Enantioselective and Synergetic Toxicity of Axial Chiral Herbicide Propisochlor to SP2/0 Myeloma Cells

ARTICLE *in* JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY · AUGUST 2015

Impact Factor: 2.91 · DOI: 10.1021/acs.jafc.5b03027 · Source: PubMed

JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Article

pubs.acs.org/JAFC

Enantioselective and Synergetic Toxicity of Axial Chiral Herbicide Propisochlor to SP2/0 Myeloma Cells

Yao Liu,[†] Xuan Zhang,[†] Chunhong Liu,[†] Ruili Yang,[†] Zhenlin Xu,[†] Lijun Zhou,*[,‡] Yuanming Sun,*[,†] and Hongtao Lei*[,†]

[†]Guangdong Provincial Key Laboratory of Food Quality and Safety, College of Food Science, South China Agricultural University, Guangzhou, Guangdong 510642, People's Republic of China

[‡]Central Laboratory, Navy General Hospital, Beijing 100048, People's Republic of China

ABSTRACT: The axial chiral herbicide propisochlor is used to control weeds. Different enantiomers of a compound usually have different biological activities. It is unclear how the toxicities of the propisochlor enantiomers differ. Propisochlor enantiomers, separated by high-performance liquid chromatography, were tested on SP2/0 myeloma cells. Cytotoxicity and apoptosis were measured, and interactions between the enantiomers were evaluated. The *rac*-propisochlor, pure *R*-(+) isomer, and pure *S*-(−) isomer inhibited cell proliferation and induced apoptosis. The *rac*-propisochlor, *R*-(+) isomer, and *S*-(−) isomer half maximal effective concentration values after 24 h of incubation were 111 ± 0.15, 68 ± 0.09, and 99 ± 0.21 μM, respectively. *R*-(+) isomer induced the most apoptosis. *R*-(+) isomer was ∼1.63 times more cytotoxic than *rac*-propisochlor and ∼1.46 times more cytotoxic than *S*-(−) isomer. Antagonistic cytotoxic interactions were found between *R*-(+) and *S*-(−) isomers. This is the first time the toxicities of these enantiomers and antagonism between the enantiomers have been reported. The antagonism indicates that the ecotoxicological effects of the enantiomers should be investigated.

KEYWORDS: *propisochlor, enantiomers, axial chirality, cytotoxicity, SP2/0*

INTRODUCTION

It is important to understand the modes of action of chiral pesticides, so that the pesticides can be used to optimum effect, meaning that less will need to be used and fewer side effects will occur.[1] Currently, about 28% of the pesticides in use are chiral.[2] Racemic mixtures of some chiral pesticides are applied, but single enantiomers of others are applied.[3] The different enantiomers of a compound can have significantly different affinities for biological receptors, intrinsic activities, and toxicities.[4−6] It is therefore important to understand the toxicity of each enantiomer of a chiral pesticide. The toxicities of different enantiomers of pesticides containing asymmetric chiral centers and the mechanisms through which the enantiomers act have previously been studied. However, the enantioselective toxicities of axial chiral pesticides have not previously been described.

Propisochlor is a chloroacetamide herbicide that contains an asymmetric axis. The two enantiomers of propisochlor are shown in Figure 1. Chloroacetamide herbicides are usually used to eradicate annual grasses and weeds in water and soil, particularly where fruit and vegetables are grown.[7,8] There are many chloroacetamide herbicides, including acetochlor, alachlor, metolachlor, propisochlor, butachlor, and pretilachlor.[9,10] It has previously been found that chloroacetamide herbicides can induce hemolysis, lipid peroxidation, and catalase activity.[11] It has been found that concentrations of *S*-metolachlor that are found in the environment can be spermiotoxic and embryotoxic.[7] Metolachlor has also been found to depress membrane potentials and respiration in rat liver mitochondria[12] and to trigger liver tumors.[13] The photodegradation products of several chloroacetamide herbicides (including acetochlor, alachlor, and metolachlor) have been found to have similar ecotoxicological effects to the parent herbicides.[14] Previous studies of propisochlor have focused on the biodegradation of propisochlor by algae,[15] the detection of propisochlor residues in agricultural products or soil by gas chromatography,[16] the methods involving molecularly imprinted polymers,[17] the photolysis of propisochlor in water, and the environmental distribution of propisochlor under field conditions.[18,19] However, the cytotoxicity of propisochlor has not yet been studied. It is important to thoroughly understand the enantioselective cytotoxicity of propisochlor.

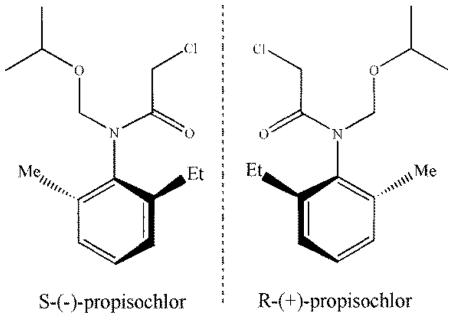

Figure 1. Enantiomers of propisochlor.

In the study presented here, we separated the propisochlor enantiomers by high-performance liquid chromatography (HPLC) using a Chiralpak AD-H column and then investigated

Received: June 19, 2015
Revised: August 18, 2015
Accepted: August 21, 2015
Published: August 24, 2015

DOI: 10.1021/acs.jafc.5b03027
J. Agric. Food Chem. 2015, 63, 7914−7920

the cytotoxicities of the enantiomers for the first time using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium (MTT) method and flow cytometry. Interactions between the propisochlor enantiomers in the cytotoxicity tests were also studied using an isobologram and a combination index (CI). The interaction studies improved our understanding of the cytotoxicities of each of the enantiomers and allowed for the toxic effects caused by interactions between the enantiomers to be identified.

MATERIALS AND METHODS

Reagents. Racemic propisochlor [98%, 2-chloro-*N*-(isopropylmethyl)-*N*-(2-ethylmethyl-6-methyl)phenylacetamide], MTT, dimethyl sulfoxide, culture medium RPMI-1640, penicillin, and streptomycin were purchased from Sigma (St. Louis, MO). Fetal bovine serum was purchased from Hangzhou Sijiqing Biological Engineering Materials Co. (Hangzhou, China). An Annexin V-fluorescein isothiocyanate (FITC)/propidium iodide (PI) apoptosis detection kit and a 4′,6-diamidino-2-phenylindole (DAPI) staining kit were purchased from the Nanjing Jiancheng Bioengineering Institute (Nanjing, China). A Chiralpak AD-H column [150 mm long, 4.6 mm inner diameter, with an amylose tris(3,5-dimethylphenylcarbamate) stationary phase] was obtained from Daicel (Tokyo, Japan). Other solvents and chemicals were of chromatographic or analytical grade.

Separating Propisochlor Enantiomers by HPLC. The propisochlor enantiomers were separated using a Shimadzu LC-20A series HPLC system equipped with a LC-20AT intelligent gradient pump, a SPD-20A ultraviolet−visible (UV−vis) light absorbance detector, a CTO-10AS vp temperature-controlled column compartment, a SIL-20A automatic sampler, and a CMB-20A data collector (Shimadzu, Kyoto, Japan). A Chiralpak AD-H column, kept at 35 °C, was used in the HPLC system. The mobile phase was a 99:1 (by volume) mixture of *n*-hexane and isopropanol. The fractions containing the separated propisochlor enantiomers were collected automatically at the outlet and then evaporated to dryness under vacuum. The separate enantiomers obtained in this way were used in the subsequent experiments.

Cell Culture. SP2/0 cells were cultured in RPMI-1640 medium containing 10% (v/v) fetal bovine serum. Cells were incubated with different concentrations of *rac*-propisochlor, *R*-(+) isomer, and *S*-(−) isomer for 12, 24, or 48 h. Interactions between *R*-(+) and *S*-(−) isomers were identified by exposing cells to mixtures of the enantiomers at different ratios for 24 h. The cells were analyzed at the end of each test using the MTT assay.

Assessing Cell Viability. The enantioselective effects of propisochlor on SP2/0 cell viability were examined by assessing the growth of cells exposed to propisochlor using the MTT assay. Aliquots of the cells were taken, and the medium in each was replaced with medium containing 0, 10, 50, 100, 200, 400, 600, or 800 μM *rac*-propisochlor, *R*-(+) isomer, or *S*-(−) isomer. The cultures were then incubated for 12, 24, or 48 h, and then 20 μL of MTT (1 mg/mL) was added to each well. The plate was incubated at 37 °C for another 4 h. The cultured cells were then collected and centrifuged. The supernatant was discarded, and then 150 μL of dimethyl sulfoxide were added to each well. The cells were then shaken for 10 min, and the absorbance at 490 nm was measured using a 1420 multilabel counter (PerkinElmer, Waltham, MA). The experiments were carried out using 96-well plates, and four replicates of each test were performed. Cell viability was calculated using the equation

$$\text{cell viability (\% of control)} = \frac{\text{OD}_{490\text{ nm}} \text{ of the test wells}}{\text{OD}_{490\text{ nm}} \text{ of the control wells}} \times 100$$

where $\text{OD}_{490\text{ nm}}$ is the optical density at 490 nm.

The half maximal effective concentration (EC_{50}) values were calculated using nonlinear statistical regressions, performed using GraphPad Prism 5.0.1 software.[20] The result for each test is later expressed as the mean ± standard deviation of the four independent replicates of the test.

Annexin V-FITC/PI Double Staining. The Annexin V-FITC/PI double staining assay was used to determine the effect of propisochlor on apoptosis in the SP2/0 cells. Aliquots of the SP2/0 cells were taken, and each was treated with a 0, 10, 50, 100, or 200 μM solution of *rac*-propisochlor, *R*-(+) isomer, or *S*-(−) isomer for 24 h. The cells were then centrifuged at 1000 rpm for 10 min, and the supernatant solutions were discarded. The precipitated cells were then washed twice with phosphate-buffered saline, resuspended in 500 μL of binding buffer, and then stained with 5 μL of Annexin V-FITC and 5 μL of PI. The cells were then stored in the dark for 5−15 min at room temperature. The stained cells were then analyzed by flow cytometry using a Cytomics TMFC500 instrument (Beckman Coulter, Brea, CA). Four replicates of each test were performed. The result for each test is later expressed as the mean ± standard deviation of the four independent replicates of the test. Statistical analyses of the apoptosis ratios of the SP2/0 cells exposed to propisochlor were performed using SPSS 19.0 software (IBM, Armonk, NY).

Observing Changes in Cellular Morphology Induced by Propisochlor. Growing SP2/0 cells were allowed to adhere to glass plates. The SP2/0 cells on the glass plates were exposed to different concentrations of *rac*-propisochlor, *R*-(+) isomer, and *S*-(−) isomer for 24 h, and then the cells were harvested and fixed with 4% polyformaldehyde for 10 min and then washed 3 times with phosphate-buffered saline (PBS). One drop of DAPI staining solution was placed on each glass plate, and the plates were stored in the dark. The morphologies of the cells on the plates were observed, and photographs were taken using a fluorescence microscope (Nikon, Tokyo, Japan).

Interactions between Propisochlor Enantiomers. A 100 μL aliquot of the RPMI-1640 medium containing SP2/0 cells (giving 3×10^5 cells/well) was added to each well of a 96-well culture plate. To each well was added a 5:1, 3:1, 1:1, 1:3, or 1:5 mixture of *R*-(+) and *S*-(−) isomers of propisochlor to give a final total propisochlor concentration of 0, 10, 50, 100, 200, 400, 600, or 800 μM. The cells were then incubated for 24 h, and then cell viability was assessed as described in the "Assessing Cell Viability" subsection above.

Interactions between *R*-(+) and *S*-(−) isomers were identified using an isobologram and using CI values.[21−23] A normalized isobologram for *R*-(+) and *S*-(−) isomers at their EC_{50} values was constructed using OriginPro 8.5.1 software (OriginLab, Northampton, MA). Interactions between the isomers were identified from the positions of the EC_{50} values for the five isomer mixture compositions that were used in the normalized isobologram. The normalized isobologram was divided into three areas, representing synergism (area A), additive effects (area B), and antagonism (area C), as shown in Figure 6. The CI values were calculated using the formula

$$\text{CI} = \frac{a}{A} + \frac{b}{B}$$

where *A* and *B* are the concentrations of the separate enantiomers required to produce the same effect as the combination tested and *a* and *b* are the proportions of the two enantiomers in the combination tested.

A CI value less than 0.9 was taken to indicate synergism; a CI value higher than 1.1 was taken to indicate antagonism; and a CI value between 0.9 and 1.1 was taken to indicate additivity.

RESULTS

Chiral Resolution of the Enantiomers. Baseline separation between the two enantiomers in the *rac*-propisochlor was achieved using the Chiralpak AD-H column at 35 °C with a 99:1 (by volume) mixture of *n*-hexane and isopropanol mobile phase at a flow rate of 1 mL/min. A resolution of 2.41 was achieved, and the results are shown in Figure 2. The retention mechanism and the enantiomer selection at the temperature used were the same as we have found previously. We concluded that the propisochlor enantiomers were separated and isolated successfully. We have previously demonstrated that *R*-(+)

DOI: 10.1021/acs.jafc.5b03027
J. Agric. Food Chem. 2015, 63, 7914−7920

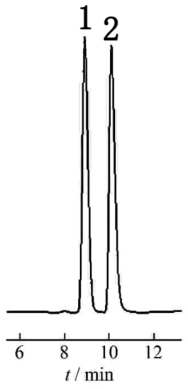

Figure 2. Chromatogram of propisochlor on the Chiralpak AD-H column with a mobile phase *n*-hexane/isopropanol (99:1, v/v), flow velocity of 1 mL/min, and column temperature at 35 °C. Peak 1 was *R*-(+) isomer enantiomer, and peak 2 was *S*-(−) isomer enantiomer.

isomer eluted first (peak 1) and *S*-(−) isomer eluted second (peak 2).

Enantioselective Cytotoxicity to SP2/0 Cells. SP2/0 cells were incubated with different concentrations of *rac*-propisochlor, *R*-(+) isomer, and *S*-(−) isomer for 12, 24, and 48 h. As shown in Figure 3 and Table 1, the cell viability decreased as the *rac*-propisochlor, *R*-(+) isomer, and *S*-(−) isomer concentrations increased. The EC_{50} values for *rac*-propisochlor, *R*-(+) isomer, and *S*-(−) isomer after 24 h of incubation were 111 ± 0.15, 68 ± 0.09, and 99 ± 0.21 μM, respectively. According to the EC_{50} values, the cytotoxicity to SP2/0 cells decreased in the order *R*-(+) isomer > *S*-(−) isomer > *rac*-propisochlor. The cells exposed to 200 μM propisochlor (as *rac*-propisochlor, *R*-(+) isomer, or *S*-(−) isomer) were 20% less viable than the control group cells after 24 h.

Apoptosis occurred in more than 70% of the cells treated with a propisochlor [as *rac*-propisochlor, *R*-(+) isomer, or *S*-(−) isomer] concentration higher than 50 μM, and apoptosis occurred in more cells treated with *R*-(+) isomer than with *rac*-propisochlor or *S*-(−) isomer, as shown in Figure 4. We concluded from the cell viability and apoptosis analyses that *R*-(+) isomer is more toxic than *rac*-propisochlor and *S*-(−) isomer to SP2/0 cells. The apoptosis ratios for the SP2/0 cells exposed to propisochlor were statistically analyzed using SPSS 19.0, and the results are shown in Figure 4, in which asterisks indicate significant differences ($p < 0.05$).

Changes in Cellular Morphology. Nuclear pyknosis is a reliable indicator of cell damage.[24] The nuclei of untreated SP2/0 cells were integrated and homogeneous, as seen in Figure 5D. The nuclei of cells exposed to 50 μM *rac*-propisochlor were similar to the nuclei of untreated cells, as seen in Figure 5A1. Pyknosis occurred in cells exposed to *rac*-propisochlor at concentrations higher than 50 μM (Figure 5A2). Pyknosis occurred in the nuclei of cells treated with *R*-(+) isomer at a concentration of 50 μM and cells treated with *S*-(−) isomer at a concentration of 200 μM, as seen in panels B and C of Figure 5. These results suggest that *rac*-propisochlor, *R*-(+) isomer, and *S*-(−) isomer may all affect the vitalities of and apoptosis in SP2/0 cells by affecting the cell nuclei.

Interactions between the Propisochlor Enantiomers. Interactions between the propisochlor enantiomers were investigated by analyzing the effects on the SP2/0 cells of combinations of *R*-(+) and *S*-(−) isomers at five different

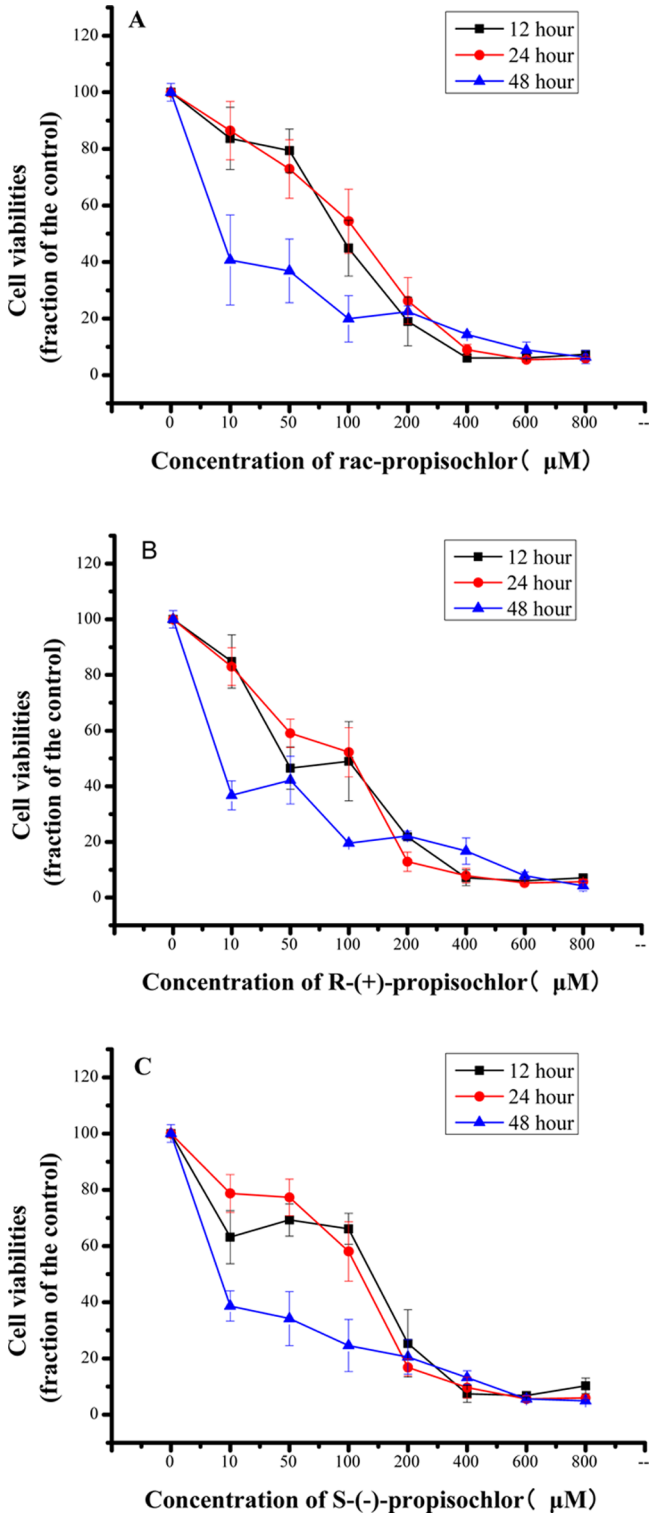

Figure 3. Effects of (A) *rac*-propisochlor, (B) *R*-(+) isomer, and (C) *S*-(−) isomer on the cell viabilities of SP2/0 were analyzed by the MTT assay after exposure to varying concentrations of propisochlor for 12, 24, or 48 h.

ratios. A dose−response curve and an EC_{50} value were produced for each combination. The results of the interaction analyses are shown in Figures 6 and 7, and the EC_{50} values are shown in Table 2. The CI values were less than 0.9 when the *R*-(+) to *S*-(−) isomer ratio was 5:1, 3:1, or 1:5, indicating that the enantiomers interacted synergistically. However, antago-

DOI: 10.1021/acs.jafc.5b03027
J. Agric. Food Chem. 2015, 63, 7914−7920

Table 1. EC_{50} Values of *rac*-Propisochlor, *R*-(+) Isomer, and *S*-(−) Isomer after Exposure to Varying Concentrations of Propisochlor for 24 h

propisochlor	EC_{50} (μM)	95% confidence intervals	coefficient of determination (R^2)
rac-propisochlor	111 ± 0.15	96.13−127.3	0.97
R-(+) isomer	68 ± 0.09	50.73−90.49	0.93
S-(−) isomer	99 ± 0.21	86.79−112.1	0.97

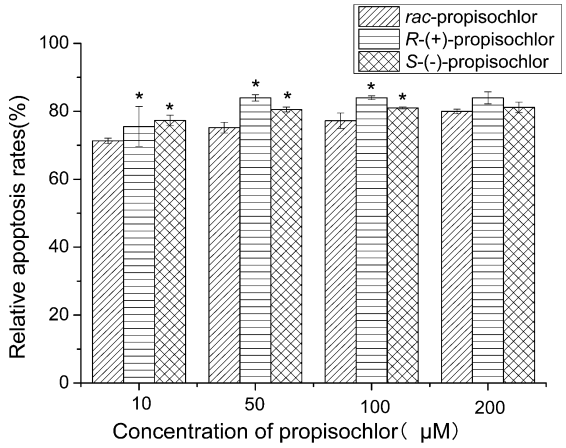

Figure 4. Apoptosis rates of SP2/0 after treated with propisochlor for 24 h. The apoptosis rates of SP2/0 cells were detected by flow cytometry after staining with Annexin V-FITC/PI. The apoptosis rates of SP2/0 after treating with *R*-(+) isomer were higher those of *rac*-propisochlor and *S*-(−) isomer. The asterisks represented significant difference ($p < 0.05$).

nistic interactions (CI > 1.1) were found when the *R*-(+) to *S*-(−) isomer ratio was 1:1 or 3:1. These results show that *R*-(+) and *S*-(−) isomers in *rac*-propisochlor could interact, resulting in less cytotoxicity occurring than if either was present alone.

DISCUSSION

We assessed the cytotoxicities of *rac*-propisochlor and both enantiomers. The EC_{50} values for *R*-(+) and *S*-(−) isomers, determined from the MTT analysis results, were 68 ± 0.09 and 99 ± 0.21 μM, respectively, as shown in Table 1. *R*-(+) isomer was found to be 1.46 times more cytotoxic than *S*-(−) isomer to the SP2/0 cells. As shown in Figure 3, the cytotoxicity increased more rapidly as the concentration of *R*-(+) isomer was increased from 0 to 400 μM than when the concentration of *S*-(−) isomer was increased from 0 to 400 μM. Less than 40% of the cells exposed to either enantiomer for 48 h remained viable, and as little as 20% of the cells remained viable at concentrations of 200 μM. Several studies focused on the enantioselective cytotoxicities of pesticides with asymmetric chiral centers have been performed, and these studies have shown that the enantiomers of chiral pesticides may have different biological activities both *in vitro* and *in vivo*.[25] Interestingly, our results showed that the cytotoxicity of the axial chiral herbicide propisochlor is also enantioselective, with *R*-(+) isomer being more toxic than *S*-(−) isomer to SP2/0 cells.

The differences between the cytotoxicities of the propisochlor enantiomers were investigated further by identifying differences between the abilities of the enantiomers to induce apoptosis in SP2/0 cells and changes in the cell nuclei. The relative amounts of apoptosis induced in SP2/0 cells by *rac*-

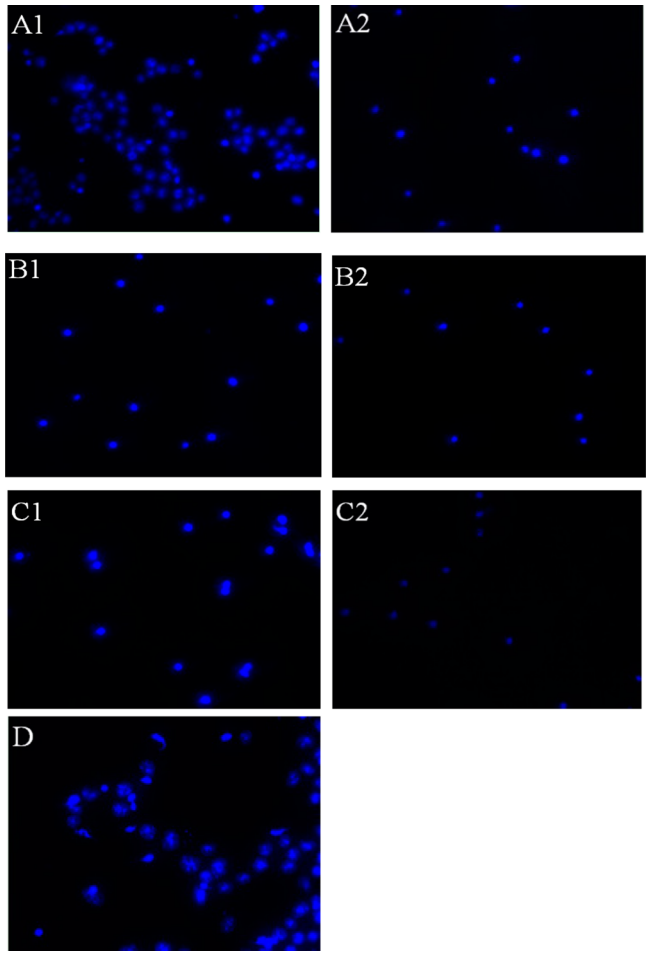

Figure 5. Cellular morphology change induced by different concentrations of propisochlor (400×): (A1 and A2) 50 and 200 μM *rac*-propisochlor, (B1 and B2) 50 and 200 μM *R*-(+) isomer, (C1 and C2) 50 and 200 μM *S*-(−) isomer, and (D) control group.

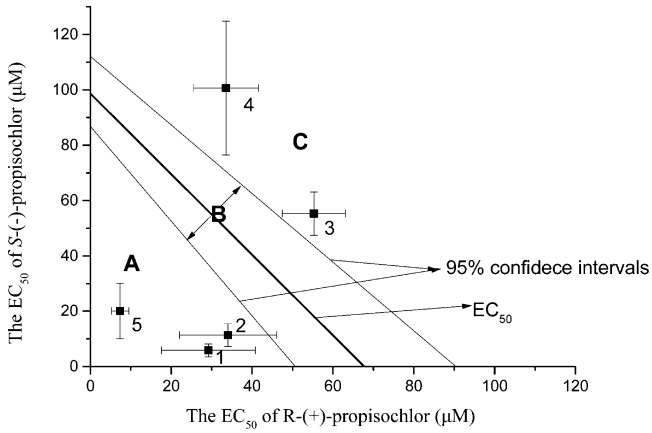

Figure 6. Normalized isobologram analyzes the interaction of the two propisochlor enantiomers. The numbers 1, 2, 3, 4, and 5 mean that the rates of *R*-(+) isomer and *S*-(−) isomer were 5:1, 3:1, 1:1, 1:3, and 1:5, respectively. The three areas of the normalized isobologram were, namely, A, B, and C, which represent synergism, additive, and antagonism, respectively. According to the positions of the five combinations, the positions of 1, 2, and 5 exhibit synergism, while the positions of 3 and 4 exhibit antagonism.

DOI: 10.1021/acs.jafc.5b03027
J. Agric. Food Chem. 2015, 63, 7914−7920

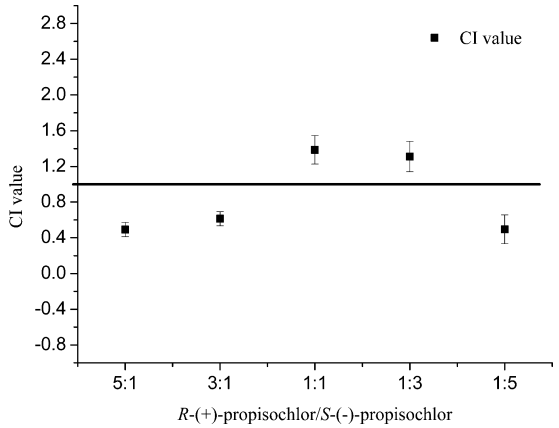

Figure 7. CI values of different rates of *R*-(+) isomer and *S*-(−) isomer. After exposure with different combinations of *R*-(+) isomer and *S*-(−) isomer (5:1, 3:1, 1:1, 1:3, and 1:5, respectively), the CI values were calculated by the formula showed as 2.7. CI values were less than 0.9, between 0.9 and 1.1, more than 1.1, which indicated synergy, additivity, and antagonism, respectively. When the ratio of *R*-(+) isomer and *S*-(−) isomer was 5:1, 3:1, and 1:5, the CI values indicated synergy. However, the CI values indicated antagonism when the ratio of *R*-(+) isomer and *S*-(−) isomer was 1:1 and 1:3.

Table 2. Interaction of Two Propisochlor Enantiomers

rates of *R*-(+) isomer and *S*-(−) isomer	EC_{50} (μM)	95% confidence intervals	coefficient of determination (R^2)	CI
1:0	67.75	50.73−90.49	0.93	
5:1	35.03	23.81−51.55	0.91	0.493 ± 0.08
3:1	45.40	32.04−64.31	0.91	0.614 ± 0.079
1:1	110.6	96.13−127.3	0.97	1.385 ± 0.16
1:3	134.2	105.7−170.3	0.92	1.31 ± 0.17
1:5	44.17	29.8−66.20	0.94	0.495 ± 0.16
0:1	98.64	86.79−112.1	0.97	

propisochlor and the separate enantiomers after 24 h of exposure are shown in Figure 4. The apoptosis rates were higher in the SP2/0 cells treated with *R*-(+) isomer than in the cells treated with *S*-(−) isomer. Changes in the cell nuclei were seen when the cells were exposed to *rac*-propisochlor, *R*-(+) isomer, and *S*-(−) isomer for 24 h. As seen in Figure 5A1, the amount of pyknosis that occurred in the cell nuclei increased as the concentration of *rac*-propisochlor or either isomer increased but similar amounts of pyknosis occurred in cells exposed to 50 μM *rac*-propisochlor and untreated cells. Various factors can influence cell vitality and apoptosis. In this study, *rac*-propisochlor and *R*-(+) and *S*-(−) isomers may have affected SP2/0 cell vitality and apoptosis by causing changes to occur in the cell nuclei.

The cytotoxic mechanisms of chiral pesticides have been described in previous publications.[26−32] Bifenthrin (BF) is a synthetic pyrethroid (SP). SPs are commonly used to control agricultural and indoor insect pests. It has been found that *cis*-BF can induce apoptosis through the enantioselective activation of the JNK/MAPK signaling pathway, causing changes in the control of intracellular reactive oxygen species (ROS) and allowing DNA damage to occur. SPs have also been found to be estrogenic endocrine-disrupting chemicals (EDCs). It has been shown that *S*-(+)-acetofenate (AF) is clearly more toxic than *R*-(−)-AF and *rac*-AF to macrophages and that *S*-(+)-AF induces the generation of intracellular ROS, causes DNA damage, and upregulates p53 gene expression.[6] Some chiral herbicides have been found to induce the generation of ROS, increase the malondialdehyde (MDA) concentration, increase superoxide dismutase (SOD) activity, and trigger toxin production.[28] Chiral pesticides and herbicides have been found to cause enantioselective ecotoxicological effects.[33−36] The effects of the chiral herbicide diclofop-methyl (DM) and its major metabolite diclofop acid (DA) on *Microcystis aeruginosa* have been studied, and *rac*-DA was found to be more toxic than DM, *R*-DA, and *S*-DA.[37] The results described above significantly improved our understanding of the cytotoxicities of pesticides with asymmetric chiral centers and the risks that they pose to ecological and human health. The enantioselective cytotoxicities of pesticides with asymmetric chiral axes have seldom been studied. We have shown here that the cytotoxicity of the axial chiral herbicide propisochlor is enantioselective, although we have not identified the specific toxic mechanisms involved.

The EC_{50} values showed that *rac*-propisochlor, *R*-(+) isomer, and *S*-(−) isomer had different cytotoxicities to SP2/0 cells; therefore, we supposed that a combination effect could have occurred when SP2/0 cells were exposed to both isomers simultaneously. We therefore used an isobologram and CI values to identify interactions between *R*-(+) and *S*-(−) isomers.[21,22] At least three types of interactions (synergism, additive, and antagonism) can occur between two different chemicals interacting with a cell line.[38] Analysis of our isobologram and our CI values showed that antagonism occurred when the *R*-(+) to *S*-(−) isomer ratio was 1:1 (Figures 6 and 7). To the best of our knowledge, this is the first time an interaction between *R*-(+) and *S*-(−) isomers of propisochlor has been found.

Propisochlor is an axial chiral herbicide with two enantiomers, *R*-(+) and *S*-(−) isomers. Propisochlor belongs to the chloroacetamide group of herbicides, which is the most widely used group of herbicides around the world. It has been shown in several studies that applying mixtures of different chloroacetamide herbicides markedly increases the effectiveness with which weeds are killed. Metolachlor is another chloroacetamide herbicide. Applying high doses of dimethenamid-p and *S*-metolachlor together has been found to cause transient injuries to but not to interfere with the maturities and yields of pinto and small red Mexican beans.[39] However, the effects of the co-exposure of non-target organisms to different herbicides and specific interactions between different herbicides have not previously been reported. In this study, we co-exposed SP2/0 cells to both propisochlor isomers and found that some *R*-(+) to *S*-(−) isomer ratios gave synergistic cytotoxic effects and other ratios gave antagonistic cytotoxic effects. A relatively environmentally benign herbicide must be a broad-spectrum herbicide, interfere little with the crops that it is applied to, and must have few toxic effects on non-target organisms. Mixtures of different herbicides, including with different isomer ratios, may prove to be useful treatments.

We investigated the cytotoxicities of *rac*-propisochlor and *R*-(+) and *S*-(−) isomers of propisochlor and the interactions between the two enantiomers for the first time. We found that *R*-(+) isomer is more toxic than *S*-(−) isomer and *rac*-propisochlor. The apoptosis rates that we found showed that *R*-(+) and *S*-(−) isomers may have different biological activities. More importantly, we found that *R*-(+) and *S*-(−) isomers can interact antagonistically, meaning that *rac*-propisochlor is less cytotoxic than either enantiomer. Our results suggest that interactions between enantiomers should be considered when

DOI: 10.1021/acs.jafc.5b03027
J. Agric. Food Chem. 2015, 63, 7914−7920

developing and using chiral pesticides and that this may result in a decrease in pesticide application rates and reduced harm to the environment.

AUTHOR INFORMATION

Corresponding Authors

*E-mail: hzzhoulj@126.com.
*E-mail: ymsun@scau.edu.cn.
*Telephone: +86-20-8528-3448. Fax: +86-20-8528-0270. E-mail: hongtao@scau.edu.cn.

Funding

This work was supported by Guangdong Natural Science Foundation (S2013030013338), Natural Science Foundation of China (U1301214), Guangdong Planed Program in Science and Technology (2013B051000072, 2014TX01N250, 2012A020100002), and National Basic Research Program of China (973 Program, 2012CB720803).

Notes

The authors declare no competing financial interest.

ABBREVIATIONS USED

HPLC, high-performance liquid chromatography; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium; EC_{50}, half maximal effective concentration; CI, combination index; BF, bifenthrin; SP, synthetic pyrethroid; EDC, endocrine-disrupting chemical; AF, acetofenate; ROS, reactive oxygen species; MDA, malondialdehyde; SOD, superoxide dismutase; DM, diclofop-methyl; DA, diclofop acid

REFERENCES

(1) Nillos, M. G.; Gan, J.; Schlenk, D. Chirality of organophosphorus pesticides: analysis and toxicity. *J. Chromatogr. B: Anal. Technol. Biomed. Life Sci.* **2010**, *878*, 1277−1284.

(2) Ulrich, E. M.; Morrison, C. N.; Goldsmith, M. R.; Foreman, W. T. Chiral pesticides: identification, description, and environmental implications. *Rev. Environ. Contamt.* **2012**, *217*, 1−74.

(3) Leone, A. D.; Amato, S.; Falconer, R. L. Emission of chiral organochlorine pesticides from agricultural soils in the cornbelt region of the U.S. *Environ. Sci. Technol.* **2001**, *35*, 4592−4596.

(4) Kurt-Karakus, P. B.; Bidleman, T. F.; Jones, K. C. Chiral organochlorine pesticide signatures in global background soils. *Environ. Sci. Technol.* **2005**, *39*, 8671−8677.

(5) Zhao, M.; Wang, C.; Zhang, C.; Wen, Y.; Liu, W. Enantioselective cytotoxicity profile of o,p′-DDT in PC 12 cells. *PLoS One* **2012**, *7*, e43823.

(6) Zhao, M.; Liu, W. Enantioselectivity in the immunotoxicity of the insecticide acetofenate in an in vitro model. *Environ. Toxicol. Chem.* **2009**, *28*, 578−585.

(7) Mai, H.; Morin, B.; Pardon, P.; Gonzalez, P.; Budzinski, H.; Cachot, J. Environmental concentrations of irgarol, diuron and S-metolachlor induce deleterious effects on gametes and embryos of the Pacific oyster, Crassostrea gigas. *Mar. Environ. Res.* **2013**, *89*, 1−8.

(8) Hladik, M. L.; Bouwer, E. J.; Roberts, A. L. Neutral chloroacetamide herbicide degradates and related compounds in Midwestern United States drinking water sources. *Sci. Total Environ.* **2008**, *390*, 155−165.

(9) Zhang, J.; Zheng, J. W.; Liang, B.; Wang, C. H.; Cai, S.; Ni, Y. Y.; He, J.; Li, S. P. Biodegradation of chloroacetamide herbicides by Paracoccus sp. FLY-8 in vitro. *J. Agric. Food Chem.* **2011**, *59*, 4614−4621.

(10) Park, S. G.; Jin, H.; Thangadurai, D. T.; Yoo, Y. J.; Lee, Y. I. Detoxification of cytotoxic alachlor by glutathione: characterization of conjugated adducts by electrospray ionization tandem mass spectrometry. *J. Agric. Food Chem.* **2009**, *57*, 9838−9847.

(11) Bernasinska, J.; Duchnowicz, P.; Koter-Michalak, M.; Koceva-Chyla, A. Effect of safeners on damage of human erythrocytes treated with chloroacetamide herbicides. *Environ. Toxicol. Pharmacol.* **2013**, *36*, 368−377.

(12) Pereira, S. P.; Fernandes, M. A.; Martins, J. D.; Santos, M. S.; Moreno, A. J.; Vicente, J. A.; Videira, R. A.; Jurado, A. S. Toxicity assessment of the herbicide metolachlor comparative effects on bacterial and mitochondrial model systems. *Toxicol. In Vitro* **2009**, *23*, 1585−1590.

(13) Coleman, S.; Linderman, R.; Hodgson, E.; Rose, R. L. Comparative metabolism of chloroacetamide herbicides and selected metabolites in human and rat liver microsomes. *Environ. Health Perspect.* **2000**, *108*, 1151−1157.

(14) Souissi, Y.; Bouchonnet, S.; Bourcier, S.; Kusk, K. O.; Sablier, M.; Andersen, H. R. Identification and ecotoxicity of degradation products of chloroacetamide herbicides from UV-treatment of water. *Sci. Total Environ.* **2013**, *458*−*460*, 527−534.

(15) Rauch, R.; Foldenyi, R. The effect of alginite on the decomposition of the herbicide propisochlor. *J. Environ. Sci. Health, Part B* **2012**, *47*, 670−676.

(16) Hu, J. Y.; Zhen, Z. H.; Deng, Z. B. Simultaneous determination of acetochlor and propisochlor residues in corn and soil by solid phase extraction and gas chromatography with electron capture detection. *Bull. Environ. Contam. Toxicol.* **2011**, *86*, 95−100.

(17) Hu, X.; Dai, G.; Huang, J.; Ye, T.; Fan, H.; Youwen, T.; Yu, Y.; Liang, Y. Molecularly imprinted polymer coated on stainless steel fiber for solid-phase microextraction of chloroacetanilide herbicides in soybean and corn. *J. Chromatogr. A* **2010**, *1217*, 5875−5882.

(18) Huang, Q.; Li, F.; Wang, M.; Wang, Y.; Gu, Q.; Wang, Q. Photolysis behavior of herbicide propisochlor in water media and preliminary analysis of photoproducts under different light sources. *J. Environ. Sci. Health, Part B* **2006**, *41*, 253−267.

(19) Konda, L. N.; Pasztor, Z. Environmental distribution of acetochlor, atrazine, chlorpyrifos, and propisochlor under field conditions. *J. Agric. Food Chem.* **2001**, *49*, 3859−3863.

(20) Xu, X.; Jiang, J.; Wang, X.; Shen, Z.; Li, R.; Zhou, Z. Stereoselective metabolism and toxicity of the herbicide fluroxypyr methylheptyl ester in rat hepatocytes. *Chirality* **2011**, *23*, 860−866.

(21) Chou, T. C.; Talalay, P. Quantitative analysis of dose-effect relationships: the combined effects of multiple drugs or enzyme inhibitors. *Adv. Enzyme Regul.* **1984**, *22*, 27−55.

(22) Tsakalozou, E.; Eckman, A. M.; Bae, Y. Combination effects of docetaxel and Doxorubicin in hormone-refractory prostate cancer cells. *Biochem. Res. Int.* **2012**, *2012*, 1−10.

(23) Park, S. O.; Yoo, Y. B.; Kim, Y. H.; Baek, K. J.; Yang, J. H.; Choi, P. C.; Lee, J. H.; Lee, K. R.; Park, K. S. Effects of combination therapy of docetaxel with selenium on the human breast cancer cell lines MDA-MB-231 and MCF-7. *Ann. Surg. Treat. Res.* **2015**, *88*, 55−62.

(24) Wohlan, K.; Goy, S.; Olling, A.; Srivaratharajan, S.; Tatge, H.; Genth, H.; Gerhard, R. Pyknotic cell death induced by Clostridium difficile TcdB: chromatin condensation and nuclear blister are induced independently of the glucosyltransferase activity. *Cell. Microbiol.* **2014**, *16*, 1678−1692.

(25) Liu, H.; Zhao, M.; Zhang, C.; Ma, Y.; Liu, W. Enantioselective cytotoxicity of the insecticide bifenthrin on a human amnion epithelial (FL) cell line. *Toxicology* **2008**, *253*, 89−96.

(26) Hu, F.; Li, L.; Wang, C.; Zhang, Q.; Zhang, X.; Zhao, M. Enantioselective induction of oxidative stress by permethrin in rat adrenal pheochromocytoma (PC12) cells. *Environ. Toxicol. Chem.* **2010**, *29*, 683−690.

(27) Liu, H. J.; Cai, W. D.; Huang, R. N.; Xia, H. L.; Wen, Y. Z. Enantioselective toxicity of metolachlor to Scenedesmus obliquus in the presence of cyclodextrins. *Chirality* **2012**, *24*, 181−187.

(28) Wen, Y.; Li, C.; Fang, Z.; Zhuang, S.; Liu, W. Elucidation of the enantioselective enzymatic hydrolysis of chiral herbicide dichlorprop methyl by chemical modification. *J. Agric. Food Chem.* **2011**, *59*, 1924−1930.

(29) Ye, J.; Zhang, Y.; Chen, S.; Liu, C.; Zhu, Y.; Liu, W. Enantioselective changes in oxidative stress and toxin release in

DOI: 10.1021/acs.jafc.5b03027
J. Agric. Food Chem. 2015, 63, 7914−7920

Microcystis aeruginosa exposed to chiral herbicide diclofop acid. *Aquat. Toxicol.* **2014**, *146*, 12−19.
(30) Wang, C.; Li, Z.; Zhang, Q.; Zhao, M.; Liu, W. Enantioselective induction of cytotoxicity by o,p′-DDD in PC12 cells: implications of chirality in risk assessment of POPs metabolites. *Environ. Sci. Technol.* **2013**, *47*, 3909−3917.
(31) Boerth, D. W.; Eder, E.; Stanks, J. R.; Wanek, P.; Wacker, M.; Gaulitz, S.; Skypeck, D.; Pandolfo, D.; Yashin, M. DNA adducts as biomarkers for oxidative and genotoxic stress from pesticides in crop plants. *J. Agric. Food Chem.* **2008**, *56*, 6751−6760.
(32) de Oliveira, J. L.; Campos, E. V.; Goncalves da Silva, C. M.; Pasquoto, T.; Lima, R.; Fraceto, L. F. Solid lipid nanoparticles co-loaded with simazine and atrazine: preparation, characterization, and evaluation of herbicidal activity. *J. Agric. Food Chem.* **2015**, *63*, 422−432.
(33) Jarman, J. L.; Jones, W. J.; Howell, L. A.; Garrison, A. W. Application of capillary electrophoresis to study the enantioselective transformation of five chiral pesticides in aerobic soil slurries. *J. Agric. Food Chem.* **2005**, *53*, 6175−6182.
(34) Cai, X.; Liu, W.; Sheng, G. Enantioselective degradation and ecotoxicity of the chiral herbicide diclofop in three freshwater alga cultures. *J. Agric. Food Chem.* **2008**, *56*, 2139−2146.
(35) Bidleman, T. F.; Leone, A. D.; Falconer, R. L.; Harner, T.; Jantunen, L. M.; Wiberg, K.; Helm, P. A.; Diamond, M. L.; Loo, B. Chiral pesticides in soil and water and exchange with the atmosphere. *Sci. World J.* **2002**, *2*, 357−373.
(36) Celis, R.; Gamiz, B.; Adelino, M. A.; Hermosin, M. C.; Cornejo, J. Environmental behavior of the enantiomers of the chiral fungicide metalaxyl in Mediterranean agricultural soils. *Sci. Total Environ.* **2013**, *444*, 288−297.
(37) Ye, J.; Wang, L.; Zhang, Z.; Liu, W. Enantioselective physiological effects of the herbicide diclofop on cyanobacterium Microcystis aeruginosa. *Environ. Sci. Technol.* **2013**, *47*, 3893−3901.
(38) Wang, H. W.; Wang, J. Q.; Zheng, B. Q.; Li, S. L.; Zhang, Y. D.; Li, F. D.; Zheng, N. Cytotoxicity induced by ochratoxin A, zearalenone, and alpha-zearalenol: effects of individual and combined treatment. *Food Chem. Toxicol.* **2014**, *71*, 217−224.
(39) Sikkema, P. H.; Robinson, D. E.; Nurse, R. E.; Soltani, N. Pre-emergence herbicides for potential use in pinto and small red mexican bean (Phaseolus vulgaris) production. *Crop Prot.* **2008**, *27*, 124−129.

DOI: 10.1021/acs.jafc.5b03027
J. Agric. Food Chem. 2015, 63, 7914−7920