effects [13]. In the past years, acetochlor degradates have become extensively present in surface and ground water as well as in soils [14–16]. Chloroacetanilide has also been detected in finished drinking water [17]. Hladik and co-authors reported the identification of 26 chloroacetanilide degradation products in drinking water sources [12]. Those authors reported that chlorinated herbicides require close attention as they may be present in the environment at significant concentration.

As a significant fraction of those herbicides released into the aquatic environment is attributed to their ineffective or incomplete removal through conventional wastewater management in addition to their leaching and runoff from agricultural areas, their treatment has become a major source of concern. A variety of water treatment procedures, rather conventional or advanced for endocrine disrupting compounds removal allowing different treatment efficiency, have been reported in the literature [18,19]. Water treatment by photochemical processes was studied for two centuries [20]. In the present study, the photodegradation process was chosen since some water treatment utilities employ this process as an alternative way for water disinfection [21,22]. Some studies have been devoted to pesticide photodegradation (direct and indirect) including the identification of photodegradation products, kinetic studies and reaction pathway establishment [23–27]. However, fewer studies were dedicated to acetochlor photodegradation behavior investigation [28,29]. Moreover, the stability and persistence of the chloroacetanilide transformation products (TPs) generated through photolysis process pointed out their potential occurrence as environmental contaminants [30,31]. Those contaminants may be released in the nature as an output of water treatment plants where UV disinfection is of current use.

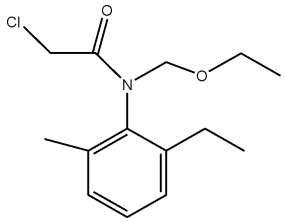
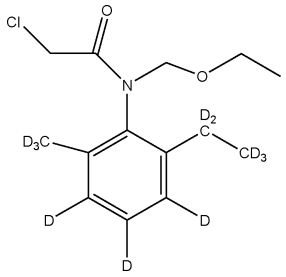
Thus, the aim of the current studies is to identify and characterize those TPs with the future aim of establishing analytical methods able to target those specific compounds and to predict their impact on the environment. The elucidation of these structures is possible combining the knowledge of the mechanisms of fragmentation of molecular ions for the compounds of reference (MH^+ in *Liquid Chromatography-Quadrupole/Time of Flight-Mass Spectrometry* LC-Q/TOF-MS and $M^{+\bullet}$ in *Gaz Chromatography-Ion Trap–Mass Spectrometry* GC-IT–MS). In a second part of the paper, we aimed at performing a comprehensive evaluation of the biological effect of the produced photoproducts. Thus, in vitro reporter gene bioassays allowing the assessment of the estrogenic and (anti)androgenic activities of the photolyzed solution as a first step before its fractioning to test each photoproduct separately were conducted. The estrogenic and (anti)androgenic activities were assessed using the MELN human reporter cell line and MDA-kb2 assay [32]. Moreover an approach of quantitative structure–activity relationship (QSAR) was used to assess the potential toxicity effect of acetochlor and of its by-products and to compare them. For that purpose we used a Toxicity Estimation Software Tool (T.E.S.T.), a U.S. Environmental Protection Agency (EPA) developed program, as it offers modules to predict mutagenicity, oral rat lethal doses, developmental toxicity as well as growth inhibition concentrations and lethal doses of aquatic organisms (*Tetrahymena pyriformis*, *Daphnia magna* and fathead minnow).

2. Materials and methods

2.1. Chemicals and reagents

The chemical structures of acetochlor, acetochlor-D_{11} (respectively referred as Ace, Ace-D_{11}) are displayed in Table 1. The chemicals, formic acid, phosphoric acid and HPLC grade solvents (water and acetonitrile) were all purchased from Sigma Aldrich (Saint-Quentin Fallavier, France) and used as received (98% purity).

Table 1
Names and chemical structures of the studied compounds.

Structure	Chemicals	MW
	Acetochlor (Ace)	269
	Acetochlor D_{11} ($AceD_{11}$)	280

2.2. Sample preparation

2.2.1. Standard solutions

Solutions of acetochlor and acetochlor-D_{11} at 1 mg mL^{-1} were prepared in water and stored at −20 °C. These solutions were diluted at 10^{-6} M in a H_2O/CH_3CN (50:50) mixture acidified with formic acid (FA) (0.1%) for fragmentation studies.

2.2.2. Procedure for photolytic experiments and sample preparation

Photolysis experiments were carried out in a photolysis reactor equipped with a high-pressure mercury lamp (HPL-N 125W/542 E27 SG, Philips, 94856 Ivry sur Seine, France). The lamp used on those experiments emits light in a broad spectra ranging from 200 to 1100 nm. Photolysis experiments were carried out in quartz glass containing 10 mL of herbicide (1 mg mL^{-1}) solution. The lamp was placed into the inner part of the reactor cooled by water circulation to avoid uncontrolled heating of the irradiated solution in maintaining a constant temperature of 25 ± 2 °C. The luminous flux emitted from the HPL-N lamp was reported by the manufacturer to be 6200 lm. The solution was stirred during radiation time with a sonicator (Bioblock Scientific 91631, Illkirch, France). Several irradiated times have been studied (5, 10, 20, 30, 60 and 90 min). In order to ensure that the photoproducts were not generated through the effect of sonication, two reference solutions dopped with acetochlor and not exposed to UV treatment were prepared. One was sonicated and the other one was not. The GC–MS and LC–MS analyzes of these two solutions showed identical chromatographic profile. The irradiated solution was concentrated using solid phase extraction. The solid phase extraction (SPE) cartridges (Atoll column 30 ATH 200 mg Interchim SA, Montluçon France) were consecutively conditioned with 3 mL of methanol and then 3 mL of deionized water. The cartridges were loaded with the photolysis solution (5 mL). The cartridge was then washed with 4 mL of the H_2O/MeOH (95/5, v/v) solution. After drying for 2 min under vacuum, the compounds were eluted with 8 mL of MeOH then 4 mL of CH_3CN 0.1% formic acid. The solvents were removed under a