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Enantioselective Degradation in Sediment and Aquatic Toxicity to *Daphnia magna* of the Herbicide Lactofen Enantiomers

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Many pesticides in use are chiral compounds containing stereoisomers. However, the environmental behavior and fate of such compounds with respect to enantioselectivity so far has received little attention. In this study, the degradation of lactofen and its main metabolites (acifluorfen, an achiral compound; desethyl lactofen, a chiral compound) in sediment were investigated under laboratory conditions using enantioselective HPLC, and the enantioselectivities of individual enantiomers of lactofen and desethyl lactofen in acute toxicity to *Daphnia magna* were studied. The calculated LC_{50} values of *S*-(+)-, *rac*-, and *R*-(−)-lactofen were 17.689, 4.308, and 0.378 μg/mL, respectively, and the calculated LC_{50} values of *S*-(+)-, *rac*-, and *R*-(−)-desethyl lactofen were 21.327, 13.684, and 2.568 μg/mL, respectively. Therefore, the acute toxicities of lactofen and desethyl lactofen enantiomers were enantioselective. In sediments, *S*-(+)-lactofen or *S*-(+)-desethyl lactofen was preferentially degraded, resulting in relative enrichment of the *R*-(−)-form. Lactofen and desethyl lactofen were both configurationally stable in sediment, showing no interconversion of *S*- to *R*-enantiomers or vice versa. Furthermore, the conversion of lactofen to desethyl lactofen proceeded with retention of configuration. These results for major differences in acute toxicity and degradation of the enantiomers may have some implications for better environmental and ecological risk assessment for chiral pesticides.

KEYWORDS: Enantioselective degradation; enantioselective toxicity; *Daphnia magna*; lactofen; metabolites

INTRODUCTION

Lactofen (**Figure 1**) is a selective, broad-spectrum herbicide that was introduced by PPG Industries in 1987. It is a light-dependent photoreactive herbicide (LDPH) and is in Environmental Protection Agency (EPA) toxicity class I. Lactofen is applied as a foliar spray on target weeds (*1*) and is commonly used to control broadleaf weeds in soybeans, cereal crops, potatoes, and peanuts (*1*, *2*).

Lactofen, 2-ethoxy-1-methyl-2-oxoethyl 5-[2-chloro-4-(trifluoromethyl)phenoxy]-2-nitrobenzoate, is C-chiral due to the presence of an asymmetrically substituted C-atom in the alkyl moiety. The compound consists of a pair of enantiomers with *S*-(+) and *R*-(−). The absolute configuration of the lactofen enantiomers was established by combined use of chemical correlation methods and chiral HPLC (*3*). Lactofen was marketed as the racemic product, although its herbicidal activity is almost entirely from the *S*-enantiomer (*4*). In theory, enantiomers have identical physical and chemical properties and abiotic degradation rates (*5*), whereas their individual toxicities, biological activities, and microbial degradation rates have been shown to differ (*5*−*12*). Thus, fate, exposure, and effects data for each enantiomer should be evaluated when the risk of chiral pesticides to human and wildlife populations is assessed (*13*).

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Lactofen is degraded in soil by cleavage of the ethyl ester side chains to form the preliminary diphenyl ether metabolites, desethyl lactofen and acifluorfen, and further metabolites are also formed via this primary hydrolysis product (*14*). Desethyl lactofen is a chiral compound, but acifluorfen is an achiral compound.

Lactofen is not persistent in the environment, and it will tend to be bound to sediment with a high binding potential (*15*). It is necessary to study the degradation of lactofen in sediment. Biodegradation of lactofen in different soils has been extensively studied (*1*, *15*−*18*), and many studies have demonstrated that the degradation of this chemical in soil is microbial (*1*). Enantioselectivity plays an important role in the environmental fate and ecological risks of a chiral compound, as many microbiologically mediated environmental processes are enantioselective (*19*, *20*). For example, studies have shown that enantiomers of α-HCH, metolachlor, metalaxyl, and benalaxyl behave significantly differently during biodegradation and bioaccumulation in the environment (*21*−*27*). However, enantioselectivity has seldom been studied for the environmental fate and ecotoxicological effects of lactofen. In our previous study (*28*), we demonstrated that residues from the application of *rac*-lactofen in soil were enriched with the *R*-enantiomer, indicating that the herbicidally active *S*-enantiomer is more rapidly degraded, but this study provided no information on the degradation of the two metabolites and the enantioselective toxicity of either the parent compound or its chiral hydrolysis product.

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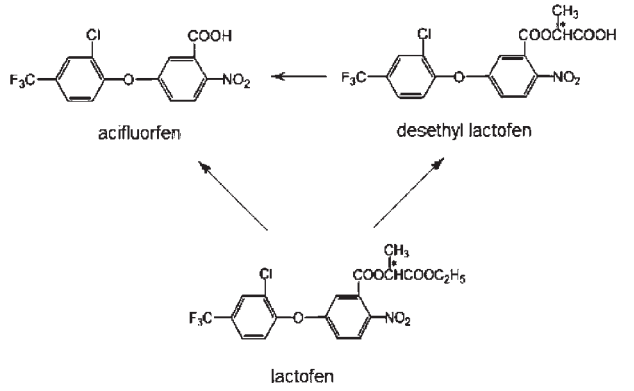

Figure 1. Structures of lactofen, desethyl lactofen, and acifluorfen.

In this study, we assayed the acute toxicity to *Daphnia magna* for lactofen and its two metabolites using 48 h static acute toxicity tests to the parent compound and its two metabolites and compared the acute toxicity with the individual enantiomers of either the parent compound or their chiral hydrolysis product. We also evaluated the enantioselectivity of lactofen and its chiral metabolite during their biodegradation in sediment.

MATERIALS AND METHODS

Chemicals and Materials. *rac*-Lactofen {2-ethoxy-1-methyl-2-oxoethyl 5-[2-chloro-4-(trifluoromethyl)phenoxy]-2-nitrobenzoate, purity ≥ 99.0%} and acifluorfen {5-[2-chloro-4-(thrifluoromethyl)phenoxy]-2-nitrobenzoic acid, purity ≥ 99.0%} were supplied by the China Ministry of Agriculture's Institute for Control of Agrochemicals. *rac*-Desethyl lactofen {1-(carboxy)ethyl 5-[2-chloro-4-(thrifluoromethyl)phenoxy]-2-nitrobenzoate, purity ≥ 98.0%} and its enantiomers (purity ≥ 99.0%, optical purity ≥ 97.5%) and a pair of enantiomers of *rac*-lactofen (purity ≥ 99.0%, optical purity ≥ 97.5%) were synthesized in our own laboratory according to the procedure described in the literature (*3*). Solvesso 100 was purchased from ExxonMobil Chemical, and other chemicals or solvents were purchased from Fisher Scientific (Fair Lawn, NJ) and were of analytical or HPLC grade.

Test Solutions for Toxicity Assays. The technical grade (TG) of lactofen is only slightly soluble [0.1 mg of active ingredient (ai)/L at 20 °C to 0.97 mg of ai/L at 25 °C] in water, and studies on aquatic animals yielded questionable results. The typical end-use product (TEP) that must be used in the aquatic studies is formulated to increase its dispersal in an aqueous solution (*14*). Therefore, an emulsifiable concentrate, comprising 24% by weight of pesticidal active ingredient compound lactofen, 26% by weight of Solvesso 100, 20% by weight of dimethyl sulfoxide, and 30% by weight of Tween-80, was used in this study. To determine the emulsion stability for pesticide, we used the following test method (*29*): First, transfer into a 100 mL graduated cylinder with 100 mL of hard water (the preparation method of hard water is as follows: dissolve anhydrous calcium chloride ($CaCl_2$; 0.304 g) and magnesium chloride hexahydrate ($MgCl_2 \cdot 6H_2O$; 0.139 g) in water and make up to 1000 mL). Second, add 0.5 mL of each emulsifiable concentrate of these compounds to each cylinder. Deliver the emulsifiable concentrate using a pipet (the delivery rate should be approximately 1 mL/s), and then stopper the graduated cylinders and invert 30 times (The expression "invert the cylinder" means that the stoppered cylinder is held by two hands, one at each end of the cylinder. The upright cylinder is turned through 180° and back to its original position without any "bounce" occurring, this operation taking approximately 2 s). Third, maintain each cylinder at 25 °C and allow the cylinders to remain undisturbed. At 1, 2, and 24 h, observe and record the emulsion quality and the milliliters of cream or oil separation, or both. As a result, no cream or oil separation was present in any emulsifiable concentrate at 1, 2, and 24 h. Thus, the formula of emulsifiable concentrate was viable. Test solutions for toxicity assays were prepared by using the emulsifiable concentrate of these compounds. Stock solution of 2000 μg of ai/mL were prepared in deionized water for *S*-(+)-, *rac*-, *R*-(−)-lactofen, *S*-(+)-, *rac*-, *R*-(−)-desethyl lactofen, and acifluorfen, respectively, and stocked at 4 °C.

Aquatic Toxicity Assays. Enantioselectivity in aquatic toxicity was evaluated through 48 h acute toxicity assays using *D. magna* as the test organism. The test organisms were obtained from a continuous culture maintained at 22 ± 1 °C in M4 culture medium (*30*) with a photoperiod of 14/10 h light/dark and a density of < 50 animals/L. Stock organisms were originally obtained from the CABET (Center for Agrochemical Biological and Environmental Technology Institute, Beijing, China). The medium was renewed three times a week, and daphnids were fed daily with the alga *Scenedesmas obliquus*, which were cultured in the laboratory using a nutrient medium. The test animals used in these experiments were juveniles aged between 6 and 24 h. Prior to the test, a sensitive test for daphnids to potassium dichromate was performed as a positive control, and the LC_{50} (24 h) value was in the range of 0.6−1.7 mg/L. The overall acute toxicity test was conducted according to the Standard Protocol (*31*). Briefly, five neonates were transferred into glass beakers filled with 20 mL of test solutions. The test solutions with the highest concentrations were prepared by spiking the stock solution to the dilution water. Subsequent dilution was made from the highest concentration to derive the lower concentration solutions. The nominal concentrations were 100.00, 50.00, 25.00, 12.50, 6.25, and 3.12 μg of ai/mL for *S*-(+)-lactofen; 20.00, 10.00, 5.00, 2.50, 1.25, and 0.62 μg of ai/mL for *rac*-lactofen; 2.00, 1.00, 0.50, 0.25, 0.12, and 0.06 μg of ai/mL for *R*-(−)-lactofen; 120.00, 60.00, 30.00, 15.00, 7.50, and 3.75 μg of ai/mL for *S*-(+)-desethyl lactofen; 75.00, 37.50, 18.75, 9.38, 4.69, and 2.34 μg if ai/mL for *rac*-desethyl lactofen; 15.00, 7.50, 3.75, 1.88, 0.94, and 0.47 μg of ai/mL for *R*-(−)-desethyl lactofen; and 60.00, 30.00, 15.00, 7.50, 3.75, and 1.88 μg of ai/mL for acifluorfen. Six test concentrations and two controls [a test water control and a carrier (a mixture of Solvesso 100, dimethyl sulfoxide, and Tween-80) control] for each compound were tested with three glass beakers per concentration and control, bringing the final totals to 24 glass breakers and 120 daphnids (three replicates for each compound per concentration and control). The test animals were not further fed and were incubated at 22 ± 1 °C for 48 h. Mortality of daphnids was observed after incubation for 48 h. The LC_{50} values were determined from the survival data using a probit equation with SPSS 16.0. Tests were considered to be valid if control mortality was < 10%.

Sediment Samples. A sediment was used in the laboratory incubation experiments. The sediment was taken from a location along the Liao River and collected from the 0−10 cm surface layer. No lactofen or any of its metabolites were found at detectable levels in the sediment. After collection, the sediment samples were air-dried at room temperature, homogenized, passed through a 2 mm sieve, and kept in the dark until used within a few days. Physicochemical properties of the sediment were as follows: organic matter, 3.02 ± 0.15%; clay, 2.71 ± 0.11%; sand, 72.60 ± 1.42%; silt, 24.69 ± 1.15%; pH, 7.2 ± 0.2.

Incubation in Sediment. Separate incubation experiments were carried out with the racemic and with the pure *S*-(+)- and *R*-(−)-compounds, respectively, using 250 mL Erlenmeyer flasks covered with aluminum foil. Approximately 20 g of sediment (dry weight equivalent) was spiked with 100 μg of *rac*-, *S*-(+)-, or *R*-(−)-lactofen and desethyl lactofen, respectively. A portion of the sediment (5 g) was first spiked with 100 μL of acetone and stirred for 5 min. The spiked sediment was then allowed to air-dry for 10 min, before the remaining sediment (15 g) was added and mixed thoroughly for another 10 min, yielding a fortification level of 5 μg/g (experiments SE1, SE2, and SE3, respectively). Adequate amounts of distilled water were added to each sample to immerse the sediment and form a 0.5 cm water layer. All of the samples were incubated at 25 °C in the dark. Samples were checked regularly for water content by weighing and were frequently mixed for aeration. Similar experiments were carried out with *rac*-, *S*-(+)-, and *R*-(−)-desethyl lactofen (experiments SE4, SE5, and SE6, respectively). Three replicate samples were removed from each treatment at different time intervals for further sample treatment and immediately transferred into a freezer (−20 °C) to stop degradation. Triplicate samples were taken immediately after fortification and mixing ($t = 0$) to determine the recovery and reproducibility of extraction in the sediment (see below). Blank determinations of the sediment prior to fortification revealed no lactofen, desethyl lactofen, or acifluorfen present.

Extraction of Lactofen, Desethyl Lactofen, and Acifluorfen from Sediment. Samples were first thawed at room temperature. Then 5 g samples (dry weight basis) were then removed and placed into 50 mL polypropylene centrifuge tubes. For extraction, ethyl acetate (20 mL) was added to each tube. The tube was stirred for 3 min on a vortex mixer,

Table 1. Calculated LC_{50} Values for Lactofen, Desethyl Lactofen, and Acifluorfen

	S-(+)-enantiomer			*rac*-compound			*R*-(−)-enantiomer		
compound	LC_{50} (μg/mL)	$R^{2\,a}$	p^b	LC_{50} (μg/mL)	R^2	p	LC_{50} (μg/mL)	R^2	p
lactofen	17.689	0.97	0.0003	4.308	0.99	0.0003	0.378	0.96	0.0005
desethyl lactofen	21.327	0.97	0.002	13.684	0.94	0.001	2.568	0.93	0.008
acifluorfen[c]				20.027	0.96	0.0005			

[a] Correlation coefficient. [b] Probability (associated with the *t* test). A p value < 0.05 indicates that the correlation of the linear equation is significant, and it is calculated using a Linear Regression Model of SPSS 16.0. [c] Acifluorfen is an achiral compound.

ultrasonic for 10 min, and then centrifuged at 4000 rpm for 5 min. The same extraction step was repeated once with 20 mL of ethyl acetate, and the solvent extracts were filtered through 5 g of anhydrous sodium sulfate for dehydration. The combined extract was evaporated to near dryness on a vacuum rotary evaporator at 45 °C and reconstituted in 1.0 mL of hexane. An aliquot (20 μL) was injected into the HPLC.

Enantioselective HPLC Analysis. Chromatography in this study was performed using an Agilent 1200 series HPLC (Agilent Technology) equipped with a G1322A degasser, a G1311A quat pump, a G1329A ALS, and a G1314B VWD. The signal was received and processed by Agilent Chemstation software. Cellulose-tri(3,5-dimethylphenylcarbamate)-based chiral stationary phase (CDMPC-CSP, provided by the Department of Applied chemistry, CAU, Beijing) was used to separate lactofen and its two major metabolites desethyl lactofen and acifluorfen in this study. The CSP was prepared according to the procedure described by Zhou et al. (*32*). CSP was packed into a 250 × 4.6 mm (i.d.) or 150 × 4.6 mm (i.d.) stainless steel column. Although CDMPC-CSP has been widely used and has already separated many pesticides including lactofen (*33*), to date no method based on the use of CDMPC-CSP has been described in the literature to simultaneously determine the acifluorfen and the lactofen and desethyl lactofen enantiomers. Thus, in the present work, this column was employed in the development of a new method for the simultaneous determination of the lactofen and its two major degradates in sediment samples. A simultaneous baseline separation of these compounds was obtained with a mobile phase of *n*-hexane/2-propanol/TFA (98:2:0.1, v/v/v) with a flow rate of 1.0 mL/min. Trifluoroacetic acid (TFA) was a mobile phase acidic additive, and it was added into the mobile phase for the determination of desethyl lactofen and acifluorfen. Chromatographic separation was conducted at a temperature of 15 °C and UV detection at 230 nm. For *S*-(+)- and *R*-(−)-lactofen, acifluorfen, and *S*-(+)- and *R*-(−)-desethyl lactofen, the elution order was established by comparison with the retention time of the enantiopure enantiomers of the lactofen and desethyl lactofen and the standard of acifluorfen. No enantiomerization had been observed for lactofen or desethyl lactofen under these analytical conditions. Concentrations were determined by using peak area, assuming the same response factor for enantiomers originating from the same compound.

The three compounds gave five separate peaks with retention times of 11.7 min (peak I), 18.9 min (peak II), 39.9 min (peak III), 49.9 min (peak IV), and 57.2 min (peak V) under the described conditions (**Figure 1**). The five peaks were as follow: *S*-(+)- and *R*-(−)-lactofen, acifluorfen, and *S*-(+)- and *R*-(−)-desethyl lactofen.

Recoveries of these compounds were determined immediately after fortification. Experiments showed that the recovery of the above procedure was > 92% for lactofen, desethyl lactofen, or acifluorfen in sediment. The limit of quantification (LOQ) for these compounds in all samples was found to be 0.3 μg/g on the basis of an acceptable RSD of 20%. The limit of detection (LOD) for these compounds, defined as the concentration with a signal-to-noise ratio of 3, was 0.1 μg/g for all samples.

RESULTS AND DISCUSSION

Acute Toxicity Assay. The acute aquatic toxicity was measured for lactofen and its two metabolites using *D. magna* as the test animals (**Table 1**). On the basis of the LC_{50} values of these compounds, the order of toxicity potency to *D. magna* at 48 h was lactofen > desethyl lactofen > acifluorfen. In **Table 1**, we also list the LC_{50} values for individual enantiomers of lactofen and desethyl lactofen. Significant differences were observed in LC_{50} between the individual enantiomers. For the two enantiomers of lactofen, the *R*-(−)-enantiomer exhibited acute toxicity to *D. magna* about 47 times that for the herbicidally active *S*-(+)-enantiomer, and for the two enantiomers of desethyl lactofen, the *R*-(−)-enantiomer was about 8 times more toxic than the *S*-(+)-enantiomer. It is common for the acute aquatic toxicity of chiral pesticides to be associated primarily with only one of the enantiomers. A 50-fold difference response for *D. magna* was observed for isomers of isocarbophos (*34*), and the (+)-enantiomer of fipronil is more acutely toxic to *Ceriodaphnia dubia* than the (−)-enantiomer, whereas the racemic mixture displayed intermediate toxicity (*35*).

The significant enantioselectivity strongly suggests that the aquatic toxicity of enantiomers of lactofen and desethyl lactofen

Table 2. Enantiomer Ratio (ER = *S*/*R*, Mean ± SD) of (*S*,*R*)-Lactofen and (*S*,*R*)-Desethyl Lactofen Metabolite in Experiment SE1

	ER			ER	
days after treatment	*S*,*R*-lactofen	*S*,*R*-desethyl lactofen	days after treatment	*S*,*R*-lactofen	*S*,*R*-desethyl lactofen
0	1.02 ± 0.01		7	0.10 ± 0.03	0.54 ± 0.08
0.25	0.97 ± 0.01		9	0.08 ± 0.01	0.31 ± 0.08
0.75	0.91 ± 0.01	2.33 ± 0.03	12		0.25 ± 0.07
1	0.88 ± 0.05	3.01 ± 0.04	15		0.11 ± 0.07
2	0.40 ± 0.05	4.23 ± 0.04	23		0.05 ± 0.06
3	0.23 ± 0.01	2.79 ± 0.08	25		0.04 ± 0.10
4	0.16 ± 0.01	1.77 ± 0.02			

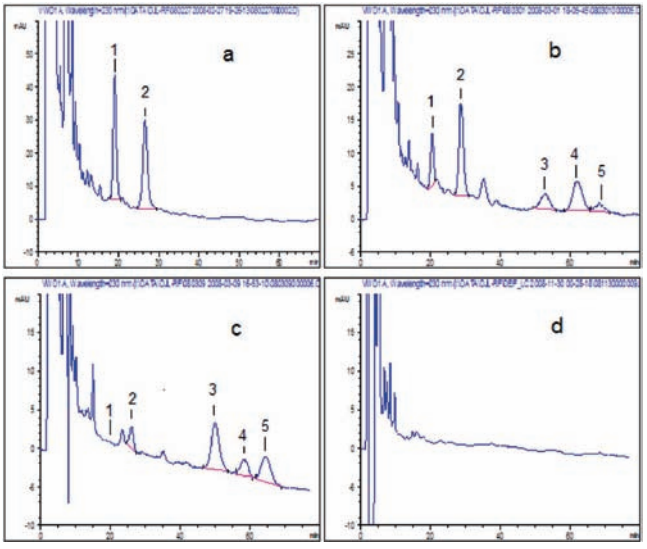

Figure 2. Chromatograms showing elution of lactofen and its two metabolites (desethyl lactofen and acifluorfen) from the incubation of *rac*-lactofen in sediment (expt SE1) after (**a**) 0, (**b**) 2, and (**c**) 7 days. Panel **d** shows the chromatogram of blank sediment. Peaks: 1, (*S*)-lactofen; 2, (*R*)-lactofen; 3, acifluorfen; 4, (*S*)-desethyl lactofen; 5, (*R*)-desethyl lactofen. *n*-hexane:2-propanol:TFA = 98:2:0.1, flow rate = 1.0 mL/min, 250 mm column.

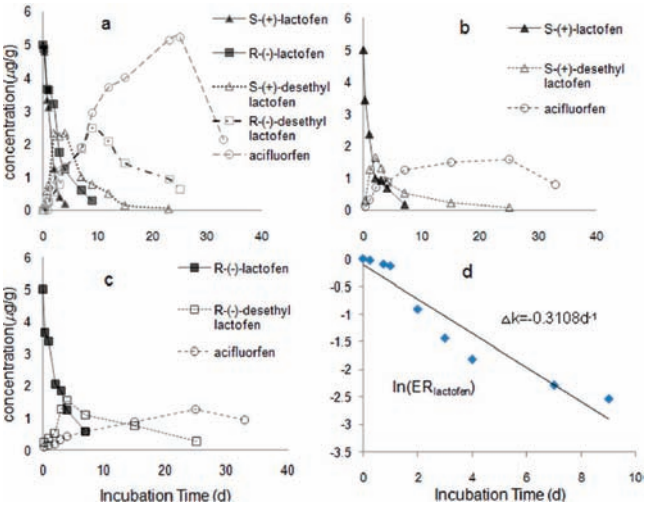

Figure 3. Degradation of lactofen in sediment experiments SE1, SE2, and SE3 with *rac*-, *S*-(+)-, *R*-(−)-lactofen, respectively: (**a**) degradation of *rac*-lactofen showing faster degradation of the *S*-(+)-lactofen (expt SE1); (**b**) degradation of *S*-(+)-lactofen (expt SE2); (**c**) degradation of *R*-(−)-lactofen (expt SE3; note the concurrent formation and degradation of acifluorfen and desethyl lactofen arising from lactofen); (**d**) plot of ln(ER) from experiment SE1 (incubation of *rac*-lactofen) versus incubation time showing a linear relationship.

Table 3. Rate Constants for the Degradation of Lactofen and the Chiral Metabolite in Sediment Experiments SE1−SE6

	rate constants (day^{-1})			
expt (incubated compound)	*S*-(+)-lactofen	*R*-(−)-lactofen	*S*-(+)-desethyl lactofen	*R*-(−)-desethyl lactofen
SE1 (*rac*-lactofen)	0.627	0.314		
SE2 [*S*-(+)-lactofen]	0.457			
SE3 [*R*-(−)-lactofen]		0.294		
SE4 (*rac*-desethyl lactofen)			1.404	0.217
SE5 [*S*-(+)-desethyl lactofen]			1.417	
SE6 [*R*-(−)-desethyl lactofen]				0.262

should be considered individually. Currently, the environmental risk of most chiral pesticides is evaluated on the basis of their racemates, but enantioselective toxicity assessment of currently used pesticides to nontarget organisms has been limited (*11*, *35*). In theory, enantiomers often exhibit differential toxicity. One enantiomer of a pesticide may have an adverse effect on some nontarget species, whereas the other enantiomer may not have such an effect. The toxicity of lactofen to *D. magna* is predicted to be acute but might be underestimated by 4-fold if only the *S*-(+)-enantiomer is present. However, it might cause an 11-fold overestimated toxicity if only the *R*-(−)-enantiomer is present in the residue. Therefore, the present data available on the toxicity of the racemic mixtures of these chiral pesticides are not reliable, and the study of enantioselective toxicity of chiral pesticides has been very popular for environmental safety issues (*11*, *34*, *36*).

Enantioselective Degradation of Lactofen in Sediment. In general, the residues of both enantiomers of lactofen decreased with time in experiments SE1, SE2, and SE3, when the racemic and the enantiopure *S*-(+)- and *R*-(−)-compounds were incubated. The data of the residual concentrations of the two enantiomers were

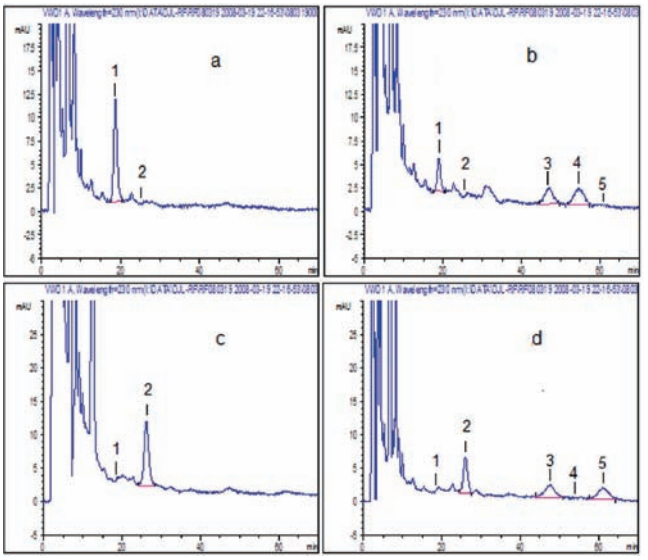

Figure 4. Chromatograms showing elution of lactofen and its two metabolites (desethyl lactofen and acifluorfen) from the incubation of *S*-(+)-lactofen in sediment (expt SE2) after (**a**) 0 and (**b**) 2 days and from *R*-(−)-lactofen (expt SE3) after (**c**) 0 and (**d**) 3 days. Note the absence of enenantiomerization with respect to *S*-(+)- and (*R*)-(−)-lactofen and formation of the respective desethyl lactofen with retention of configuration (panels **b** and **d**). Peaks: 1, (*S*)-lactofen; 2, (*R*)-lactofen; 3, acifluorfen; 4, (*S*)-desethyl lactofen; 5, (*R*)-desethyl lactofen. *n*-hexane/2-propanol/TFA = 98:2:0.1, flow rate = 1.0 mL/min, 250 mm column.

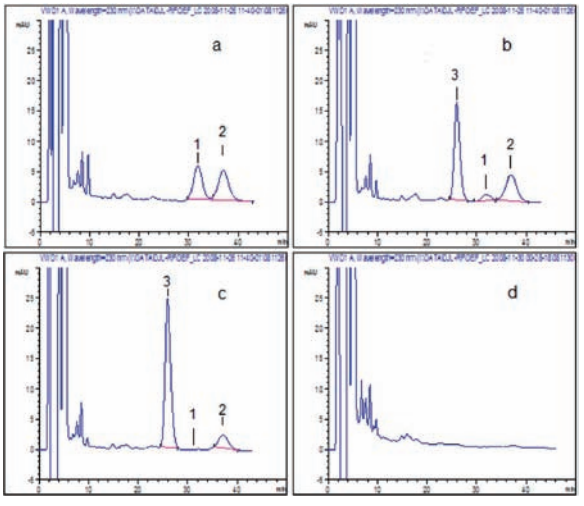

Figure 5. Chromatograms showing elution of *S*-(+)- and *R*-(−)-desethyl lactofen from the incubation of *rac*-desethyl lactofen in sediment (expt SE4) after (**a**) 0, (**b**) 1, and (**c**) 3 days. Panel **d** shows the chromatogram of blank sediment. Peaks: 1, (*S*)-desethyl lactofen; 2, (*R*)-desethyl lactofen; 3, acifluorfen. *n*-hexane/2-propanol/TFA = 98:2:0.1, flow rate = 0.5 mL/min, 150 mm column.

used for estimating the enantiomeric ratio (ER) values during these experiments. The ER was defined as the peak aera of the first eluting *S*-(+)-enantiomer divided by the peak aera of the later eluting *R*-(−)-enantiomer (*5*). The ER of lactofen consistently decreased with time in experiment SE1 (**Table 2**). The initial ER for lactofen in this experiment was 1.02, but it gradually decreased to 0.08 after 9 days of incubation. This steady decrease of *S*-(+)-enantiomer concentration in comparison to the *R*-(−)-enantiomer could indicate a preferential biotransformation of the

S-(+)-enantiomer by the sediment's enzymatic systems when this isomer is applied as a racemic mixture. The selective degradation resulted in relative enrichment of the *R*-(−)-enantiomer that was more toxic to *D. magna* than the *S*-(+)-enantiomer, as shown in **Figure 2a**−**c**. A *t* test between the means of the values of spiked sediment and ER = 1.0 yielded a *p* value of 0.006. Degradation thus showed an enantioselectivity in the same sense as in a previous study (*28*).

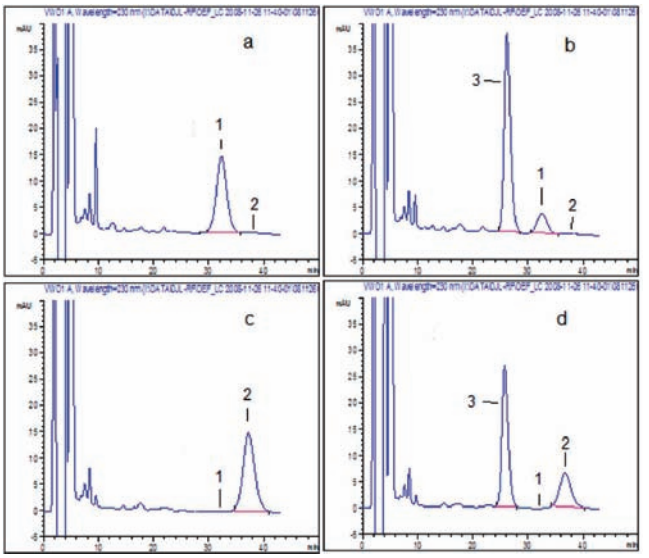

Figure 6. Chromatograms showing elution of desethyl lactofen and acifluorfen from the incubation of *S*-(+)-desethyl lactofen in sediment (expt SE5) after (**a**) 0 and (**b**) 2 days and from *R*-(−)-desethyl lactofen (expt SE6) after (**c**) 0 and (**d**) 3 days. Note the absence of enantiomerization with respect to *S*-(+)- and (*R*)-(−)-lactofen and formation of the respective desethyl lactofen with retention of configuration (panels **b** and **d**). Peaks: 1, (*S*)-desethyl lactofen; 2, (*R*)-desethyl lactofen; 3, acifluorfen. *n*-hexane/2-propanol/TFA = 98:2:0.1, flow rate = 0.5 mL/min, 150 mm column.

Data from experiment SE1 were plotted with *rac*-lactofen, and those from experiments SE2 and SE3 with *S*-(+)- and *R*-(−)-lactofen show the data fit a first-order decay model with a R^2 ranging from 0.97 to 0.99 (**Figure 3a**−**c**). Rate constants are listed in **Table 3**.

Assuming that degradation of enantiomers followed first-order kinetics with a rate constant of k_S for the *S*-(+)-enantiomer and a rate constant of k_R for the *R*-(−)-enantiomer, ER may be expressed as a function of time (*t*) in the relationship (*24*)

$$\mathrm{ER} = [\mathrm{S}]/[\mathrm{R}] = \mathrm{ER}_0 \times e^{(k_R - k_S)} = \mathrm{ER}_0 \times e^{\Delta k} \quad (1)$$

where ER_0 is the initial ER valure (e.g., $\mathrm{ER}_0 = 1.02$ for racemic lactofen), [S] is the concentration of the *S*-(+)-enantiomer at time *t*, [R] is the concentration of the *R*-(−)-enantiomer at time *t*, and Δk is the difference between k_R and k_S, which reflects the rate at which ER deviates from ER_0 over time. The above relationship can be further expressed in a linear form after logarithmic transformation of ER:

$$\ln(\mathrm{ER}) = \ln(\mathrm{ER}_0) + \Delta k \quad (2)$$

Equation **2** indicates a linear relationship between ln(ER) and *t*. A plot of ln(ER) versus *t* can be used to determine the rate difference Δk and thus enantioselectivity.

The rates k_S and k_R for *rac*-lactofen from experiment SE1 were 0.627 and 0.314 day^{-1}, respectively, and the rate k_S for *S*-(+)-lactofen from experiment SE2 and the rate k_R for *R*-(−)-lactofen from experiment SE3 were 0.457 and 0.294 day^{-1}, respectively (**Table 3**). The reason for the difference of k_S in experiments SE1 and SE2 and of k_R in experiments SE1 and SE3 is unknown. Apparently, the conditions in these experiments were not exactly the same, and it is possible that there is some influence of one enantiomer [*S*-(+)-] on the degradation of the other [*R*-(−)-], and vice versa, such as when the racemic compound was incubated.

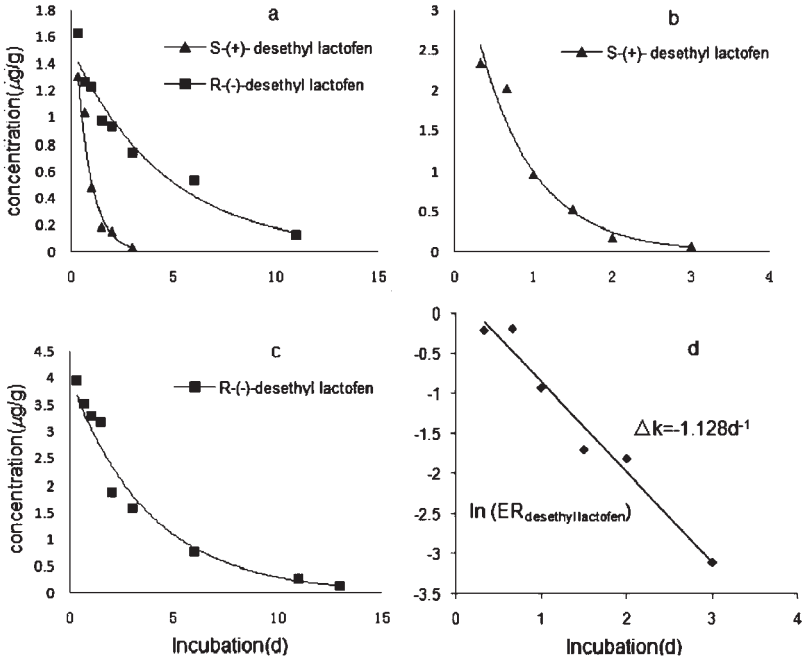

Figure 7. Degradation of desethyl lactofen in sediment experiments SE4, SE5, and SE6 with *rac*-, *S*-(+)-, *R*-(−)-desethyl lactofen, respectively: (**a**) degradation of *rac*-desethyl lactofen showing faster degradation of the *S*-(+)-desethyl lactofen (expt SE4); (**b**) degradation of the *S*-(+)-desethyl lactofen (expt SE5); (**c**) degradation of the *R*-(−)-desethyl lactofen (expt SE6). (**d**) Plot of ln(ER) from expt SE4 (incubation of *rac*-desethyl lactofen) versus incubation time showing a linear relationship .

The ER values of lactofen from experiment SE1 showed a continuous decrease from the initial value of 1.02 (racemic) to 0.08 (composition [S] < [R], **Table 2**). Fitting the measured ER values in **Figure 3d** to incubation time t using eq **2** yielded a linear form for lactofen (incubation of the *rac*-lactofen). The rate difference Δk calculated from this plot is -0.311 day^{-1}, which is in agreement with above data ($k_R - k_S = -0.313$ day^{-1}). The half-lives of the two enantiomers of lactofen estimated from the data of experiment SE1 are 1.1 and 2.2 days, respectively. The half-lives of individual enantiomers estimated from the data of experiments SE2 and SE3 are 1.5 and 2.4 days, respectively.

ES, which is a measure of enantioselectivity, was defined as $ES = (k_S - k_R)/(k_S + k_R)$ in a previous study (*37*). Positive values ($0 < ES \leq 1$) indicate a more rapid degradation of the (*S*)-enantiomer, whereas negative values ($-1 < ES \leq 0$) indicate a more rapid dissipation of the (*R*)-enantiomer. At an ES value of 0, dissipation is not enantioselective, and at an ES value of 1, degradation is fully enantioselective. In experiment SE1, the ES value was 0.332, which suggested that the degradation of lactofen in sediment is enantioselective and that *S*-(+)-lactofen degraded more rapidly than *R*-(−)-lactofen.

The incubation experiments SE2 and SE3 spiked with enantiopure *S*-(+)-lactofen, respectively, revealed no formation of the *S*-form from *R*-(−)-lactofen throughout the incubation time and vice versa (**Figure 4**). This result indicated lactofen configuration was stable in sediment.

Formation of the Two Metabolites of Lactofen. Desethyl lactofen and acifluorfen are the main breakdown products for lactofen in soil, arising from ester hydrolysis (*15*). Therefore, the two metabolites were detected in sediment in this study. The identities of the two metabolites of lactofen were confirmed by comparing the residue retention time with authentic standards. In **Figure 4**, the chromatograms document the high enantiomeric purity of *S*-(+)- and *R*-(−)-lactofen prior to incubation and indicate degradation of *S*-(+)- and *R*-(−)-lactofen and the concurrent formation of acifluorfen and desethyl lactofen. The chromatograms in **Figures 2** and **4** and the data plotted in **Figure 3a−c** show that the decrease in the unchanged parent compound was accompanied by an increase in the concentration of the two metabolites. From *rac*-lactofen (expt SE1), the formation of a chiral compound of desethyl lactofen is observed, whereas from *S*-(+)- and *R*-(−)-lactofen (expts SE2 and SE3, respectively) only the formation of the respective desethyl lactofen is observed. These data indicate that ester cleavage of *S*-(+)- and *R*-(−)-lactofen proceeded with retention of configuration. During the initial day of incubation, the amounts of acifluorfen and desethyl lactofen detected were about 0.26 and 1.04 μg/g, respectively, in sediment samples spiked with *rac*-lactofen. After further incubation, the concentration of acifluorfen reached a maximum of 5.22 μg/g at day 25 and then decreased to 2.14 μg/g at the end of incubation. However, the concentration of desethyl lactofen reached a maximum of 2.84 μg/g at day 9 and then decreased to 0.26 μg/g at day 25.

Enantioselective Degradation of the Chiral Metabolite of Desethyl Lactofen in Sediment. In **Figures 5** and **6**, chromatograms show the elution of desethyl lactofen in the sediment samples from experiments SE4, SE5, and SE6. The chromatogram from the incubation of the *rac*-desethyl lactofen (expt SE4, **Figure 5a−c**) illustrated the more rapid degradation of the first-eluted *S*-(+)-enantiomer, leading to residues enriched in the *R*-(−)-enantiomer, and it also indicated that desethyl lactofen degraded to acifluorfen. This result is consistent with a previous study, in which lactofen degrades to desethyl lactofen and acifluorfen in soil, and desethyl lactofen will also degrade to acifluorfen (*14*). The chromatograms from the incubation of *S*-(+)- and *R*-(−)-desethyl lactofen (expts SE5 and SE6, respectively) reavealed degradation of the compounds but no formation to the respective antipodes (**Figure 6c,d**). Experiments SE5 and SE6 indicated negligible (< 1%) conversion of *S*-(+)- to *R*-(−)-desethyl lactofen during incubation and vice versa. *S*-(+)- and *R*-(−)-desethyl lactofen are thus both configurationally stable, and enantiomerization is thus unimportant compared to degradation.

In **Figure 7a−c**, we plot the data from experiments SE4, SE5, and SE6 with *rac*-, *S*-(+)-, *R*-(−)-desethyl lactofen, respectively, as normalized concentrations versus time. The plots show significant degradation of both enantiomers but with a clearly more rapid degradation of *S*-(+)- than *R*-(−)-desethyl lactofen. A t test between the means of the values of spiked sediment and ER = 1.0 yielded a p value of 0.008, and this indicated that the degradation of desethyl lactofen in sediment was enantioselective. The plots also include simulated data using the rates listed in **Table 3**. Reasonable fits were obtained with $K_{S\text{-enantiomer}} = 1.404$ and 1.417 day^{-1} in experiments SE4 and SE5, respectively, and with $K_{R\text{-enantiomer}} = 0.217$ and 0.262 day^{-1} in experiments SE4 and SE6, respectively. The respective rates thus showed reasonable agreement in these experiments. The ERs from the incubation of *rac*-desethyl lactofen (expt SE4) showed a continuous decrease with time from the initial value of (racemic) to about 0.04, indicating eventual compositions $R > S$. A plot of ln(ER) verus time was linear. The slope indicated a rate difference Δk ($k_R - k_S$) of -1.128 day^{-1} (**Figure 7d**), which is in reasonable agreement with the above data ($k_R - k_S = -1.187$ day^{-1}). The fact that the two enantiomers of desethyl lactfen degraded at different rates ($k_S > k_R$) and the ES value of 0.73 calculated from these rates suggest that degradation is clearly biotic (*24*).

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