Environmental Pollution 230 (2017) 107–115

Contents lists available at ScienceDirect

Environmental Pollution

journal homepage: www.elsevier.com/locate/envpol

Multiple spectroscopic analyses reveal the fate and metabolism of sulfamide herbicide triafamone in agricultural environments☆

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ARTICLE INFO

Article history:
Received 4 April 2017
Received in revised form
20 May 2017
Accepted 8 June 2017

Keywords:
Triafamone
Paddy environment
Fate
Transformation pathway
Multi-spectroscopic analyses

ABSTRACT

Triafamone, a sulfamide herbicide, has been extensively utilized for weed control in rice paddies in Asia. However, its fate and transformation in the environment have not been established. Through a rice paddy microcosm-based simulation trial combined with multiple spectroscopic analyses, we isolated and identified three novel metabolites of triafamone, including hydroxyl triafamone (HTA), hydroxyl triafamone glycoside (HTAG), and oxazolidinedione triafamone (OTA). When triafamone was applied to rice paddies at a concentration of 34.2 g active ingredient/ha, this was predominantly distributed in the paddy soil and water, and then rapidly dissipated in accordance with the first-order rate model, with half-lives of 4.3–11.0 days. As the main transformation pathway, triafamone was assimilated by the rice plants and was detoxified into HTAG, whereas the rest was reduced into HTA with subsequent formation of OTA. At the senescence stage, brown rice had incurred triafamone at a concentration of 0.0016 mg/kg, but the hazard quotient was <1, suggesting that long-term consumption of the triafamone-containing brown rice is relatively safe. The findings of the present study indicate that triafamone is actively metabolized in the agricultural environment, and elucidation of the link between environmental exposure to these triazine or oxazolidinedione moieties that contain metabolites and their potential impacts is warranted.

1. Introduction

Rice paddy fields form the largest agricultural environment in Asia and act as an important resource to provide food for an increasing population and forage for livestock (Seck et al., 2012). In rice cultivation, in addition to pest management, weed control has been deemed a serious challenge. To resolve yield loss caused by weeds, a variety of chemical herbicides, especially those with broad-spectrum herbicidal activity, have been extensively applied in rice paddies in the past century (Opena et al., 2014). Recently, a novel sulfamide herbicide triafamone was isolated for weed control in rice paddies (Rosinger et al., 2012). Field efficacy trials have shown that this pre- and post-emergence herbicide, at rates of 20–50 g active ingredient/ha by using sprayer granular formulations, could be effectively applied in directly seeded or transplanted rice from sowing or transplanting to late post-emergence (Rosinger et al., 2012). Triafamone is thus considered as a promising herbicide (Rosinger et al., 2012) because it is capable of controlling a wide array of weeds such as *Echinochloa crus-galli*, *Echinochloa colonum*, *Echinochloa oryzicola*, *Paspalum distichum*, *Isachne globosa*, sedges, and even including some strains that are resistant to acetolactate synthase. Interestingly, the mode of action of triafamone against weeds has been attributed to an unidentified metabolite of triafamone instead of triafamone itself, by which the activity of acetolactate synthase of weeds is drastically repressed (Rosinger et al., 2012).

Despite alleviated crop yield loss, the input of large amounts of synthetic herbicides into the agricultural environment not only poses side effects on non-target organisms (McCallum et al., 2013; Pareja et al., 2012a), but also lead to public health concerns relating to food safety (Cao et al., 2015; Lu et al., 2014). Within the past few decades, endocrine disrupting compounds (EDCs) that result in side effects involving the endocrine system of aquatic organisms and

☆ This paper has been recommended for acceptance by Charles Wong.
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http://dx.doi.org/10.1016/j.envpol.2017.06.046
0269-7491/

even mammals have been reported (Dodgen et al., 2013; Liu et al., 2009; Sun et al., 2015). Environmental contaminants and synthetic substances, including agrochemicals, pharmaceuticals, industrial chemicals, and heavy metals, have been classified as EDCs (Dodgen et al., 2015; Giesy et al., 2002; Orton et al., 2009). Among the currently available herbicides, triazines and amides are two major classes of herbicides that impart EDC effects in fish and mammals (Maggioni et al., 2013; Orton et al., 2009; Zhang et al., 2016).

The triazine herbicide atrazine disrupts normal endocrine development and function in lower and higher vertebrates via non-steroidal NR5A nuclear receptors, whereas the amide herbicide acetochlor accelerates T-3-induced metamorphosis of *Xenopus laevis* by inducing the upregulation of thyroid hormone beta receptor genes (Crump et al., 2002; Suzawa and Ingraham, 2008). A recent report has shown that atrazine elicits an antagonistic effect on glucocorticoid receptors in mammals (Zhang et al., 2016). Because triafamone belongs to a subfamily of amides and a class of sulfamides, it comprises triazine and sulfonanilide moieties, and could also be classified as a triazine homolog. However, whether this herbicide exert EDC effects toward non-target organisms remains unclear (Orton et al., 2009), despite the results of conventional toxicology and environmental behavior tests that show that triafamone has low toxicity to non-target organisms and pose a low risk for groundwater contamination (Rosinger et al., 2012). More importantly, little is known about the fate and transformation pathway of triafamone in rice paddies, which are considered to be highly linked to environmental effects posed by residues from environmental exposure and daily food ingestion (Cao et al., 2015; Rosinger et al., 2012).

To address this issue, we developed an efficient and convenient approach based on a laboratory-scale microcosm combined with multiple spectroscopic analyses for screening of triafamone metabolites in a typical agricultural environment. Accordingly, the distribution, kinetics, and incurrence of triafamone associated with the formation of its metabolites in the rice paddy environment were also investigated, particularly in terms of health risk after long-term rice consumption. Our findings provide further insights into the environmental impacts exerted not only by residual triafamone but also by the metabolites formed *in situ*.

2. Materials and methods

2.1. Materials

Methanol (MeOH), acetonitrile (ACN), ethyl acetate (EtOAc), dichloromethane (DCM), acetone, hexane, and other solvents of high performance liquid chromatography (HPLC) grade were purchased from Sigma-Aldrich. NaCl, $MgSO_4$, Na_2SO_4, and other reagents were of residue analysis grade (Sigma-Aldrich). Standard triafamone and penoxsulam were of analytical standard grade (Sigma-Aldrich). Triafamone 19% suspension concentrate (SC) is a commercially available herbicide in China (Bayer Crop Science). A modified QuEChERS (Quick, Easy, Cheap, Effective, Rugged, and Safe) approach was applied by using a DisQuE kit (Waters).

2.2. Rice paddy microcosm-based simulation trial

To trace triafamone metabolism in rice paddies, a rice paddy microcosm was constructed, as previously reported (Fu et al., 2015), with minor modifications. A polyvinyl chloride pot (height: 30 cm, width: 20 cm, depth: 20 cm) was used to simulate a rice paddy. The paddy soil (triafamone-free, pH 6.6, organic matter content: 1.95%) used for the rice paddy microcosm trial was collected from the 0–15 cm layer of a rice paddy field (29°48.467′N, 120°17.867′E) in Zhejiang Province, China. In each pot, 3.0 kg of dried soil (10 mesh) was used to form a 10-cm soil layer and then flooded with 1 L of ultrapure water to form a water layer of ≈ 3 cm. To cultivate the rice seedlings, healthy rice seeds (*Oryza sativa* cv. Ning88) were immersed in water and incubated at 25 °C for 2 days in the dark until germination; the germinated seeds were then transplanted into each pot (150 seeds per pot). The constructed rice paddy microcosms were maintained in a plant growth incubator (25 °C, 12-h photoperiod). After the rice seedlings reached the early tillering stage, triafamone 19% SC was diluted with water and evenly dripped into the water layer at 180 mL/ha (equivalent to 34.2 g active ingredient/ha). For the control group, the same volume of water was applied.

2.3. Preliminary screening of triafamone metabolites

To screen triafamone metabolites, representative samples, including rice seedlings, soil, and water were collected at 1, 3, 5, 7, and 10 days after application from the triafamone-treated and control groups. At each sampling interval, three pots from each group, respectively, were subjected to diverse extraction approaches using various organic solvents and HPLC-based profiling (Texts S1-S4).

2.4. Isolation and identification of triafamone metabolites

To obtain large quantities of the putative metabolites, the remaining rice paddy microcosm samples relevant to the analyte solution, whose chromatographic profiles indicated putative metabolites, were combined and subjected to extraction. The resulting concentrates were further subjected to a PHPLC system (Agilent 1260 Infinity) equipped with an Agilent Prep-C_{18} column (250 mm × 30.0 mm, with 10-μm particle size). The mobile phases consisted of a 60% ACN–ultrapure water solution. To maintain the same isolation performance of the analytical HPLC, the PHPLC was amplified linearly at a flow rate of 34 mL/min and an injection volume of 850 μL.

Multiple spectroscopic analyses were conducted as follows. FD-HR-MS was conducted in a JMS-SX-102 (JEOL). ESI-MS/MS was carried out in a Quattro Premier XE (Waters). One-dimensional NMR was conducted using DMSO-d_6 as a solvent in a DMX-500 (Bruker) for ^{1}H NMR (nondecoupling) and ^{13}C NMR (bilevel complete decoupling and distortionless enhancement by polarization transfer [DEPT]) to elucidate chemical structure.

2.5. Rice paddy field trial

To investigate the initial deposition, kinetics, and incurrence of triafamone, field trial was conducted in a typical rice production region in Zhejiang Province (29°48.467′N, 120°17.867′E), China. The rice paddy showed a pH of 6.6 and organic matter content of 1.95%, and were classified as paddy soil according to the Chinese Soil Taxonomy (ISS, 2001). The treatment group consisted of nine adjacent replicated plots following a complete randomized block design; each plot was 30 m^2 in size and included one inlet and one outlet. *Oryza sativa* cv. Ning88 was selected and cultivated in the seedling bed until the early tillering stage. One week after transplantation into the designed plots, triafamone 19% SC was diluted with water and evenly dripped into the paddy water at 34.2 g active ingredient/ha. A blank control (nine plots) was treated with water only. Rice plants (500 g), paddy soil (500 g), and water (2 L) were collected from each plot at 0 (2 h), 1, 3, 5, 7, 14, 21, and 28 days after triafamone application using a five-point sampling method for the analysis of deposition and kinetics of triafamone and the formation of metabolites. During this period, the paddy water layer was maintained at a height of 5 cm by daily irrigation. At the senescence stage (pre-harvest interval was set at 103 days), rice grains (500 g)