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Enantioselective toxicity of lactofen and its metabolites in *Scenedesmus obliquus*

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ABSTRACT

In this study, the acute toxicity of lactofen and its two metabolites desethyl lactofen and acifluorfen was determined in the aquatic algae *Scenedesmus obliquus*. After exposure for 96 h, the EC_{50} values for S-(+)-, rac-, and R-(−)-lactofen were 0.896, 0.784, and 0.649 μg/L, respectively; the EC_{50} values for S-(+)-, rac-, and R-(−)-desethyl lactofen were 0.837, 0.822, and 0.696 μg/L, respectively; and the EC_{50} value for acifluorfen was 340.838 μg/L. The results indicate that the toxicities of the transformation products are lower than that of the parent compound and that the acute toxicity (96 h EC_{50}) of these compounds is enantioselective. The photosynthetic pigment content (Chl a, Chl b, total chlorophyll and carotenoids), antioxidant enzyme activity (CAT, SOD), and malondialdehyde content (MDA) were investigated to assess the different toxic effects when *S. obliquus* was exposed to lactofen and its two metabolites for 96 h. On the basis of these data, the acute toxicity and toxic effects of lactofen and its two metabolites against *S. obliquus* were concluded to be enantioselective, indicating that such enantiomeric differences should be taken into consideration in the study of pesticide risk assessment.

1. Introduction

Organic contaminants in ecosystems such as pesticides and their residues have become environmental concerns because the human and ecosystem health can be adversely affected by these compounds [1,2]. Previous studies indicate that most applied pesticides are lost into the environment via surface runoff and infiltration rather than by reaching their targets [3]. As a consequence, the massive use of organic pesticides, both in agriculture and for domestic purposes, has caused a severe accumulation of these pesticides in the aquatic environment including rivers, lakes, underground streams and even coastal sea waters [1,4]. This raises concerns about the potentially detrimental effects on ecologically relevant organisms, such as microalgae [5–7]. Microalgae are the dominant primary producers in many aquatic systems, and effects on phytoplankton communities may likely impact higher trophic levels [8]. Pesticides may be degraded either via chemical processes or by microorganisms, when they are released to the environment. For example, lactofen could be degraded quickly to desethyl lactofen and acifluorfen primarily via aerobic soil metabolism and hydrolysis [9], and these two metabolites are apt to be more persistent than the parent lactofen [9,10]. Generally, transformation products differ from their parent compounds in chemical and toxicological properties and exhibit potential and ambiguous ecological risks [11]. In addition, many studies have found that pesticide transformation products had a lower toxicity to organisms than their parent compounds [12]. However, under some circumstances, they may cause a greater risk than their parent compound to the environment. Consequently, it is critical to consider the toxicity of lactofen and its two metabolites against algae in the environmental risk assessment process.

Chiral pesticides, accounting for more than 40% of the currently used pesticides in China [13], incorporate two or more enantiomers with the same physicochemical properties. However, enantiomers may vary in their toxicity, bioactivity, excretion, metabolism, and environmental behaviors [14]. In practical application, several enantiomers of chiral pesticides may possess less efficacious or no effects on target organisms, with only one enantiomer exhibiting active effects [15]. The existing knowledge of racemate pesticides cannot adequately describe their ecological risks and actual environmental fate [15]. Many chiral pesticides are released as racemates into the environment, and they transform into chiral or achiral breakdown products that could also be formed from achiral pollutants [11]. Lactofen {2-ethoxy-1-methyl-2-oxoethyl 5-[2-chloro-4-(trifluoromethyl)phenoxy]-2-nitrobenzoate}, a chiral pesticide, is such a case, as of its two main metabolites, desethyl lactofen {1-(carboxy)ethyl 5-[2-chloro-4-(trifluoromethyl)phenoxy]-2-nitrobenzoate} is a chiral compound, and acifluorfen {5-[2-chloro-4-(trifluoromethyl)phenoxy]-2-nitrobenzoic acid} is an achiral compound. Therefore, it is necessary to study the enantioselective behaviors of lactofen and its two

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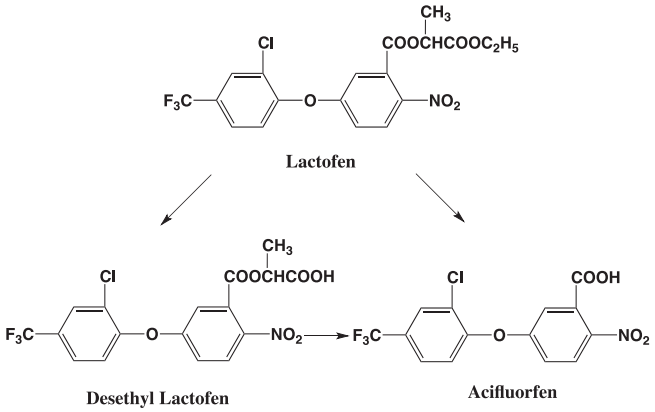

Fig. 1. Chemical structures and the main metabolic pathways of lactofen, desethyl lactofen and acifluorfen.

metabolites in the environment to more comprehensively understand the effects of chiral agrochemicals and their chiral or achiral transformation products on the environmental safety and public health [8].

Lactofen (Fig. 1) is C-chiral because there exists an asymmetrically substituted C-atom in the alkyl moiety. It consists of two enantiomers S-(+)-lactofen and R-(−)-lactofen [10]. Lactofen was developed by PPG Industries in 1987, as a broad-spectrum, selective diphenylether herbicide. The major target of this class of herbicides is protoporphyrinogen oxidase in the porphyrin biosynthetic pathway [16]. It is applied preemergence and/or postemergence by ground or air spray and is generally used to control broadleaved weeds in peanuts, potatoes, cereals and soybeans [17]. Research on the transformation of lactofen has focused on the plants [18], soil [19], water [9], sediment [10], and primary hepatocytes of rats [17]. When lactofen is degraded in the soil, the preliminary transformation products (desethyl lactofen and acifluorfen) can be formed by the fracture of the ethyl ester side chains, and further metabolites would also be formed via these primary hydrolysis products [20]. Nevertheless, due to the lack of knowledge on the bioactivities and toxicities of the individual enantiomers, the possible risks of this chiral herbicide and its metabolites are still not clear. Therefore, studies investigating the potential effects of lactofen and its metabolites are particularly significant.

In this study, we assessed the acute toxicity of lactofen, desethyl lactofen and acifluorfen in *Scenedesmus obliquus*. Different biomarkers such as the EC_{50}, chlorophyll and carotenoid contents, antioxidant enzyme activities (CAT, SOD) and malondialdehyde (MDA) in *S. obliquus* were investigated when the algae were treated by lactofen and its two metabolites for 96 h. It is useful to apply a multiparametric approach incorporating photosynthetic and physiological–biochemical indexes in the evaluation of enantioselective toxic effects.

2. Materials and methods

2.1. Chemicals and reagents

rac-Lactofen (purity ≥ 99.0%) and acifluorfen (purity ≥ 99.0%) were provided by the China Ministry of Agriculture's Institute for Control of Agrochemicals. rac-Desethyl lactofen (purity ≥ 98.0%), its enantiomers (purity ≥ 99.0%, optical purity ≥ 97.5%) and rac-lactofen's enantiomers (purity ≥ 99.0%, optical purity ≥ 97.5%) were synthesized in our own laboratory [10]. All analytical grade reagents were purchased from Yili Fine Chemicals (Beijing, China). Water was purified by a Millipore Purification System (Milli-Q system).

2.2. Algal culture

The freshwater microalgae *S. obliquus* were obtained from the Institute of Hydrobiology of the Chinese Academy of Sciences. It was cultured in 250-mL flasks with 100 mL liquid HB-4 medium. The preparation of HB-4 medium for *S. obliquus* growth is followed by the protocol provided by the Chinese National Environmental Protection Agency Guideline 201 [21]. The incubation of axenic cultures used standard temperature and lighting conditions in an environmental chamber (25 °C; illumination 3000–4000 lx; 16:8 light:dark cycle). For preparation for subsequent bioassays, the algae were inoculated into fresh media every 4 days.

2.3. Growth inhibition test

A test of algae growth inhibition was carried out to detect the effective concentration of pesticide that reduced the population growth rate of algae cells by 50% (EC_{50}), in accordance with the updated Organisation for Economic Co-operation and Development (OECD) guideline 201 for a freshwater algae growth inhibition test [22]. Lactofen and its two metabolites were dissolved in acetone as a stock solution, and this solution was added into the HB-4 media to achieve a series of desired concentrations. For the growth inhibition test, 100 mL HB-4 medium containing exponentially growing algae cells was distributed into sterile 250-mL flasks. The flasks were inoculated with an initial concentration of 20,000 cells/mL. The algal cells were exposed to a series of concentrations (S-(+)-lactofen 3, 2.5, 2, 1.5, 1, and 0.5 μg/L; rac-lactofen 3, 2, 1.5, 1, 0.7, and 0.5 μg/L; R-(−)-lactofen 2, 1.5, 1, 0.7, 0.5, and 0.3 μg/L; S-(+)-, rac-, R-(−)-desethyl lactofen 3, 1.5, 0.75, 0.5, 0.25, and 0.1 μg/L; and acifluorfen 1, 0.5, 0.25, 0.15, 0.075, 0.04, 0.02 mg/L). Each treatment was performed in triplicate. Each flask was shaken three times and repositioned daily to prevent the clumping of algal cells. Under continuous illumination (3000–4000 lx) on a 16:8 light:dark cycle, the algae were incubated at 25 ± 0.5 °C in an incubator. The cell count of *S. obliquus* was monitored by measuring the optical density at 650 nm (OD_{650}) at 48 h, 72 h and 96 h and applying linear equations.

Table 1
Calculated EC_{50} values for lactofen, desethyl lactofen, and acifluorfen.

Compound	S-(+)-Enantiomer			rac-Compound			R-(−)Enantiomer		
EC_{50}[a](μg/L)	48 h	72 h	96 h	48 h	72 h	96 h	48 h	72 h	96 h
Lactofen	0.426	0.736	0.943	0.279	0.559	0.790	0.287	0.464	0.645
Confidence intervals[b]	0.345–0.494	0.676–0.796	0.670–1.178	0.151–0.367	0.313–0.709	0.727–0.853	0.245–0.321	0.430–0.496	0.598–0.694
Desethyl lactofen	0.619	0.679	0.823	0.559	0.666	0.821	0.475	0.599	0.691
Confidence intervals[b]	0.474–0.810	0.519–1.017	0.670–1.067	0.386–0.766	0.463–0.958	0.624–1.145	0.428–0.524	0.485–0.732	0.570–0.846
Acifluorfen				205.7	335.1	340.5			
Confidence intervals[b]				155.4–281.3	254.6–470.3	258.9–478.1			

[a] EC_{50}, the effective concentration that results in a 50% reduction in population growth relative to the control.
[b] 95% confidence intervals surrounding each estimated EC_{50} are bracketed.