Bull Environ Contam Toxicol (2009) 83:85–91
DOI 10.1007/s00128-009-9773-x

Enantioselective Effects of Chiral Herbicide Diclofop Acid on Rice *Xiushui 63* Seedlings

Jing Ye · Qiong Zhang · Anping Zhang · Yuezhong Wen · Weiping Liu

Received: 13 September 2008 / Accepted: 5 May 2009 / Published online: 19 May 2009
© Springer Science+Business Media, LLC 2009

Abstract In this study, the acute toxicity (72-h EC50 values) of chiral diclofop acid towards rice *Xiushui 63* seedlings and its effects on the Hill reaction activities of chloroplasts were determined. Significant differences were observed between the two enantiomers in 72-h EC50 values and in both in vivo and in vitro relative Hill reaction activities. These observations indicate that the enantiomers of diclofop acid pose different toxicities to rice seedlings: the *S*-enantiomer is more toxic to leaves and the *R*-enantiomer is more toxic to roots. These enantioselective toxic effects on rice seedlings should be taken into account in chiral herbicide application.

Keywords Enantioselectivity · Diclofop · Toxicity · Rice *Xiushui 63*

Diclofop acid ((*R*,*S*)-2-[4-(2,4-dichlorophenoxy)phenoxy] propanoic acid), which is hydrolyzed from diclofop methyl via cleavage of the ester bond, is an herbicidal form of diclofop methyl. Diclofop methyl is a post-emergence herbicide registered in the United States in 1982 by Farbwerke Hoechst AG (Frankfurt, Germany) for the control of wild oats and annual grasses in wheat and barley. The total annual usage of diclofop methyl in the USA between 1987 and 1996 was approximately 340,200 kg of active ingredient (a.i.), and the total usage in Canada in 1986 was over one million kilograms (EPA 2000a). In China, the usage of diclofop methyl was one to five million kilograms in 2006 (Su and Shao 2005). The widespread application of diclofop methyl may bring about deleterious environmental effects, because residues of the parent compound and the acid degradation product may become common in the environment. It has been reported that up to 73% of the active ingredient may fall onto soil surfaces upon application (Smith et al. 1986). Under alkaline aquatic conditions, diclofop methyl rapidly hydrolyzes into diclofop acid, which is a polar compound and has a relatively high solubility (23 mg/L at pH 7, 20°C) in water compared to diclofop methyl (0.8 mg/L at pH 7, 25°C) (EPA 2000b). Therefore, diclofop acid is likely to be present in surface water in significant amounts (Liu et al. 1991) and is more likely to contaminate soil and water than the parent compound. Many studies have reported the risks of exposure to diclofop methyl for birds, mammals, freshwater fish, invertebrates and terrestrial plants (EPA 2000b). However, there is little information on either the toxicity of diclofop methyl to non-target aquatic plants or the environmental behaviors of diclofop acid. There is little information on the environmental risks of diclofop acid.

Diclofop methyl and diclofop acid are both chiral herbicides with one stereogenic center, and therefore they have two enantiomers (Fig. 1). About 25% of pesticides currently in use are chiral, and this ratio is increasing as compounds with more complex structures are introduced (Williams 1996). A study on the enantioselective activity of diclofop has shown that *R*-diclofop is herbicidally active (Matell 1953) and is approximately twice as active as the racemic mixture (Kurihara et al. 1997). However, the racemic mixture remains the form of diclofop in wide commercial use. Previous studies on the environmental

J. Ye · Y. Wen
Institute of Environmental Science, Zhejiang University, 310027 Hangzhou, China

Q. Zhang · A. Zhang · W. Liu (✉)
Research Center of Environmental Science, College of Biological and Environmental Engineering, Zhejiang University of Technology, 310032 Hangzhou, China
e-mail: wliu@zjut.edu.cn

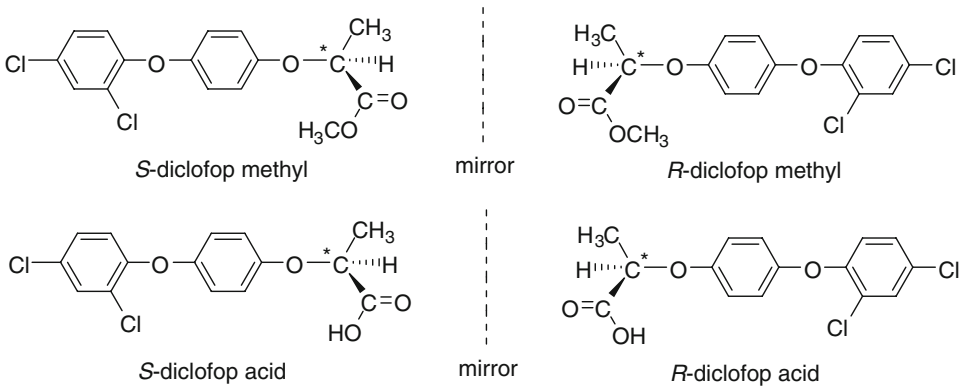

Fig. 1 Enantiomers of chiral diclofop acid ("*" indicates chiral position)

behavior of diclofop methyl or diclofop acid have generally considered the enantiomers as if they were identical compounds (Palut et al. 2001; Ditomaso 1993; Petit et al. 1997; Headley et al. 1998). The enantiomers of both diclofop methyl and diclofop acid are present simultaneously in the environment. Therefore, investigation of both chiral diclofop methyl and diclofop acid is needed. Diclofop methyl is the commercial product and is the original source of pollution. In the present study, however, we investigated only diclofop acid, because we could obtain the acid form more conveniently than the methyl form. We have recently investigated the ecotoxicities of the enantiomers of diclofop acid under laboratory conditions (Cai 2006; Cai et al. 2008). The degradation process and aquatic ecotoxicities of diclofop acid on three freshwater algae were found to be enantioselective.

In the south of China, dryland (wheat) and aquatic (rice) crops are usually cultivated alternately with season. The residues of diclofop used in wheat crops may contaminate the soil and pose ecotoxicity to rice. Rice *Xiushui 63*, a monocotyledonous aquatic non-target plant, is widely cultivated in South China. Therefore, rice *Xiushui 63* was chosen to investigate the phytotoxicity of chiral diclofop acid in the present study. Investigating the toxicity of diclofop acid to this crop can facilitate the proper application of this chiral herbicide and protect non-target plants.

Materials and Methods

Rac-diclofop acid {(±)-2-[4-(2,4-dichlorophenoxy)phenoxy]propanate} with a chemical purity ≥97.0% was prepared from diclofop-methyl {(±) methyl 2-[4-(2,4-dichlorophenoxy)phenoxy]propionate} according to Smith (1976) and identified by high-performance liquid chromatography (HPLC) according to Lin et al. (2006). Diclofop methyl was kindly provided by Iprochem Co., Ltd (Shenzhen, Guangdong Province, China). *R*- and *S*-diclofop acid (purity ≥ 99.0%, optical purity ≥ 94.0%) were synthesized in our laboratory (Cai 2006).

Rice seeds (*Xiushui 63*) were obtained from The National Rice Research Institute of China, Hangzhou, Zhejiang Province, China. The seeds were immersed in tap water for 1–3 days, washed several times with Milli-Q water, and germinated in the dark for 48 h at 25°C. Uniformly germinated seedlings were selected and placed in growth medium for subsequent studies. The growth medium was a modified Hoagland nutrient solution with a pH of 5.0–5.1, containing 914 mg/L NH_4NO_3, 403 mg/L $NaH_2PO_4 \cdot 2H_2O$, 714 mg/L K_2SO_4, 3,240 mg/L $MgSO_4 \cdot 7H_2O$, 886 mg/L $CaCl_2$, 800 mg/L Na_2SiO_4, 3 mg/L $MnCl_2 \cdot 4H_2O$, 0.15 mg/L $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$, 1.87 mg/L H_3BO_3, 0.07 mg/L $ZnSO_4 \cdot 7H_2O$, 0.06 mg/L $CuSO_4 \cdot 5H_2O$, and 15.40 mg/L $FeCl_3 \cdot 6H_2O$.

The rice growth-inhibition tests were carried out according to the OECD guidelines for the testing of chemicals (OECD 2002). Seedlings that had produced two leaves, with the primary leaf being 1.5–2.0 cm long, were chosen for the leaf growth-inhibition test. We used five seedlings per replicate per glass beaker. Three replicates were tested in three independent glass beakers (250 mL), each containing 100 mL of growth medium, for each concentration treatment. The concentrations of *rac*-, *R*- and *S*-diclofop acid ranged from 1 to 20 mg/L (1% v/v acetone). The glass beakers were kept at 25 ± 0.5°C in a culture chamber with light (20,000 Lux)/dark alternation (16 h/8 h) and 80% humidity for 72 h, and were repositioned daily to minimize spatial differences in illumination and temperature.

Seedlings that had produced three roots 48 h after germination, with the middle root measuring 1.5–1.8 cm in length, were chosen for the root growth-inhibition test. We used ten seedlings per replicate per Petri dish. Three replicates were tested in three independent 9-cm Petri dishes, each containing 6 mL of growth medium, for each concentration treatment. The concentration of *rac*-, *R*- and *S*-diclofop acid ranged from 1.67 to 1,000 μg/L (1% v/v acetone). The Petri dishes were kept in the dark at 28°C and 100% humidity for 72 h.

The mean leaf and root lengths of the seedlings were measured, and the growth rate was expressed as a

percentage of the control value. The concentration of each chemical that caused 50% inhibition (EC50 value) was determined from the dose-response regression curve by a Probit analysis.

The plant growth medium was extracted every day during incubation. The samples were filtered through a 0.45-μm filter, and then extracted three times with 1:1 (v:v) diethyl ether. Fractions were combined, evaporated completely, dissolved in an HPLC mobile phase, and then analyzed by chiral HPLC. The chiral analyses were performed on a Jasco LC-2000 series HPLC system (Jasco, Tokyo, Japan) with a PU-2089 quaternary gradient pump, a CO-2060 column temperature control compartment, and a UV-2075 plus UV/VIS detector. The operation conditions were a Chiralcel OJ column (4.6 mm × 250 mm, Daicel Chemical Industries, Tokyo, Japan), a flow rate of 0.5 mL/min, a mobile phase of *n*-hexane/IPA (90:10 v/v), a detection wavelength of 254 nm, an injection volume of 10 μL, and an oven temperature of 20°C (Lin et al. 2006). The enantiomers were identified according to Lin et al. (2006) by comparing the chromatograms of the samples with those of optically pure isomer standards. Peak retention time was used as the evaluation criterion.

One of the first steps of photosynthesis is the splitting of water to donate two electrons to the reaction center, P680. This reaction is known as the Hill reaction. Seven-day-old chloroplasts from rice seedlings treated with different concentrations of *rac*-, *R*- or *S*-diclofop acid were isolated after treatment by grinding five grams of fresh leaves in 20 mL cold STN solution [0.4 M sucrose, 0.05 M *tris*–hydrochloride (pH 7.6), and 0.01 M NaCl]. The extract was filtered through four layers of cheesecloth into a flask and centrifuged at 200*g* for five min at 4°C, and the precipitate was discarded. The chloroplasts were collected by centrifuging at 1,000*g* for 20 min at 4°C and resuspended in 10 mL of cold STN solution. The chloroplast suspension was kept in the dark and on ice before use.

Chlorophyll content in the acetone extracts was determined by the method of Arnon (Arnon 1949), using a Jasco V-550 UV/VIS spectrophotometer (Jasco V-550, Japan) at 663 and 432 nm. The concentration was expressed as mg chlorophyll per g fresh weight.

The Hill reaction mixture contained 0.1 mL of the chloroplast suspension from each treatment, 0.1 mL of 0.5 M Tris–hydrochloride (pH 7.6), 0.1 mL of 0.05 M $MgCl_2$, 0.1 mL of 0.1 M NaCl, 0.1 mL of 0.01 M $K_3Fe(CN)_6$, and 0.5 mL of distilled water. A reaction mixture containing a suspension of chloroplasts without diclofop acid was used as a control. The tests were grouped into two sets, the light-dependent ("light") reactions and the light-independent ("dark") reactions. The cuvettes for illumination testing were put in a water bath using a glass beaker at 20°C. Two 500 W outdoor-type spot lamps with a controlled light intensity of 30,000 Lux were used as the light source. They were placed 20 cm away from the beaker. After illumination for 1 min, 0.2 mL of 10% trichloroacetic acid was added to terminate the reaction. The mixtures were then centrifuged at 1,000*g* for 2 min, and 0.7 mL supernatant was removed for the color reaction, using a mixture of 2 mL of 0.2 M sodium citrate, 0.1 mL of 0.01 M $FeCl_3$, 0.2 mL of 0.05 M *o*-Phenanthroline hydrochloride, and 1 mL of distilled water. The optical density at 520 nm was measured after keeping the color reaction in the dark for 10 min (Ye and Qian 1985). Three replicates were made for each treatment, and the experiment was repeated twice.

Chloroplasts from 7-day-old healthy cultivated rice seedlings were isolated and then treated with diclofop acid at different concentrations (0, 0.5, 1, and 2 mg/L in 1% v/v acetone), as described above.

EC50 values were determined using the LD50 Data Processing Program (Version 1.01) (Blue Cosmos Studio, Guangzhou, China), based on the Probit analysis method. Statistical analysis was performed using Origin 6.0 (Microcal Software, Northampton, MA, USA) to determine the significance of the effects of diclofop acid on the Hill reaction activity. Differences in Hill reaction activity values were considered to be statistically significant with $p < 0.05$.

Results and Discussion

The inhibitory effect of *rac*-, *R*- and *S*-diclofop acid on the growth rate (I_r) of leaves and roots of rice seedlings was calculated for each test concentration according to the following formula:

$$\%I_r = \frac{(\mu_C - \mu_T)}{\mu_C} \times 100$$

where $\%I_r$ represents percent inhibition relative to the average specific growth rate, μ_C represents the mean value for leaf (root) length in the control, and μ_T represents the mean value for the leaf (root) length in the treatment group.

The inhibition results are presented in Fig. 2. The EC50 values (Table 1) were calculated using linear regression analyses, indicated by the 72-h EC50 value. The 72-h EC50 values of *rac*-, *R*- and *S*-diclofop acid for rice roots were 5.03 μg/L [95% fiducial limits (FL): 3.37–7.51 μg/L], 2.46 μg/L (95% FL: 1.42–4.24 μg/L) and 11.19 μg/L (95% FL: 6.57–19.03 μg/L), respectively. Leaf tissue is not as sensitive as root tissue. The 72-h EC50 values of *rac*-, *R*- and *S*-diclofop acid for rice leaves were 3.41 mg/L (95% FL: 1.40–8.28 mg/L), 9.31 mg/L (95% FL: 4.38–19.82 mg/L) and 3.86 mg/L (95% FL: 1.54–9.66 mg/L), respectively. Comparing the two enantiomers, we found

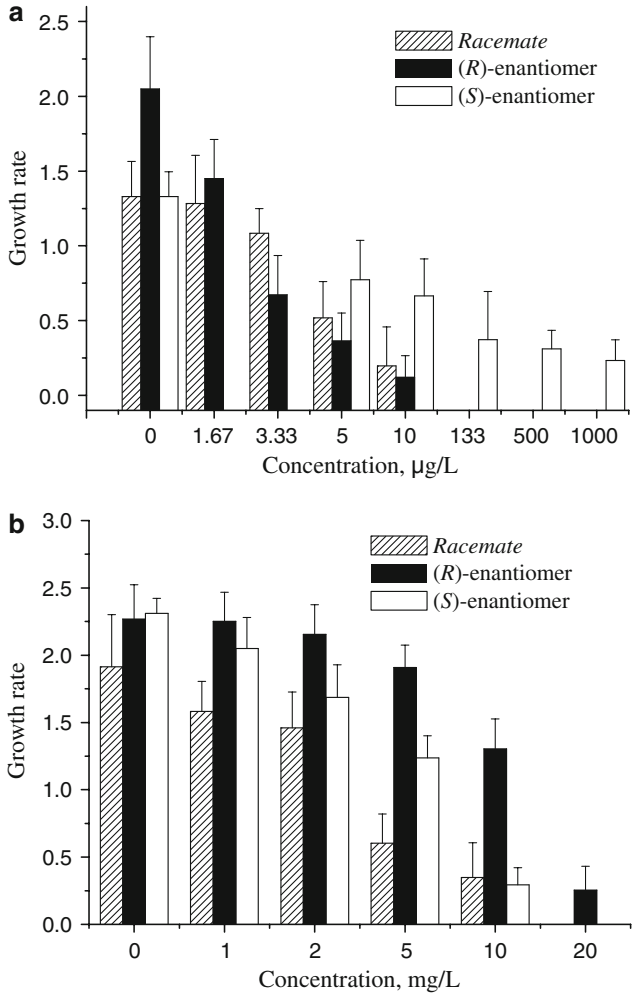

Fig. 2 Growth rate of roots in rice seedlings treated with *rac*-, (*R*)- and (*S*)-diclofop acid (**a**), and growth rate of leaves in rice seedlings treated with *rac*-, (*R*)- and (*S*)-diclofop acid (**b**). Some data are missing because different concentrations of the chemicals were used for different parts of the plant. For subfigure **a**, when the concentrations of the racemate and the *R*-enantiomer exceeded 10 ppb, the roots showed no growth. However, when the concentration of the *S*-enantiomer exceeded 5 ppb, the chemical began to affect the root growth. The situation was similar for subfigure **b**

that the sensitivity and enantioselectivity differed between roots and leaves: the chemicals were 1,000-fold more active toward root growth than toward leaf growth, and the *R*-enantiomer was more active toward root growth while the *S*-enantiomer was more active toward leaf growth. The results indicate that *R*-diclofop acid is 4.5 times more toxic than the *S*-enantiomer to roots, whereas the *S*-enantiomer is 2.4 times more toxic than the *R*-enantiomer to leaves. The inhibition time differs between roots and leaves; root damage happens earlier than leaf damage, since roots grow earlier than leaves. Inhibition of leaf growth would be recognized as a secondary or tertiary effect.

Shimabukuro and Hoffer (1995) have demonstrated that oat root growth is inhibited significantly by *R*-diclofop but

Table 1 Seventy-Two-Hour EC50 (median effective treatment) values of *Rac*-, *R*-, and *S*-diclofop acid for rice *Xiushui 63* seedlings

Seedling	Compound	*r*	EC50 (95% fiducial limits) (μg/L)
Root	*Racemic*-diclofop	0.9853	5.03 (3.37–7.51) (A)[a]
	(*R*)-diclofop	0.9930	2.46 (1.42–4.24) (B)
	(*S*)-diclofop	0.9950	11.19 (6.57–19.03) (C)
Leaf	*Racemic*-diclofop	0.9785	3.41 (1.40–8.28) × 10^3 (A)
	(*R*)-diclofop	0.9696	9.31 (4.38–19.82) × 10^3 (B)
	(*S*)-diclofop	0.9706	3.86 (1.54–9.66) × 10^3 (A)

[a] Different capitalized letters behind the values indicate significant differences ($p < 0.05$) between individual enantiomers and racemate, while the same letter indicates no significant difference

only slightly by *S*-diclofop, owing to the role of oxidative membrane catabolism by free radical lipid peroxidation and its coupling to the effect of diclofop on the transmembrane proton gradient. This result is consistent with those of the present study. The roots of rice seedlings may be tolerant to *S*-diclofop acid because of an unknown inherent mechanism that enables cells to repolarize the E_m and restore the cytoplasmic pH to near homeostasis (Holtum et al. 1994). AOPP herbicides modulate cytosolic pH by increasing the permeability of the plasmalemma to protons (Wright and Shimabukuro 1987). Damage to only a few cells or to apical initials in meristems may be all that is necessary to cause the eventual demise of whole plants (Shimabukuro and Hoffer 1995; Donald et al. 1982).

It is worth noting that the enantioselectivity is reversed between roots and leaves (Table 1). Differential enantiomer-specific responses in roots and leaves have also been observed for other chiral herbicides (Lenton et al. 1994; Omokawa and Tabei 2002). (2*S*,3*S*)-paclobutrazol reduces shoot growth more effectively than root growth, whereas (2*R*,3*R*)-paclobutrazol reduces root growth more effectively than shoot growth (Lenton et al. 1994). Another study has found that *R*-2,4-diamino-6-chloro-*s*-triazine is more effective in controlling barnyard grass at the newly-germinated stage, while the *S*-enantiomer is more effective at the early-middle growth stage (Omokawa and Tabei 2002). The enantioselective toxicity observed in roots in the present study probably indicates that rice roots are tolerant to *S*-diclofop acid. However, the mechanism is uncertain and merits further research.

The enantioselective dissipation of diclofop acid was determined by an enantioselective HPLC system. The chromatogram is given in Fig. 3. The enantiomer fractions [EFs, $EF = A_1/(A_1 + A_2)$, where A_1 and A_2 correspond to the peak areas of the enantiomers] of undegraded diclofop in plant growth medium were measured each day during the incubation process. The extraction recoveries were

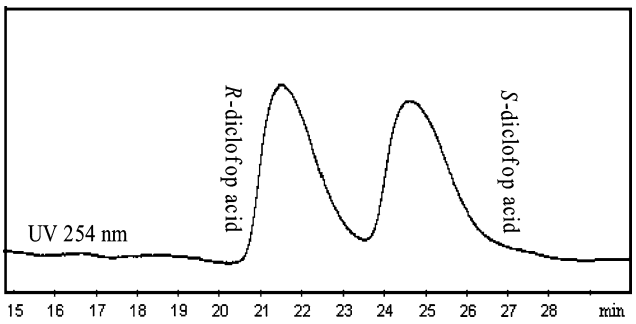

Fig. 3 HPLC chromatogram of enantiomeric separation of racemic diclofop on a Chiralcel OJ column (detection wavelength 254 nm)

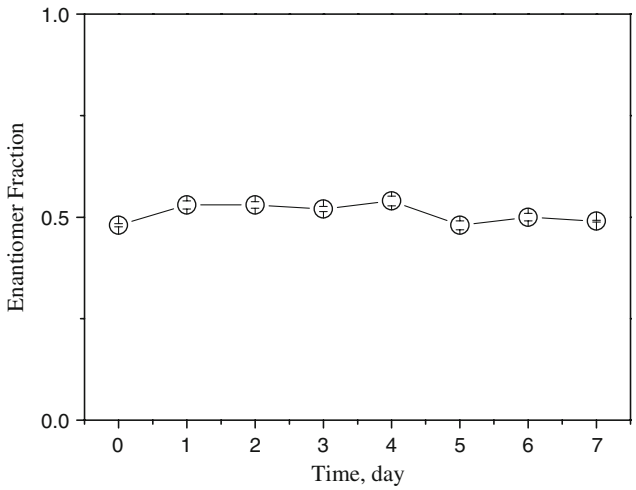

Fig. 4 The EFs of enantiomers of diclofop acid with 5 mg/L in the plant growth medium

between 88% and 110%, and the detection limit was 0.025 mg/L. The EF of the racemic standard was 0.51. EFs of the samples ranged from 0.48 to 0.54 (Fig. 4). Statistical analyses showed that the EFs of the samples were not significantly different from that of the racemate. This indicates that the dissipation of diclofop is non-enantioselective. Therefore, the enantioselective responses of rice seedlings to chiral diclofop are not due to selective uptake of enantiomers of the herbicide.

The Hill reaction activity was expressed as μM $K_4Fe(CN)_6$/(mg chlorophyll·hour). The μM $Fe(CN)_6^{4-}$ was calculated from the OD-$C_{K4Fe(CN)6}$ standard curve ($y = 0.9514x + 0.0167$, where y represents OD and x represents $C_{K4Fe(CN)6}$), and the Hill reaction activity was calculated as follows:

$$\text{Hill reaction activity} = \mu\text{M Fe(CN)}_6^{4-} \times \frac{1.2}{0.7} \times \frac{1}{\text{mg chlorophyll} \cdot \text{hour}} \times 60.$$

Every Hill reaction activity measurement of each treatment was expressed as the activity relative to the control, which was set to be 1. As shown in Table 2, at a concentration of 0.5 mg/L, the *rac*-, *R*-, and *S*-enantiomers did not significantly affect the Hill reaction activities compared to the control. At a concentration of 1 mg/L, the *S*-enantiomer significantly inhibited the Hill reaction activity, whereas the racemate and the *R*-enantiomer did not significantly affect the Hill reaction activity. In addition, the Hill reaction activities for samples treated with the *S*-enantiomer were significantly different from those of samples treated with either the *rac*- or the *R*-enantiomer. At a concentration of 2 mg/L, the Hill reaction activities of samples treated with the *rac*- and *R*-enantiomer were not significantly different from those of the control, but the *S*-enantiomer completely inhibited the Hill reaction activity. This indicates that the *S*-enantiomer is more inhibitory than the *R*-enantiomer at the concentrations of 1 and 2 mg/L. This result is in accordance with the EC50 test described above, which indicated that *S*-diclofop acid is more toxic than *R*-diclofop acid to rice leaves. The Hill reaction activity in leaf tissue treated with the *S*-enantiomer is also lower than that in leaf tissue treated with the *R*-enantiomer. These results confirm that diclofop probably inhibits

Table 2 Relative hill reaction activity (mg chlorophyll hour) in chloroplasts isolated from rice seedlings treated with chiral diclofop acid

Concentration[a] (mg/L)	Racemate	*R*-enantiomer	*S*-enantiomer
Control	1 ± 0.41[b]	1 ± 0.41	1 ± 0.41
0.5	1.17 ± 0.13 (A, a)	0.69 ± 0.47 (AB, a′)	0.33 ± 0.37 (B, a″)
1	1.01 ± 0.33 (A, a)	0.89 ± 0.21 (AB, a′)	0.04 ± 0.28* (C, a″)
2	0.58 ± 0.03 (A, b)	0.75 ± 0.48 (A, a′)	Null[c]

* Indicates $p < 0.05$ relative to the control. Different capitalized letters behind the values indicate significant differences ($p < 0.05$) between individual enantiomers and racemate, while the same letter indicates no significant difference. Different letters (lowercase) indicate a significant difference ($p < 0.05$) between different concentrations of the same analyte (a, b, c for *rac*-diclofop acid, a′, b′, c′ for *R*-diclofop acid, and a″, b″, c″ for *S*-diclofop acid)

[a] The concentrations of chemicals used for the Hill reaction activity tests

[b] Results are presented as mean ± SD of three independent assays

[c] The Hill reaction activity cannot be tested at this concentration

Table 3 Relative Hill reaction activity in isolated chloroplasts treated with chiral diclofop acid

Concentration[a] (mg/L)	Racemate	*R*-enantiomer	*S*-enantiomer
Control[b]	1 ± 0.87[d]	1 ± 0.87	1 ± 0.87
Acetone[c]	1.11 ± 0.26	1.11 ± 0.26	1.11 ± 0.26
0.5	2.59 ± 0.52 (A, a)	4.99 ± 0.55* (B, a′)	1.73 ± 0.49 (AC, a″)
1	2.54 ± 0.47 (A, a)	4.70 ± 0.76* (B, a′)	0.61 ± 0.06 (C, b″)
2	1.07 ± 0.33 (A, a)	1.89 ± 0.64 (A, c′)	0.93 ± 0.24 (A, b″)

* Indicates $p < 0.01$ relative to the control. Different capitalized letters behind the values indicate significant differences ($p < 0.05$) between individual enantiomers and racemate, while the same letter indicates no significant difference. Different letters (lowercase) indicate significant differences ($p < 0.05$) between different concentrations of the same analyte (a, b, c for *rac*-diclofop acid, a′, b′, c′ for *R*-diclofop acid, and a″, b″, c″ for *S*-diclofop acid)

[a] The concentrations of chemicals used for the Hill reaction activity tests

[b] A reaction mixture containing no pesticide was used as a control

[c] A reaction mixture containing acetone instead of herbicide was used as a solvent control

[d] Results are presented as mean ± SD of three independent assays

photosynthesis, in accordance with Nojavan and Evans (1980) and Yao et al. (1993), who have reported that diclofop inhibits the growth of weeds by inhibiting photosynthesis and destroying cell membranes, resulting in the death of the weed.

The in vitro Hill reaction activities of isolated chloroplasts treated with herbicides at different concentrations are shown in Table 3, and the solvent effect is negligible. At the concentrations of 0.5 and 1 mg/L, neither *rac*- nor the *S*-enantiomer significantly affected the Hill reaction activity. However, the *R*-enantiomer increased the Hill reaction activities by 4.99- and 4.70-fold relative to the control at concentrations of 0.5 and 1 mg/L, respectively. In addition, at these two concentrations, the Hill reaction activities were significantly different between the two enantiomers ($p < 0.01$). At a concentration of 2 mg/L, none of the three chemicals significantly affected the Hill reaction activity. Moreover, the Hill reaction activities were not affected by the increasing concentration of the racemate, but they were affected by increasing concentrations of the *R*- and *S*-enantiomers. The statistical analyses are presented in Table 3.

Comparing the Hill reaction activities from in vivo and in vitro experiments (Tables 2, 3), we found that the *S*-enantiomer did not affect the Hill reaction activity at a concentration of 0.5 mg/L in either context. However, at a concentration of 1 mg/L, the *S*-enantiomer inhibited the Hill reaction activity in the in vivo experiment but showed no effect in the in vitro experiment. At a concentration of 2 mg/L, the *S*-enantiomer completely inhibited the Hill reaction activity in the in vivo experiment but showed no effect in the in vitro experiment. Thus, the effects of the *S*-enantiomer may be quite complex when it is transported in plants.

For the *R*-enantiomer, the in vivo Hill reaction activities did not change significantly with increasing concentration. However, the in vitro Hill reaction activities increased at the concentrations of 0.5 and 1 mg/L, and then declined at the concentrations of 2 and 4 mg/L (data not shown for the concentration of 4 mg/L). The hormesis that we observed for the *R*-enantiomer may be quite complex, and it lacks a reasonable interpretation based on the available literature (Calabrese and Baldwin 2003).

Our results indicate that the *S*-enantiomer is more effective in vivo in inhibiting rice seedlings than the *R*-enantiomer at the same concentration. In the in vitro experiment, the two enantiomers also demonstrated different effects on the Hill reaction activity.

The enantioselective effects of the chiral herbicide diclofop acid on rice seedlings occur in a non-target plant. The EC50 values of *rac*-, *R*- and *S*-diclofop acid indicate that the *R*-enantiomer is more toxic to roots than the *S*-enantiomer, whereas the latter is more toxic to leaves than the former. In addition, roots were much more strongly affected than leaves by diclofop. The Hill reaction activity test also indicates that the two enantiomers have different effects on chloroplasts from rice *Xiushui 63*. In conclusion, the chiral herbicide diclofop acid has been shown to pose enantioselective phytotoxicity to non-target plants (i.e., rice).

Acknowledgments This study was supported by the Program for Changjiang Scholars and Innovative Research Team in University to W.L. (No. IRT 0653), the National Basic Research Program of China (No. 2009CB421603), the National Natural Science Foundations of China (No. 20837002, 30771255), and the Natural Science Foundation of Zhejiang Province, China (No.Y507220).

References

Arnon DI (1949) Copper enzymes in isolated chloroplasts: polyphenoloxidase in beta vulgaris. Plant Physiol 24:1–15. doi:10.1104/pp.24.1.1

Cai XY (2006) Effects of MCD and humic acid on aquatic toxicity and bioavailability of the chiral herbicide diclofop methyl. Ph.D. Dissertation Zhejiang University, Hangzhou, P. R. China

Cai XY, Liu WP, Sheng GY (2008) Enantioselective degradation and ecotoxicity of the chiral herbicide diclofop in three freshwater alga cultures. J Agric Food Chem 56:2139–2146. doi:10.1021/jf0728855

Calabrese EJ, Baldwin LA (2003) Toxicology rethinks its central belief – hormesis demands a reappraisal of the way risks are assessed. Nature 421:691–692. doi:10.1038/421691a

Ditomaso JM (1993) Membrane response to diclofop acid is pH dependent and is regulated by the protonated form of the herbicide in roots of Pea and resistant and susceptible rigid Ryegrass. Plant Physiol 102:1331–1336

Donald WW, Parke RV, Shimabukuro RH (1982) The effects of diclofop-methyl on root growth of wild oat. Plysiol Plant 54:467–474. doi:10.1111/j.1399-3054.1982.tb00710.x

Headley JV, Gandrass J, Kuballa J, Peru KM, Gong YL (1998) Rates of sorption and partitioning of contaminants in river biofilm. Environ Sci Technol 32:3968–3973. doi:10.1021/es980499l

Holtum JAM, Häusler RE, Devine MD, Powles SB (1994) Recovery of transmembrane potentials in plants resistant to aryloxyphenoxypropanoate herbicides: a phenomenon awaiting explanation. Weed Sci 42:293–301

Kurihara N, Miyamoto J, Paulson GD, Zeeh B, Skidmore MW, Hollingworth RM, Kuiper HA (1997) Chirality in synthetic agrochemicals: bioactivity and safety consideration. Pure Appl Chem 69:1335–1348. doi:10.1351/pac199769061335

Lenton JR, Appleford NEJ, Temple-Smith KE (1994) Growth retardant activity of paclobutrazol enantiomers in wheat seedlings. Plant Growth Regul 15:281–291. doi:10.1007/BF00029901

Lin KD, Cai XY, Chen SW, Liu WP (2006) Simultaneous determination of enantiomers of rac-diclofop methyl and rac-diclofop acid in water by high performance liquid chromatography coupled with fluorescence detection. Chin J Anal Chem 34:613–616

Liu WP, Chen ZW, Xu HQ, Shi YY (1991) Determination of diclofop-methyl and diclofop residues in soil and crops by gas chromatography. J Chromatogr A 547:509–515. doi:10.1016/S0021-9673(01)88681-7

Matell M (1953) Stereochemical studies on plant growth regulators. VII. optically active α-(2-methyl-4-chlorophenoxy)-propionic acid and α-(2, 4-dichlorophenoxy)-n-butyric acid and their steric relations. Ark Kemi 6:365–373

Nojavan AM, Evans JO (1980) Absorption and translocation of ^{14}C-diclofop-methyl in wild oat and barley. Proc West Soc Weed Sci 33:113–116

Omokawa H, Tabei A (2002) Enantioselective effects of optically active α-methybenzyl-*s*-triazine on the root growth of rice and echinochloa plants and their herbicidal activity. Biosci Biotechnol Biochem 66:1959–1962. doi:10.1271/bbb.66.1959

Organization for Economic Cooperation and Development (2002) OECD Guidelines for the testing of chemicals. Draft test guideline 221: *Lemna* sp. Growth Inhibition Test

Palut D, Ludwicki JK, Kostka G, Kopeć-Szlęzak J, Wiadrowska B, Lembowicz K (2001) Studies of early hepatocellular proliferation and peroxisomal proliferation in Wistar rats treated with herbicide diclofop. Toxicology 158:119–126. doi:10.1016/S0300-483X(00)00371-1

Petit F, Le Goff P, Cravedi JP, Valotaire Y, Pakdel F (1997) Two complementary bioassays for screening the estrogenic potency of xenobiotics: recombinant yeast for trout estrogen and trout hepatocyte cultures. J Mol Endocrinol 19:321–335. doi:10.1677/jme.0.0190321

Shimabukuro RH, Hoffer BL (1995) Enantiomers of diclofop-methyl and their role in herbicide mechanism of action. Pestci Biochem Physiol 51:68–82. doi:10.1006/pest.1995.1008

Smith AE (1976) Etherification of the hydrolysis product of the herbicide diclofop-methyl in methanol. J Agric Food Chem 24:1077–1078. doi:10.1021/jf60207a021

Smith AE, Grover R, Cessna AJ, Shewchuk SR, Hunter JH (1986) Fate of diclofop-methyl after application to a wheat field. J Environ Qual 15:234–238

Su F, Shao ZR (2005) Chinese pesticide market in 2006: 300 thousand ton active ingredient. Chinese Chem Eng News 5 (Nov 29, 2005)

United Stated Environmental Protection Agency. Prevention, Pesticides and Toxic Substance (7508C) (2000) EPA-738-F-00-007

United Stated Environmental Protection Agency. Prevention, Pesticides and Toxic Substance (7508C) (2000) EPA-738-R-00-009

Williams A (1996) Opportunities for chiral agrochemicals. Pestic Sci 46:3–9. doi:10.1002/(SICI)1096-9063(199601)46:1<3::AID-PS337>3.0.CO;2-J

Wright JP, Shimabukuro RH (1987) Effects of diclofop and diclofop-methyl on the membrane potentials of wheat and oat coleoptiles. Plant Physiol 85:188–193. doi:10.1104/pp.85.1.188

Yao D, Zhen X, Xue Y, Huang J, Li Y (1993) A comparative study on the tolerance to diclofop-methyl between daenel and wheat. Jiangsu J Agr Sci 9:22–25 (in Chinese)

Ye JY, Qian YQ (1985) Spectrophotometry for the hill reaction activity test. In: Xue YL, Xia ZO (eds) Handbook of plant physiological experiment. Shanghai scientific & Technical publisher, Shanghai, pp 104–107