Science of the Total Environment 626 (2018) 860–866

Contents lists available at ScienceDirect

Science of the Total Environment

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

Enantioselective effects of chiral amide herbicides napropamide, acetochlor and propisochlor: The more efficient *R*-enantiomer and its environmental friendly

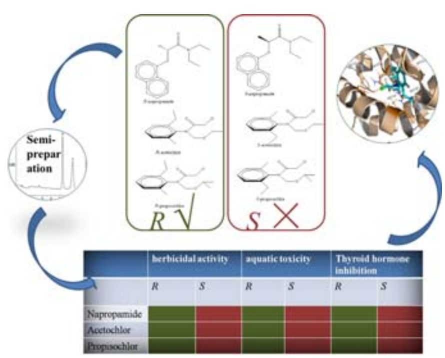
Jingqian Xie [a,b], Lu Zhao [b], Kai Liu [c], Fangjie Guo [b], Weiping Liu [b,*]

[a] *College of Marine Ecology and Environment, Shanghai Ocean University, Shanghai 201306, China*
[b] *MOE Key Laboratory of Environmental Remediation and Ecosystem Health, College of Environmental and Resource Sciences, Zhejiang University, Hangzhou 310058, China*
[c] *Department of Environmental Science and Engineering, California Institute of Technology, 1200 East California Blvd., Pasadena, CA 91125, USA*

HIGHLIGHTS

- *R*-amide herbicides had stronger activity and lower toxicity.
- Herbicides at certain low concentrations could be utilized to promote plant growth.
- Using napropamide can be better to control root growth while acetochlor and propisochlor were better at foliage control.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 18 December 2017
Received in revised form 13 January 2018
Accepted 15 January 2018
Available online xxxx

Editor: Jay Gan

Keywords:
Chiral
Target enantio-activity
Non-target enantio-toxicity
Molecule docking

ABSTRACT

Amide herbicides, which are used extensively worldwide, are often chiral. Enantiomeric selectivity comes from the different effects of the enantiomers on target and non-target organisms. In this study, the enantiomers of three amide herbicides were purified by the semi-preparative column and were used to investigate the enantioselective effects on target *Echinochloa crusgalli* (*lowland rice weeds*), and non-target *Microcystis aeruginosa*, and the yeast transformed with the human TRβ plasmid organisms. The results showed that (*i*) the *R*-enantiomers of the three amide herbicides exhibited the strongest activity toward weed inhibition and the lowest toxicity toward non-target organisms; (*ii*) napropamide was better suited for controlling root growth, while acetochlor and propisochlor were better for leaves control; (*iii*) herbicides at certain low concentrations (0.01 mg L^{-1} for acetochlor and propisochlor) could be utilized to promote plant growth. These findings encourage the use of *R*-amide herbicides instead of their racemates to increase the efficiency of weed control and reduce the risk to non-target organisms. On the other hand, the adverse effects are caused mostly by *S*-enantiomer, using *R*-enantiomer-enriched products may offer great environmental/ecological benefits.

1. Introduction

Herbicides are chemicals used for controlling weeds and increasing crop yields. Currently, global herbicide sales reach US $ 17 billion annually (Kraehmer, 2012), and the market is growing in both developed

* Corresponding author.
E-mail address: wliu@zju.edu.cn (W. Liu).

https://doi.org/10.1016/j.scitotenv.2018.01.140

and developing countries (Gianessi, 2013). However, only a fraction of herbicides has substantiated effects on weeds, while the majority of herbicides applied would eventually enter environment. There are 19 known types of herbicides (Duke, 2012). In particular, amide herbicides have a large market share just below that of amino acid and sulfonylurea herbicides. They are highly effective and selective. And amide herbicides are usually applied at high concentration such as liters/ha (E-Phy, 2015). The mechanism of action of amide herbicides has no consistent pattern. Some of them are applied only into the soil to inhibit roots or seeds, while others applied only to foliage. They can enter the environment through point and non-point sources and are distributed in water, sediment soil and biota (Liu et al., 2005). Their concentrations in the environment range from ng/l to μg/l (Fenner et al., 2013).

Some of the most widely used amide herbicides are chiral, resulting in pairs of enantiomers due to the presence of a chiral center. The enantiomers share the same physical and chemical properties in achiral environments, but they usually display different physiochemical and biochemical properties in chiral environment resulting from their different interactions with enzymes or other chiral molecules (Smith, 2009). Therefore, the different roles of each enantiomer of a chiral herbicide cannot be ignored.

Some studies (Liu et al., 2005; Na et al., 2006; Liu et al., 2008) have already paid attention to the chiral effects of insecticides and fungicides, but for chiral herbicides it was still fewer than those two types of pesticide. The inhibition abilities of (±)-imazethapyr to the root growth of maize seedlings differ between *S*-(+) - and *R*-(−)-imazethapyr (Qian et al., 2011). *S*-diclofop-methyl was more toxic to the leaves of rice seedlings, while *R*-diclofop acid was more toxic to the roots (Ye et al., 2013). The acute toxicities of lactofen and desethyl lactofen enantiomers to *Daphnia magna* were also enantioselective (Diao, 2010). (−)-napropamide was more toxic than the racemate and (+)-napropamide to soybean and cucumber (Qi et al., 2015). Unfortunately, little is known regarding the correlation between the toxicity and activity of the chiral herbicide enantiomers. Therefore, concerted effort is urgently required in order to develop herbicides with optimal efficiency on target and minimal side effects on non-target organisms (Kōhler and Triebskorn, 2013).

Echinochloa crusgalli (*E. crusgalli*), an annual grass, has been noted to cause problems in at least 61 countries and for 36 different crops (Holm et al., 1991). To minimize the inhibitory effects of *E. crusgalli* on other plants, herbicides are regularly applied to control it. Not surprisingly, the herbicides entered the water and the residues have been detected in various waters (Oehmichen et al., 1987; Hu et al., 2001; Thurman et al., 1992; Squillace and Thurman, 1992). *Microcystis aeruginosa* (*M. aeruginosa*) is a prokaryotic alga and key primary producer. Although it may cause cyanobacterial bloom, the toxicity is significantly reduced at laboratory growth condition, thus it has been used as a model organism to assess potential toxicity of herbicides in the aquatic environment (Wang et al., 2011). Most significantly, humans may be exposed either directly or indirectly to the herbicides, which often display some level of toxicity. There are considerable side effects that herbicides may have on humans. However, few references have reported the risks. Kojima et al. (2004) used Chinese hamster ovary cells to screen for estrogen and androgen receptor activities in 200 pesticides and found that a majority of herbicides have little or no estrogenic and agonistic activities. However, researchers have found that acetochlor accelerated T_3-dependent metamorphosis in ranid species (Crump et al., 2002), indicating that other herbicides may also have thyroxin interference effects. Later, Xu et al. (Jin et al., 2008) reported that acetochlor had the thyroid hormone disrupting effect on zebra fish. And Turque et al. (2005) also gave a warning that acetochlor was a thyroid disruptor.

Here, the enantiomers of three chiral amide herbicides, napropamide, acetochlor and propisochlor were prepared and purified by the semi-preparative column and were used. The target activity to *E. crusgalli* and adverse effects on aquatic organisms, and yeast thyroid receptor plasmid evaluated.

2. Materials and methods

2.1. Chemicals and reagents

Napropamide was supplied by Jiangsu Rudong pesticide factory (China), and acetochlor and propisochlor were provided by Shandong Qiaochang Chemical (China). The stereochemical structures of the three herbicide enantiomers are in Fig. S1. In separation experiments, the stock solutions were stored in ethanol, and in active and adverse effect experiments, the stock solutions were prepared in acetone or dimethyl sulfoxide (DMSO).

2.2. Chromatographic separation and characterization

The stereoisomers of the three amide herbicides were isolated using a Waters 2535 semi-preparative HPLC system (Waters Corp., Milford, USA) under the conditions shown in Table S1. The configurations of the herbicides were determined by comparison of measured and calculated electronic circular dichroism (ECD) and vibrational circular dichroism (VCD) values. The solvent stabilities in isometric ethanol, isopropanol, acetone, ethyl acetate, *n*-hexane and water were measured. The thermostabilities at 4 and 30 °C were evaluated as well. The purity of the enantiomers was also verified. The more detail procedure was showed in supporting information.

2.3. Target activity

The enantioselective herbicidal activity was investigated with *E. crusgalli* (obtained from Zhejiang Research Institute of Chemical Industry, China). Since amide herbicides are pre-emergence herbicides, tests were performed according to the Chinese national standards on petri dish test of herbicide bioactivity (Pesticides guidelines for laboratory bioactivity tests, n.d). Uniformly germinated seedlings were selected and placed in 9 cm petri dishes (10 seedlings in each dish) at 30 °C with 85% air humidity and 16 h light/8 h dark cycle. *Rac*-, *R*- and *S*-herbicide stereoisomers were added at both low concentration (0.01 mg L^{-1}) and high concentration (0.05 mg L^{-1}). The treated seedlings were analyzed after 7 days. The lengths of roots (shoots) and the fresh weight were recorded. The growth inhibition tests were performed according to the OECD guidelines (OECD Organization for economic cooperation and development, 2002) and the experiment also designed as Huan et al. (2011), Chung et al. (2002) and Poonpaoboonpipat et al. (2013) reported studying the same weed. Detailed procedure is shown in supporting information. The herbicide ingredient was tested after 7d by SFC-MS/MS.

$$\text{Relative inhibition } (\%) = (\text{control}-\text{treatment})/\text{control} \times 100\%$$

The root morphological parameters were measured with a root scanner Microtek MRS-6400A3, and the images were analyzed by WinRHIZO Pro 2002c (Regent Instruments Inc.).

2.4. Aquatic toxicity assay

M. aeruginosa FACHB 912 (bought from the Institute of Hydrobiology of Chinese Academy of Sciences) cultured in BG-11 medium and stored in the climatic cabinet with standard temperature and lighting conditions (12:12 light:dark cycle), was used as an indicator of aquatic toxicity. The algal growth inhibition test was performed according to OECD guideline 201 for freshwater algal and cyanobacterial growth inhibition test (Chemistry (General), 2006). According to our preliminary experiment, stereoisomers of herbicides dissolved in acetone were added to the medium at various concentrations (0.1, 1, 5, 10 mg L^{-1}). Each assay was conducted in triplicate. Cell densities were monitored at 680 nm at 24 h intervals for 96 h. EC_{20} was calculated. The more detail