JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

J. Agric. Food Chem. **2008,** *56,* 2139–2146 **2139**

Enantioselective Degradation and Ecotoxicity of the Chiral Herbicide Diclofop in Three Freshwater Alga Cultures

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Aryloxyphenoxypropanoates are a class of chiral herbicides. They have a pair of enantiomers, only the *R*(+) form of which is herbicidally active. Diclofop, the model compound of these herbicides, is commercialized as the racemate of the ester form, diclofop-methyl, consisting of a 1:1 mixture of the enantiomers. This study evaluated the enantioselectivity in aquatic toxicity and biodegradation of diclofop and diclofop-methyl. The herbicidally inactive *S*(−) enantiomers of both diclofop-methyl and diclofop were similar to or higher than the corresponding *R*(+) forms in toxicity to algae, depending on specific species. Although no enantiomeric conversion occurred for diclofop-methyl and diclofop, the difference in the enantioselective degradation of these herbicides observed in algae cultures suggested that their application forms were an important factor determining their enantioselective environmental behavior. The cell permeability and heat treatment of algae revealed that the enantioselective degradation of diclofop in algae cultures was governed primarily by the facilitated uptake by algae, whereas the enantioselective toxicity was primarily governed by the passive uptake. These results suggested that the acute toxicity test such as the 96 h EC_{50} was insufficient to assess the ecological risk of chiral pesticides because of the differential degradation as well as possibly differential action sites of enantiomers. From this study, it was concluded that the enantioselective degradation and toxicity of chiral herbicides may result in their ecotoxicological effects being difficult to predict and that specific attention should thus be paid to currently used racemic pesticides as less active or inactive enantiomers may pose higher ecological risks.

KEYWORDS: Enantioselectivity; degradation; diclofop; freshwater alga

INTRODUCTION

About 25% of pesticides currently in use are chiral, and this ratio is increasing as more structurally complex compounds are introduced into use (*1*). Chiral pesticides consist of enantiomers (*1, 2*), which have identical physicochemical properties, and are mostly commercialized as racemates, that is, mixtures of enantiomers. Enantiomers may selectively interact with biological systems that are usually enantioselective and behave as drastically different compounds (*1, 3*). Apart from different biological activities to target biological objects, some chiral pesticides have enantioselective toxicity, such as cytotoxicity, endocrine disruption, and carcinogensis, to nontarget biology (*4–6*). Hegeman and Laane (*7*) and Müller and Kohler (*8*) have reviewed that chiral pesticides selectively enrich and degrade in natural environments with various enantiomer ratios (ER). Enantioselectivity in these processes is predicted to result in ecotoxicological effects that cannot be predicted from our existing knowledge. The enantioselectivity in environmental safety of current chiral pesticides should thus be taken into account in risk assessment and regulatory decisions (*9*).

A wide variety of 2-aryloxyphenoxypropanoic (APP) acids and their esters, consisting of a pair of enantiomers, have been developed as commercial herbicides and plant growth regulators after the release of the model compound, diclofop, by Hoeschst Co. (*10*). They inhibit acetyl-CoA carboxylase in chloroplasts and thus exhibit herbicidal activities. In general, the *R*(+) enantiomers of these herbicides are herbicidally active and are approximately twice as active as respective racemic mixtures (*3*). The commercial forms of APP herbicides are the esters of parent acids to enhance their uptake into plants (*11, 12*). Conversion of esters to free acids occurs rapidly in vivo (*12, 13*), which is the form inhibiting the enzyme in vivo (*12, 14*). Although several APP herbicides such as fluazifop and haloxyfop have been converted from their racemic mixtures to their eutomers, diclofop-methyl, the commercial form of diclofop,

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10.1021/jf0728855 CCC: $40.75
Published on Web 03/05/2008

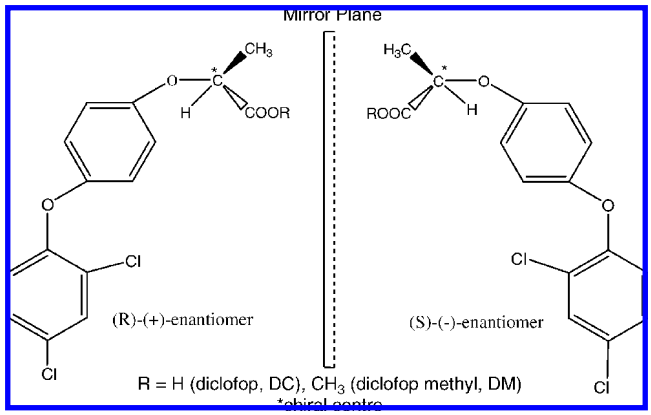

Figure 1. Chemical structures of chiral herbicide diclofop (DC) and its ester form diclofop-methyl (DM).

is still in wide use as the racemate (*15, 16*), which is known to undergo rapid hydrolysis to diclofop (*17*). Most studies on the environmental behavior of diclofop-methyl and diclofop considered their enantiomers as a single compound (*15, 18–20*). The chemical structures of the two chiral herbicides are shown in **Figure 1**. The enantioselectivity in their environmental behavior and toxicity in aquatic systems is almost unknown (*21*).

In this study, the racemates and individual enantiomers of diclofop and diclofop-methyl were used to investigate their enantioselective interactions with three freshwater algae, *Chlorella pyrenoidosa*, *Chlorella vulgaris*, and *Scenedesmus obliquus*, in terms of their toxicity and degradation. Measuring algal cell permeability was helpful for further confirmation of enantioselective interactions between diclofop and algae. Heat treatment, initially inhibiting algal growth but not killing them, was performed to evaluate the role of facilitated uptake by algae in the enantioselective interactions. Besides, the configuration stability of diclofop was detected to fully assess the enantioselective interactions.

MATERIALS AND METHODS

Chemicals and Materials. Rac-diclofop-methyl {(±)-methyl 2-[4-(2,4-dichlorophenoxy)phenoxy] propionate, Rac-DM, purity ≥ 98.0% after purification} and 4-(2,4-dichlorophenoxy)phenol (DP, purity ≥ 96.0%) were supplied by Ningbo Jiema Chemical Engineering Co., Ltd. Rac-diclofop {(±)2-[4-(2,4-dichlorophenoxy)phenoxy] propionic acid, Rac-DC, purity ≥ 98.0%} and its enantiomers (purity ≥ 99.0%, optical purity ≥ 94.0% and a pair of enantiomers of Rac-DM (purity ≥ 99.0%, optical purity ≥ 94.0%) were synthesized in our own laboratory. Fluorescein diacetate (FDA) and reagent grade fluorescein were purchased from Sigma and Amresco, respectively.

Alga Cultures. The microalgae, *C. pyrenoidosa*, *C. vulgaris*, and *S. obliquus*, were obtained from Institute of Hydrobiology of Chinese Academy of Sciences. The algae, commonly used for toxicity testing, were maintained in algal growth media (HB IV) at 24 ± 0.5 °C in an incubator under continuous illumination of 3000–4000 lx. Each culture was shaken four times per day to prevent settling of the alga and ensure its optimal growth. The algae were periodically inoculated into fresh media to keep cells at the logarithmic growth phase.

Instrumentation. Diclofop-methyl and its transformation products were resolved on a Shimadzu LC-10ADvp high-performance liquid chromatograph equipped with an SPD-10Avp photodiode array detector, a SIL-10ADvp automatic injector, two HPLC pumps, a CTO-10Avp oven, and the Class-vp data system. The HPLC conditions were as follows: Kromstar C18 column, 4.6 mm × 250 mm; flow rate, 0.8 mL/min; mobile phase, methanol/water (80:20, v/v); detection wavelength, 240 nm; injection volume, 10 μL; oven temperature, 40 °C.

Aquatic Toxicity Tests. The algal growth inhibition test was carried out according to updated OECD guideline 201 for freshwater algal and cyanobacterial growth inhibition test. The algae were taken from the exponentially growing preculture and inoculated into the test solution in flasks at the density of 7.4×10^5 cell/mL. DCs, that is, the racemate and enantiomers of diclofop, ranged from 0.10 to 30 mg/L in the solutions. Three replicates were made for the test. All flasks were repositioned daily within the cultural chamber to minimize possible spatial differences in illumination and temperature. According to the linear equation between direct cell counts and optical density at 680 nm (OD_{680}), inhibition of algal growth relative to control was determined by measuring OD_{680} using a Shimadzu UVPC-2401 spectrophotometer. The EC_{50} values were calculated as the concentration of chemicals inhibiting the growth of algae by 50% that of the control.

Interactions between Chiral Herbicides and Algae. The alga was inoculated into flasks containing fresh growth medium. All flasks were spiked with appropriate herbicide stock solutions in acetone to obtain an initial concentration of 5.0 mg/L (racemic herbicides and their enantiomers), respectively. Uninoculated medium served as a control. All flasks were sealed with four layers of pledges. Both uninoculated and inoculated media were incubated under the same conditions. All tests were conducted in triplicate. At regular intervals, algal medium was transferred from flasks to a cuvette and the algal growth was determined spectrophotometrically. Two milliliters of algal medium in the cuvette was centrifuged at 4000 rpm (about 2970*g*) for 10 min, and the supernatant was analyzed directly by HPLC to monitor the herbicides and their transformation products.

To investigate the effect of herbicide concentration on their enantioselective degradation, three levels of DCs (2.0, 5.0, and 10.0 mg/L) were chosen in *C. vulgaris* culture. Chiral stability needs to be taken into account during degradation. To resolve the enantiomers of both diclofop and its ester form, the supernatant of algal medium was pretreated and measured via the same procedure as reported by Lin et al. (*22*). In brief, the supernatant was extracted three times by ethyl ether. The combined ether extracts were dried and evaporated. After the addition of 1.0 mL of *n*-hexane/IPA (90:10, v/v), the samples were sealed in vials and stored at −20 °C prior to HPLC analysis. The HPLC conditions were as follows: Chiracel OJ-H column, 4.6 mm × 250 mm; flow rate, 0.5 mL/min; mobile phase, *n*-hexane/IPA/acetic acid (90:10:0.2, v/v/v); fluorescence detector, excitation wavelength, 298 nm, and emission wavelength, 350 nm; injection volume, 20 μL; oven temperature, 20 °C.

Permeability of Algal Cells. In vivo permeability experiments with three algae were carried out according to the method of Vigneault et al. (*23*). In brief, cells were transferred to obtain a final cell density of 7.4×10^5 cells/mL. Fresh FDA solution was prepared daily in acetone, and small aliquots were added to obtain a final FDA concentration of 3.0×10^{-6} mol/L. Algal cell permeability was measured by monitoring nonfluorescent FDA intracellular hydrolysis by nonspecific intracellular esterases to produce the fluorescent molecule fluorescein. Hydrolysis was monitored during the initial 10 min using a Hitachi F-2500 fluorescence spectrophotometer. Instrument parameters were labeled as follows: measure type, time scan; excitation wavelength, 485.0 nm; emission wavelength, 530.0 nm; excitation slit, 10.0 nm; emission slit, 10.0 nm; PMT voltage, 400 V. In the absence of cells, no hydrolysis of FDA was measured. All tests were conducted in triplicate. Initial hydrolysis rates for FDA, representing algal cell permeability, were obtained from the slope of the linear regression of fluorescence intensity versus time ($r^2 \geq 0.95$), that is, the mean rate of conversion of FDA to fluorescein. Relative cell permeability was expressed as the ratio of the slope with DCs to the control. To observe the effects of DCs, the algal permeability of the control was set at 1.0.

To minimize the effect of temperature variation on fluorescence measurement, the algal medium incubating at 24 °C was taken from the chamber 2 h in advance and kept out of light at room temperature (18−20 °C). During the 7 h measuring period, the algal growth in terms of OD_{680} and cell permeability increased by less than 15 and 12%, respectively. The increase in algal permeability caused by its self-reproduction was thus neglected.

Heat Treatment of Algal Cells. Temperature is one of the most important environmental factors affecting algal growth. Photosynthetic green algae are known to be thermophilic when they grow at constant temperatures as high as about 50 °C (*24–26*). Studies have revealed that algae can survive at temperatures as high as about 70 °C, although

Table 1. 96 h EC_{50} Values of Rac-Diclofop (Rac-DC) and Its Enantiomers

alga	compd	regression eq	*R*	*P*	EC_{50} (mg/L)
Chlorella pyrenoidosa	Rac-DC	$Y = 0.0378 + 0.2115$ Ln C	0.99	0.00	8.89 ± 0.75
	S-DC	$Y = -0.1350 + 0.2929$ Ln C	0.94	0.02	8.74 ± 0.56
	R-DC	$Y = 0.0113 + 0.2149$ Ln C	0.97	0.00	9.72 ± 0.82
Chlorella vulgaris	Rac-DC	$Y = 0.1642 + 0.2153$ Ln C	0.96	0.00	4.76 ± 0.31
	S-DC	$Y = 0.0032 + 0.4137$ Ln C	0.93	0.03	3.32 ± 0.33
	R-DC	$Y = -0.3921 + 0.3044$ Ln C	0.85	0.02	18.74 ± 2.48
Scenedes mus obliquus	Rac-DC	*a*			
	S-DC	$Y = -1.9366 + 0.7957$ Ln C	0.95	0.02	21.30 ± 3.42
	R-DC	*a*			

[a] Algal growth inhibition <24% at the maximum applied concentration of 30.0 mg/L; based on their dose−activity relationship curves, the EC_{50} values of Rac-DC and R-DC were estimated to be 64.26 and 91.47 mg/L, respectively.

Table 2. Growth Dynamic Parameters of Three Algae Treated with Chiral Herbicides and Their Enantiomers[a]

alga	compd	*a*	W_{max}	R^2	compd	*a*	W_{max}	R^2
Chlorella pyrenoidosa	Rac-DC	27.72 ± 0.63	7.52 ± 0.15	0.99	Rac-DM	13.70 ± 1.00	3.15 ± 0.18	0.96
	S-DC	22.83 ± 0.47	5.49 ± 0.09	0.99	S-DM	16.49 ± 2.44	2.84 ± 0.23	0.95
	R-DC	25.98 ± 0.54	7.25 ± 0.15	0.99	R-DM	18.78 ± 0.88	4.05 ± 0.13	0.99
Chlorella vulgaris	Rac-DC	17.72 ± 2.25	2.81 ± 0.21	0.98	Rac-DM	12.18 ± 0.49	3.28 ± 0.16	0.98
	S-DC	13.47 ± 1.42	1.91 ± 0.11	0.99	S-DM	14.12 ± 2.45	2.64 ± 0.27	0.93
	R-DC	15.47 ± 0.62	3.71 ± 0.13	0.99	R-DM	13.36 ± 0.22	2.46 ± 0.02	0.99
Scenedesmus obliquus	Rac-DC	10.32 ± 0.72	2.15 ± 0.12	0.97	Rac-DM	7.13 ± 0.30	2.35 ± 0.21	0.96
	S-DC	9.93 ± 0.74	1.93 ± 0.10	0.97	S-DM	4.72 ± 0.11	1.36 ± 0.07	0.99
	R-DC	8.00 ± 0.60	1.45 ± 0.08	0.97	R-DM	6.90 ± 0.11	2.45 ± 0.09	0.99

[a] Three algae initially inoculated at the concentration of 0.74×10^6 cells/mL.

they grow very slowly and are detected with low biomass in the environment (*25, 26*). To investigate the role of facilitated algal uptake during the enantioselective degradation, the algae were heated at 70 °C for 12 h to inhibit their growth. Heated algae turned in color from light green to light yellow, accompanied by the disappearance of the absorption peak of 683 nm. This process, however, did not destroy the apparent morphological features of algal cells by microscope. It inhibited the algal growth during the first 4 days, after which the algae recovered.

The heat treatment was carried out as follows. Two portions of algal medium were taken from the same alga precultures, and their OD_{680} values were measured. One portion of algal medium was heated, and the other was kept out of light in the chamber to minimize growth. The OD_{680} of the untreated alga culture was measured again to quantify its biomass. Both algal media were inoculated into the fresh medium at the same concentration. Rac-DC and its enantiomers were 5.0 mg/L. Treatments were in three replicates. Determination of chiral herbicides and algal growth was also measured.

Data Analysis. Statistical analysis for the significance in the effect of these compounds on the algal growth inhibition was performed using Origin 6.0 (Microcal Software, Inc.). The significance between means was also assessed, and EC_{50} values were considered to be significantly different at $p < 0.05$.

RESULTS AND DISCUSSION

Enantioselectivity in Aquatic Toxicity of DCs to Algae. Data in aquatic toxicity tests show that diclofop posed low or no toxicity to algae with 96 h EC_{50} values ranging from 3.32 to >30 mg/L (**Table 1**). However, the acute aquatic toxicity of the racemate and enantiomers of diclofop indicated the enantioselectivity. For the alga *C. vulgaris*, the *S*(−) enantiomer of diclofop was more toxic than the *R*(+) enantiomer by 4.6 times (**Table 1**). Although both the racemate and the enantiomers were only weakly toxic to the alga *S. obliquus*, the inhibition of algal growth by the *S*(−) enantiomer with the EC_{50} value of 21.30 mg/L was stronger than that by the other enantiomer, which reduced its biomass by about 25% at the concentration of 30.0 mg/L. By comparison, Rac-DC and its enantiomers posed a similar toxicity to the alga *C. pyrenoidosa* with the EC_{50} values ranging from 8.74 to 9.72 mg/L. From the EC_{50} values, the activity of the racemate was attributable primarily to the herbicidally less active *S*(−) enantiomer for both *C. vulgaris* (85.0%) and *S. obliquus* (81.2%), whereas both enantiomers were equally toxic to the alga *C. pyrenoidosa*.

The growth of oat, a target plant, was significantly inhibited by the *R*(+) enantiomer but only slightly by the *S*(−) enantiomer (*28*). Although some algae could encode ACCase, the action site of the herbicide (*29*), the inverse toxicity of the enantiomers also suggested that the interaction patterns could differ greatly in different biological systems (*30*). Therefore, the herbicidally less active enantiomer may be expected to contribute significantly to the nontarget aquatic toxicity observed for the racemate.

Enantioselective Interactions between DCs and Algae. Enantioselectivity in algal growth inhibition was evaluated for DCs by studying algal growth dynamic parameters (**Table 2**). These parameters were obtained by fitting algal growth curves to the logistic equation

$$y = a/[1 + (a - y_0)/y_0 \times e^{-(4W_{max}/t)}]$$

where y is the biomass (10^6 cells/mL) at time t, a is the amplitude (i.e., the final biomass, 10^6 cells/mL), y_0 is the initial biomass (10^6 cells/mL), and W_{max} is the maximum growth rate (10^6 cells/mL/day). The obtained parameters for both *C. pyrenoidosa* and *C. vulgaris* indicated the enantioselective toxicity of diclofop. On the basis of the decrease of the final biomass and the maximum growth rate, the *S*(−) enantiomer posed a stronger toxicity to both algae than the *R*(+) enantiomer (**Table 2**). There was no significant difference in *S. obliquus* growth inhibition by the enantiomers, suggesting the dependence

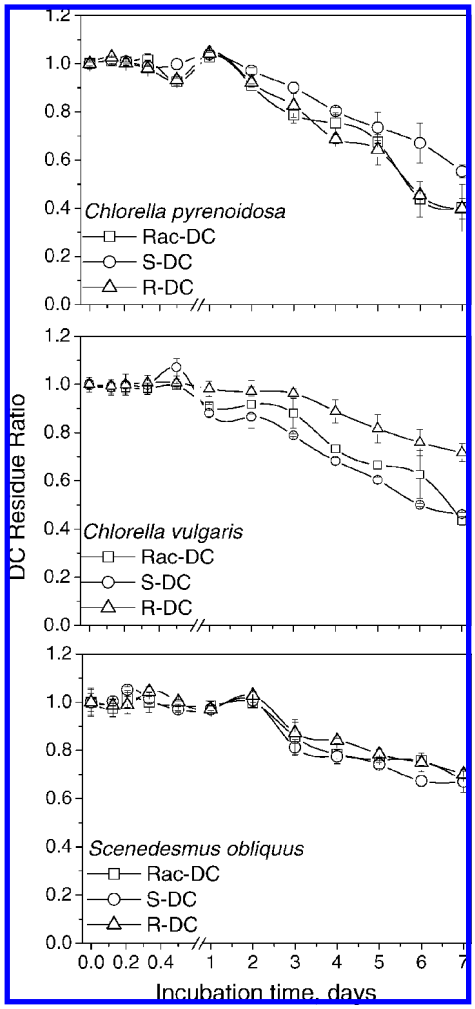

Figure 2. Degradation of DC in alga cultures.

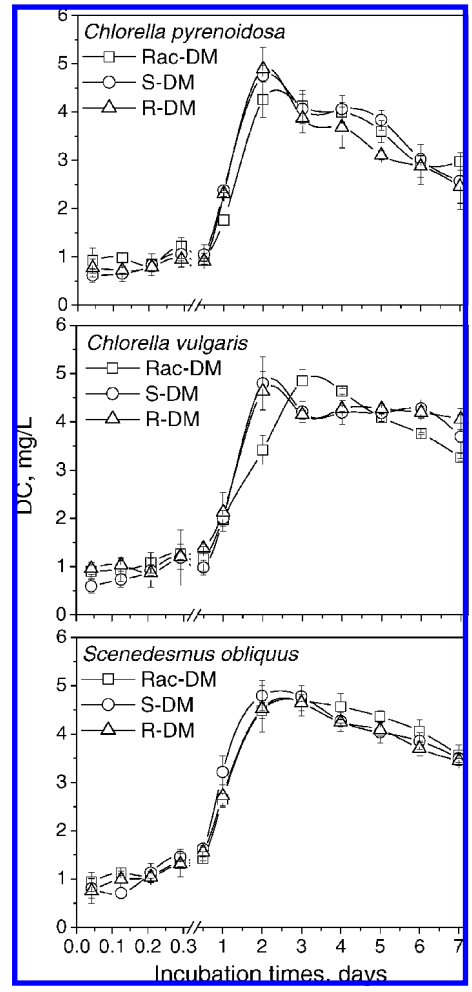

Figure 3. Degradation of DM in alga cultures.

of the enantioselective toxicity of chiral herbicides on plant species.

DC did not degrade in algal cultures within the initial 24 h, which suggested that DC was poorly absorbed by algal cells (**Figure 2**). It degraded after 2 days when algae were at the exponential or log phase, compared to the uninoculated media in which DC did not degrade during incubation. Preferential degradation of either the *R*(+) or *S*(−) isomer occurred in algal cultures (**Figure 2**). Moreover, both enantiomers of DC maintained their configuration. For *C. pyrenoidosa*, DC decreased in the order R-DC ≥ Rac-DC > S-DC, with the mean degradation rates being 0.430, 0.427, and 0.319 mg/L/day, respectively, indicating the preferential degradation of the *R*(+) enantiomer. The degradation of DC in *C. vulgaris* was in a different order: Rac-DC (0.404 mg/L/day) > S-DC (0.385 mg/L/day) > R-DC (0.202 mg/L/dat). Although they rather slowly degraded in *S. obliquus*, a relatively small difference in DC degradation still existed with the mean degradation rates of DC, being 0.236 (S-DC), 0.234 (Rac-DC), and 0.214 mg/L/day (R-DC), respectively. Thus, the *S*(−) isomer was preferentially taken up by both *C. vulgaris* and *S. obliquus*, whereas *C. pyrenoidosa* absorbed more *R*(+) isomer.

The postemergence herbicidal activity of DC comes exclusively from the *R*(+) isomer. However, both *R*(+) and *S*(−) enantiomers show a similar activity when applied as preemergence herbicides (*3*). The *S*(−)-to-*R*(+) microbial conversion in soil was observed, which may explain why the herbicidal activity of the two enantiomers was the same in soil (*31, 32*). Such a conversion did not occur in algal cultures, in agreement with a previous report (*28*). These results suggested extra action sites of the herbicide because the *S*(−) isomer was inactive to ACCase.

Enantioselective Interactions between DMs and Algae. The differential toxicity of DM to algae was also observed on the basis of the algal growth dynamic parameters (**Table 2**). The parameters obtained for both *C. pyrenoidosa* and *S. obliquus* indicated the enantioselective toxicity of DM. The *S*(−) enantiomer posed a stronger toxicity to both algae, in terms of the decrease of the final biomass and the maximum growth rate, than the *R*(+) enantiomer (**Table 2**). There was no significant difference in *C. vulgaris* growth inhibition by both enantiomers. Furthermore, these results revealed that the growth inhibition by DM was stronger than that by DC.

Less than 10% of DM was hydrolyzed to DC in uninoculated neutral media (data not shown). Differently, no detection of DM in the inoculated media after 30 min was attributable to its strong absorption to algal cells due to its high lipophilicity. DC, the hydrolytic product of DM, appeared in algal cultures after 3 h with a change in its stereochemistry, rapidly increased to the maximum after 2–3 days, and finally degraded (**Figure 3**). The major degradation product was identified as a phenol compound, 4-(2,4-dichlorophenoxy)phenol (DP). Thus, the differential formation of DC during the initial 2 days was introduced to describe the degradation of DM in algal cultures in which the degradation rates of DM were equal to the formation rates of DC. For the enantiomers of DC, the mean formation rates varied from 2.26 ± 0.24 to 2.42 ± 0.04 mg/L/day and showed no

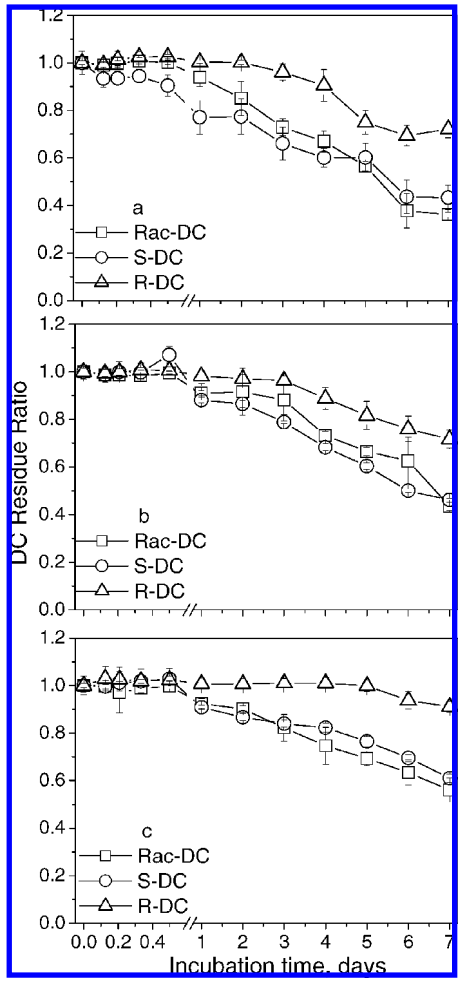

Figure 4. Effects of concentration on degradation of DC in *Chlorella vulgaris* culture: **a**, 2.0 mg/L; **b**, 5.0 mg/L; **c**, 10.0 mg/L.

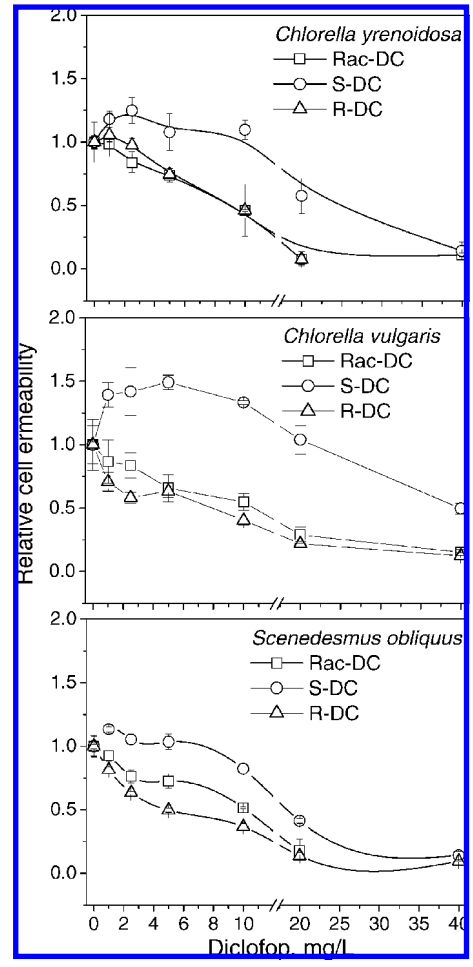

Figure 5. Influence of DC on algal cell permeability.

significant difference, which indicated the identical degradation of DM enantiomers. Due to its rapid disappearance, DM was not found to racemize in soil and plants, thus leading to no enantioselectivity in its hydrolysis to DC (*28, 30*).

Subsequent DC degradation showed an enantioselectivity in algal cultures (**Figure 3**). For *C. pyrenoidosa*, the mean degradation rates of DC within the initial 5 days decreased in the orde: R-DC (0.478 mg/L/day) > S-DC (0.436 mg/L/day) > Rac-DC (0.256 mg/L/day). In *S. obliquus* culture, the corresponding order was S-DC (0.310 mg/L/day) ≥ R-DC (0.300 mg/L/day) > Rac-DC (0.226 mg/L/day). In *C. vulgaris*, Rac-DC degraded the most rapidly with the mean degradation rate of 0.316 mg/L/day, followed by S-DC (0.220 mg/L/day) and R-DC (0.116 mg/L/day). Compared to the degradation of DC initially added, the formed DC from DM showed a relatively smaller difference in stereochemistry and the order of their degradation rates changed. Thus, the differences in the degradation of DC were primarily attributed to the rapid interaction between DM and algae, which not only inhibited the algae growth but also changed the relative degradability of the racemate and enantiomers of DC.

Influence of Concentration on Enantioselective Degradation of DC. Preferential degradation of the *S*(−) enantiomer in *C. vulgaris* at all concentrations of DC tested existed (**Figure 4**). S-DC reduced by 56.7% (2.0 mg/L), 53.9% (5.0 mg/L), and 39.0% (10.0 mg/L), respectively, in 7 days, whereas the *R*(+) enantiomer dissipated only by 27.9, 28.3, and 8.9%, respectively. This showed that the degradability of the herbicide was inversely correlated with its initial concentration. When expressed as the mean degradation rate, differential influences of initial concentration were observed. For the *S*(−) enantiomer the mean degradation rates were 0.162, 0.385, and 0.557 mg/L/day, respectively, in algal cultures treated by S-DC of 2.0, 5.0, and 10.0 mg/L during incubation, as compared to 0.080, 0.202, and 0.127 mg/L/day for the *R*(+) enantiomer. These results indicated a higher uptake at higher concentrations than at lower concentrations, in agreement with the dose−activity relationship.

Influence of DC on Algal Cell Permeability. Fluorescein diacetate (FDA) is readily absorbed by cells and metabolized by in vivo esterases. The resulting fluorescein fluorescence reflects both esterase activity and cell membrane integrity, the two parameters of cell viability (*33*). The rate of FDA conversion to fluorescein is correlated with microalgae photosynthesis (*34–36*) and nutrient-limited growth (*36*), which validates the use of this assay to test the metabolic viability of phytoplankton cells. It is generally assumed that FDA diffuses freely into intact cells (*23, 37–40*). In in vivo experiments, algal cell permeability is the potential of passive uptake of FDA into algae, representing the passive uptake by cells.

The responses of algal cell permeability differed when the racemate and two enantiomers of DC interacted with algae (**Figure 5**). A drastic decrease in algal cell permeability occurred when treated by Rac-DC and the *R*(+) isomer. Both resulted in a positive correlation between the inhibition of algal cell permeability and the level of DC. **Figure 5** shows that the *R*(+) enantiomer was a stronger inhibitor than the racemate. In the case of S-DC, low levels ranging from 1.0 to 5.0 mg/L increased algal cell permeability to different extents, and the effect

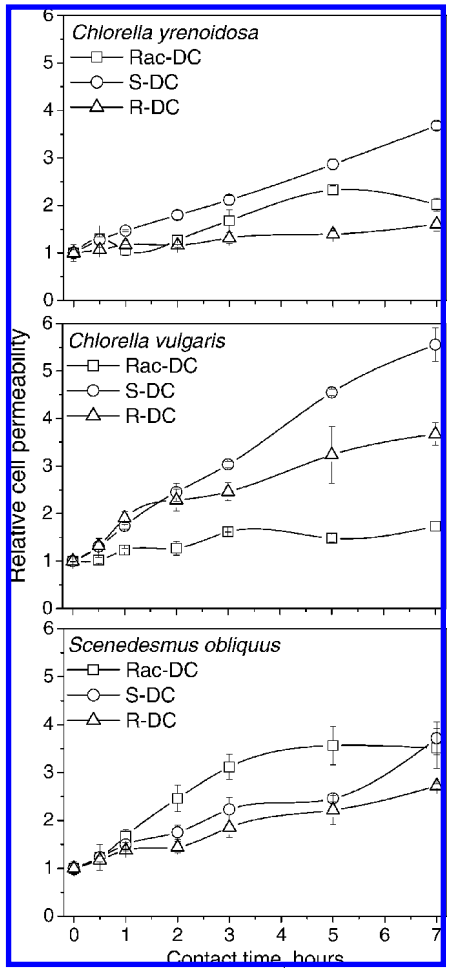

Figure 6. Time course of algal cell permeability treated with DC of 5.0 mg/L.

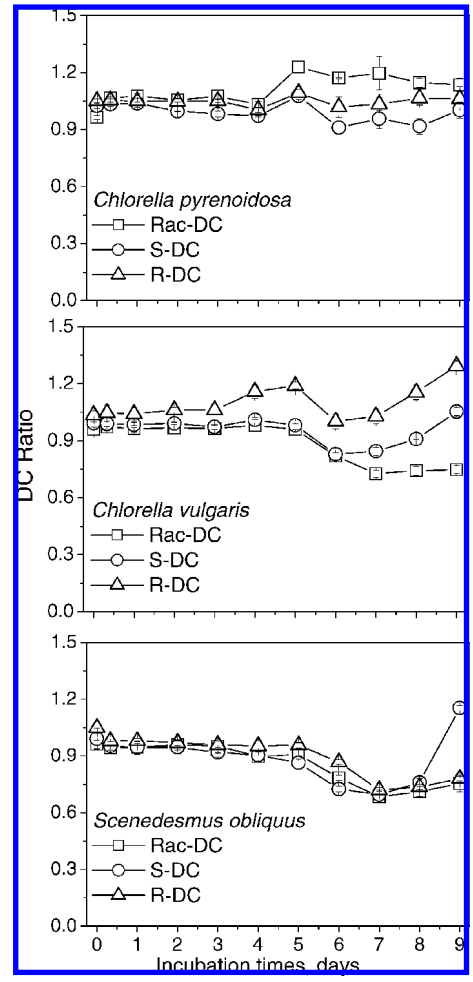

Figure 7. Differential degradation of DC in alga cultures with and without heat treatment.

increased with concentration for both *C. pyrenoidosa* and *C. vulgaris*. It reduced algal cell permeability at higher levels (over 10 or 20 mg/L), but the reduction by S-DC was less than those by the racemate and the *R*(+) enantiomer. These results showed that the pair of enantiomers resulted in a differential effect on cell permeability under the same conditions, manifesting the enantioselectivity in the interactions between chiral herbicides and algae.

Algal cell permeability treated by DC increased with contact time, and S-DC resulted in a more rapid increase in algal cell permeability than the *R*(+) enantiomer (**Figure 6**). For example, the mean increase rates of cell permeability of *C. pyrenoidosa*, defined as the slope of the time course curve, were 0.21 /h (Rac-DC), 0.36 /h (S-DC), and 0.08 /h (R-DC). Due to high initial cell permeability and high increase rate of cell permeability, the *S*(−) enantiomer of DC greatly increased cell permeability of *C. pyrenoidosa*, which implied that the algal cells assimilated more the herbicide bypassive uptake. However, these results were in contradiction with preferential degradation of the *R*(+) enantiomer in the same systems, which indicated that passive uptake of DC was not the major degradation pathway in algae and did not play a role in the enantioselective degradation. In contrast, for *C. vulgaris* and *S. obliquus*, higher cell permeability and mean increase rates of cell permeability in the presence of the *S*(−) enantiomer were consistent with its preferential degradation, indicating the importance of passive uptake in the degradation of DC. Nevertheless, algal cell permeability is a helpful parameter for a preliminary evaluation of enantioselective toxicity of chiral herbicides.

Role of Facilitated Uptake by Algae. Heated algae virtually did not grow during the initial 4 days of incubation. Thereafter, algae grew, with the color of the cultures turning light green, and the absorption peak at 683 nm reappeared. After 9 days of incubation, heated *C. vulgaris* had a similar or even higher biomass as compared to the control. The biomasses of the other two algae increased to about 70% (*C. pyrenoidosa*) and 80% (*S. obliquus*) of their respective control biomasses. These results indicate that thermal damage or thermal deactivation led to changes in normal algal growth kinetics, although they eventually recovered from the damage (*27*). The growth of heated algae consisted likely of an initial recovery phase when the algae survived and recovered their normal physiological functions primarily by passive uptake of fewer nutrients, followed by a rapid growth phase when the algae rapidly reproduced, predominantly by facilitated uptake of nutrients.

The DC ratio (**Figure 7**), defined as the ratio of DC in heated algae cultures to that in their respective controls, was expected to illustrate the role of algae passive uptake and facilitate uptake in DC degradation. On the basis of the above results, the DC ratio increases in the initial recovery phase if less DC degrades in heated algal cultures, indicating that the role of facilitated uptake by algae will increase DC degradation. An increase in DC ratio in the rapid growth phase represents the inverse result. During incubation, the DC ratio of the *R*(+) enantiomer of DC varied from 1.00 to 1.09 in *C. pyrenoidosa*. Although heating reduced algal biomass, similar processes of DC in both algae showed that passive uptake was a key process of DC degradation

during the initial incubation time for about 4 days. Subsequent facilitated uptake contributed greatly to the degradation. The DC ratio for the *S*(−) enantiomer after 5 days was <1.00 between 0.91 and 0.95, indicating that more DC degraded in heated cultures during the rapid growth phase. Facilitated uptake was thus more important for the degradation of the *S*(−) enantiomer than of the *R*(+) enantiomer. These results illustrate the inconsistency between the preferential degradation of individual enatiomers and the effects of algal cell permeability. For *C. vulgaris*, the DC ratio for both enantiomers showed that passive uptake and facilitated uptake together contributed to the degradation of the *R*(+) enantiomer, whereas facilitated uptake dominated the degradation of the *S*(−) enantiomer. These results were consistent with the preferential degradation of the *S*(−) enantiomer and the cell permeability. In the case of *S. obliquus*, the DC ratios for both enantiomers were similar (between about 0.70 and 0.90 after 4 days), suggesting again that facilitated uptake was the governing factor in DC degradation.

Ecotoxicological and Environmental Implications. DM was rapidly hydrolyzed to DC without racemization but posed enantioselective toxicity to aquatic algae. The enantioselectivity in toxicity and degradation of DC suggests that specific forms of a pesticide such as ester (e.g., DM) and acid (e.g., DC) may result in different environmental behaviors and toxicities. Our results also show that the enantiomers of DM and DC have modes of action in nontarget plants different from those in target plants, because the inactive enantiomer has a toxicity to nontarget plants similar to or higher than that of the herbicidal enantiomer. In addition, the toxicity of inactive enantiomers to aquatic algae suggested that these enantiomers may greatly inhibit the growth of some algae but are less toxic to other algae, thus reducing the biodiversity of ecological systems. Facilitated uptake by algae usually results in enantioselectivity in the degradation of chiral pesticides in algae cultures, although passive uptake may also play an important role.

Aryloxyphenoxypropanoate herbicides, including DM and DC, act at sites (ACCase) that can be found in other organisms such as nontarget plants, animals, humans, and parasites (*41*). In animals and humans, structurally similar chemicals such as quizalofop and haloxyfop have been reported to inhibit ACCase (*42*), disrupt lipid metabolism (*43*), and interfere with membrane transport (*44*). Zuther et al. (*45*) and Wilson (*46*) found that aryloxyphenoxypropanoate herbicides effectively inhibit ACCase of some parasites via a mechanism similar to that in grasses. Besides, some studies found that DM and DC are possible endocrine disruptors and carcinogens (*15, 19, 47*). For example, the herbicide diclofop inhibits the biosynthesis of sex pheromone in moths and precludes mating success, thereby reducing insect population (*48*). Potential enantioselectivity in these chronic processes for both DM and DC should be further explored. A well-timed chiral switch from racemates to single enantiomers is expected to not only offer enhanced biological activity and further profitability (*49*) but also immediately reduce the usage of chiral pesticides by almost 50% to reduce their ecological risks without suffering from the loss of their benefits.

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Received for review September 28, 2007. Revised manuscript received January 29, 2008. Accepted January 30, 2008. This study was supported by the Program for Changjiang Scholars and Innovative Research Teams in Universities (No. IRT 0653), the National Key Project for Basic Research (2003CB114400), the National Natural Science Foundation of China (No. 20707002, 30771255), and the Natural Science Foundation of Zhejiang Province, China (No. Z506070).

JF0728855