

# THE EFFECT OF SOME PESTICIDES ON ACETYLCHOLINESTERASE ACTIVITY FROM BOVINE BRAINE

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## ABSTRACT

Acetylcholinesterase (AChE) inhibitors are widely used for a variety of medical, agricultural and public health purposes. Consequently, exposure is highly possible during lifetime. Therefore, the aim of this study was to investigate *in vitro* the effects of pesticides such as cypermethrin, fenprothrin, 1,1-D-methylpiperidinium chloride, N-(phosphonomethyl)glycine,  $\beta$ -naphthoxyacetic acid, lambda-cyhalothrin, deltamethrin, and 2,4-dichlorophenoxy acetic acid on AChE activity obtained from bovine brain. Pesticides showed different effects on AChE activity. The AChE enzyme was inhibited by cypermethrin, fenprothrin, 1,1-D-methylpiperidinium chloride, N-(phosphonomethyl)glycine, and  $\beta$ -naphthoxyacetic acid pesticides and activated by lambda-cyhalothrin and deltamethrin pesticides. 2,4-dichlorophenoxy acetic acid pesticide did not alter the activity of AChE enzyme. AChE activity was inhibited in a concentration-dependent manner, and calculated  $IC_{50}$  values for cypermethrin ( $IC_{50}=24.45$  mM), fenprothrin ( $IC_{50}=16.95$  mM), 1,1-D-methylpiperidinium chloride ( $IC_{50}=69.09$  mM), N-(phosphonomethyl)glycine ( $IC_{50}=585$  mM), and  $\beta$ -naphthoxyacetic acid ( $IC_{50}=16.34$  mM).

## KEYWORDS:

Acetylcholinesterase, inhibition, pesticides

## INTRODUCTION

Pesticides commonly used throughout the world are chemicals used as agriculture, industry, domestic and war materials. They are highly toxic compounds. The broad use of the pesticides in agriculture leads to a serious public health issue with about 3,000,000 acute intoxications and over 200,000 fatalities annually worldwide, mostly in developing World [1]. Due to the increase in agricultural and industrial use, pesticides are among the most important environmental pollutants. Small amounts remaining in the environment after agricultural use have been detected in ground, foodstuffs, drinking water and sea life, posing a potential risk to

living systems. Consequently, people are unavoidably exposed via inhalation, skin permeation or ingestion of contaminated food [2, 3]. These chemicals cause adverse effects by affecting the physiological and behavioral systems in non-targeted organisms throughout the World [4-6]. Therefore, the presence of these chemicals in the environment is a global issue which may pose a threat to living systems. Intensive use of pesticides causes also pollution of food and groundwater. Dispersions of pesticides in air, water, soil and organisms cause various physical, chemical and biological changes. At the same time, pesticides can be easily dispersed into the tissues in the body by being easily absorbed by oral, dermal and respiratory means [7].

There are some studies related to the effect of pesticides taken in different ways into human body to antioxidant and other enzyme systems. For example, Diken et al. (2017) investigated *in vitro* the effects of some pesticides on glutathione-S transferase activity [8]; Karadağ and Kaplan (2016) the effect of deltamethrin and alpha cypermethrin pesticides on bovine liver catalase activity [9]; Gencer et al. (2012) *in vitro* the effects of some herbicides and fungicides on human erythrocyte carbonic anhydrase activity [10]; Hopa et al. (2011) the inhibitory effects of some pesticides on human erythrocyte glucose-6-phosphate dehydrogenase activity [11]; Arslan et al. (2011) *in vitro* the effects of some antibiotics on enzyme activity of carbonic anhydrase from bovine erythrocytes [12]; Nadaroğlu and Demir (2009) *in vivo* the effects of chlorpyrifos and parathion-methyl on some oxidative enzyme activities in chickpea, bean, wheat, nettle and parsley leaves [13]; Doğan et al. (2014) the effect of sodium tetraborate on antioxidant enzymes under *in vitro* conditions [14]; Doğan (2006) *in vitro* the effects of some pesticides on carbonic anhydrase activity of *Oncorhynchus mykiss* and *Cyprinus carpio carpio* fish [15].

AChE plays an important role in the cholinergic system including nerve impulse transmission in synapses, and it hydrolyses acetylcholine (ACh) into choline and acetic acid [16]. ACh is an important neurotransmitter at post synaptic membranes and neuromuscular junctions. AChE is typically synthesized in nerve, muscle and some blood related cells. The enzyme has been localized outside the cell of

both nerve and muscles in inducible tissues [7]. The AChE in the brain can be a target for toxic chemicals [17], and these chemicals cause disruption of nerve function and excessive ACh accumulation by inhibiting the AChE enzyme [18]. Therefore, AChE can be a biomarker in the evaluation of neurotoxic changes [19].

A variety of usages of AChE inhibitors (AChEIs) are common in medicine and agriculture [2]. AChE inhibitors are used in the treatment of various disorders such as Alzheimer's disease, glaucoma, smooth muscle weakness and autonomic nervous system functions. AChE's catalytic activity is very high, it converts 25,000 ACh per second into choline and acetic acid. The choline formed is transferred back to the nerve centers to form new ACh molecules [7]. AChEIs are also used as pesticides for the elimination of insects that pose a threat to public health, agriculture and gardening [2]. To our knowledge, there are few studies that show the effect of some pesticides used in agriculture on AChE activity. AChEIs inhibit cholinesterase, increasing the level and length of ACh action. For example, Gonçalves et al. (2010) studied *in vivo* acute effects of several pharmaceutical drugs (diazepam, clofibrate, clofibric acid) and detergents (sodium dodecyl-sulphate and benzalkonium chloride) on cholinesterases from gambusia holbrooki [20]; Matozzo et al. (2006) the effects of 4-nonylphenol (xenoestrogen) and chlorpyrifos (organophosphorus pesticide) on AChE activity in the clam *Tapes philippinarum* [21]; Gyori et al. (2017) the inhibitory effects of four neonicotinoid active ingredients on AChE activity [22]; Lee et al. (2015) inhibitory effects of biocides on transcription and protein activity of AChE in the intertidal copepod *Tigriopus japonicus* [23]. Therefore, the aims of this study were to investigate the inhibition and/or activator effects of pesticides such as cypermethrin, fenprothrin, 1,1-D-methylpiperidinium chloride, N-(phosphonomethyl)glycine,  $\beta$ -naphthoxyacetic acid, lambda-cyhalothrin, deltamethrin, and 2,4-dichlorophenoxy acetic acid used in agriculture on AChE activity obtained from bovine brain, to calculate  $IC_{50}$  values of pesticides showing inhibitory effect, and to compare the inhibitory powers of pesticides with that of AChE enzymes in the literature.

## MATERIALS AND METHODS

**Material.** All reagents used in this study were of analytical grade and obtained from Sigma-Aldrich. The structures of the pesticides used in the study have been given in Figure 1.

**Methods. Extraction of AChE.** The bovine brain used in the study was supplied fresh from the slaughterhouse, immediately brought to the laboratory and stored at  $-20^{\circ}C$  until use. The brain was

homogenized in tris buffer (50 mM, pH = 7.4, 300 mM sucrose) 10 times its weight. The homogenate was centrifuged at 1000 g for 10 minutes and the resulting supernatant was used for experimental purposes.

**Determination of AChE Activity.** AChE activity was assayed according to the method described by Ellman et al. (1961). The rate of hydrolysis of acetylthiocholine at pH 8.0 was measured at 412 nm with a Perkin Elmer Lambda-35 UV-Visible Spectrophotometer. For AChE activity, the reaction was started by adding of 100  $\mu$ L of 0.01 M dithiobisnitrobenzoic acid (DTNB), 20  $\mu$ L of 0.075 M acetylcholine substrate and 50  $\mu$ L of supernatant into 2.83 mL of 0.1 M phosphate buffer (pH = 8.0). The total volume was 3 mL. The same assay for kinetic analysis was repeated with the addition of different amounts of supernatant [24].

**Inhibition of AChE Activity.** Inhibition of AChE activity was measured according to the spectrophotometric method developed by Ellman et al. (1961). Briefly, in this method, 140  $\mu$ L of 0.1 mM sodium phosphate buffer (pH 8.0), 20  $\mu$ L of DTNB, 20  $\mu$ L of test solution and 20  $\mu$ L of AChE solution were added by multichannel automatic pipette in a 96-well microplate and incubated for 15 min at  $25^{\circ}C$ . The reaction was then initiated with the addition of 10  $\mu$ L of acetylthiocholine iodide. The hydrolysis of acetylthiocholine iodide was monitored by the formation of the yellow 5-thio-2-nitrobenzoate anion as a result of the reaction of DTNB with thiocholines, catalysed by enzymes at a wavelength of 412 nm utilising a 96-well microplate reader. The experiments were carried out in triplicate. The results were interpreted by calculating  $IC_{50}$  values [25].

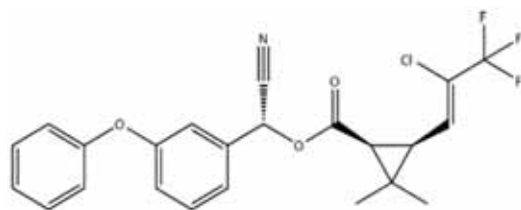
## RESULTS AND DISCUSSION

The kinetic constants of the AChE enzyme were calculated from Lineweaver-Burk equation using the acetylcholine substrate and given in Table 1. Figure 2 shows a linear relationship between points. The  $K_m$  and  $V_{max}$  values for the acetylcholine substrate were calculated as 0.33 mM and 3333 EU / mL.min, respectively.

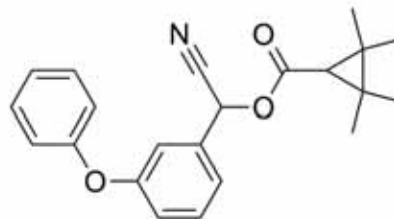
**TABLE 1**  
**Kinetic constants for AChE**

$K_m$ (mM)	0.33
$V_{max}$ (EU/mL.min)	3333

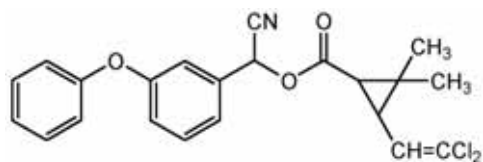
In this study, inhibition effects of pesticides used for different purposes in agriculture were investigated on AChE enzyme. AChE plays a significant role in the outgrowth of axons, synapto genesis, migration of neurons, hemopoietin stress responses and cell apoptosis. Major effects of AChEIs are due to



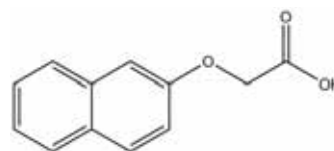
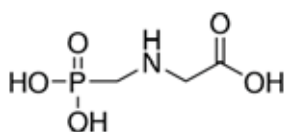
Lambda-cyhalothrin



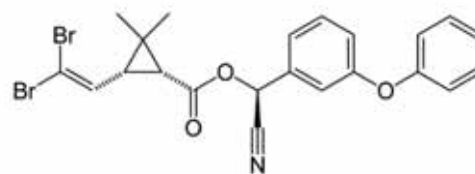
Fenpropathrin



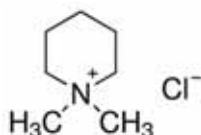
Cypermethrin

 $\beta$ -naphthoxyacetic acid

N-(phosphonomethyl)glycine



Deltamethrin



1,1-dimethylpiperidinium chloride

FIGURE 1

The structure of pesticides

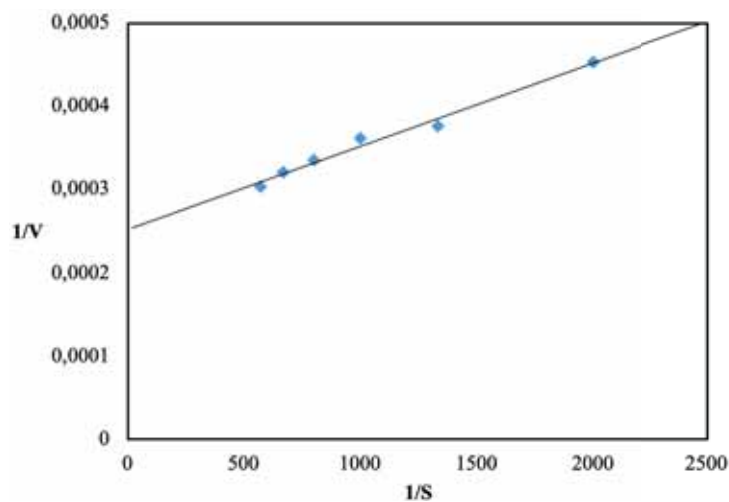


FIGURE 2

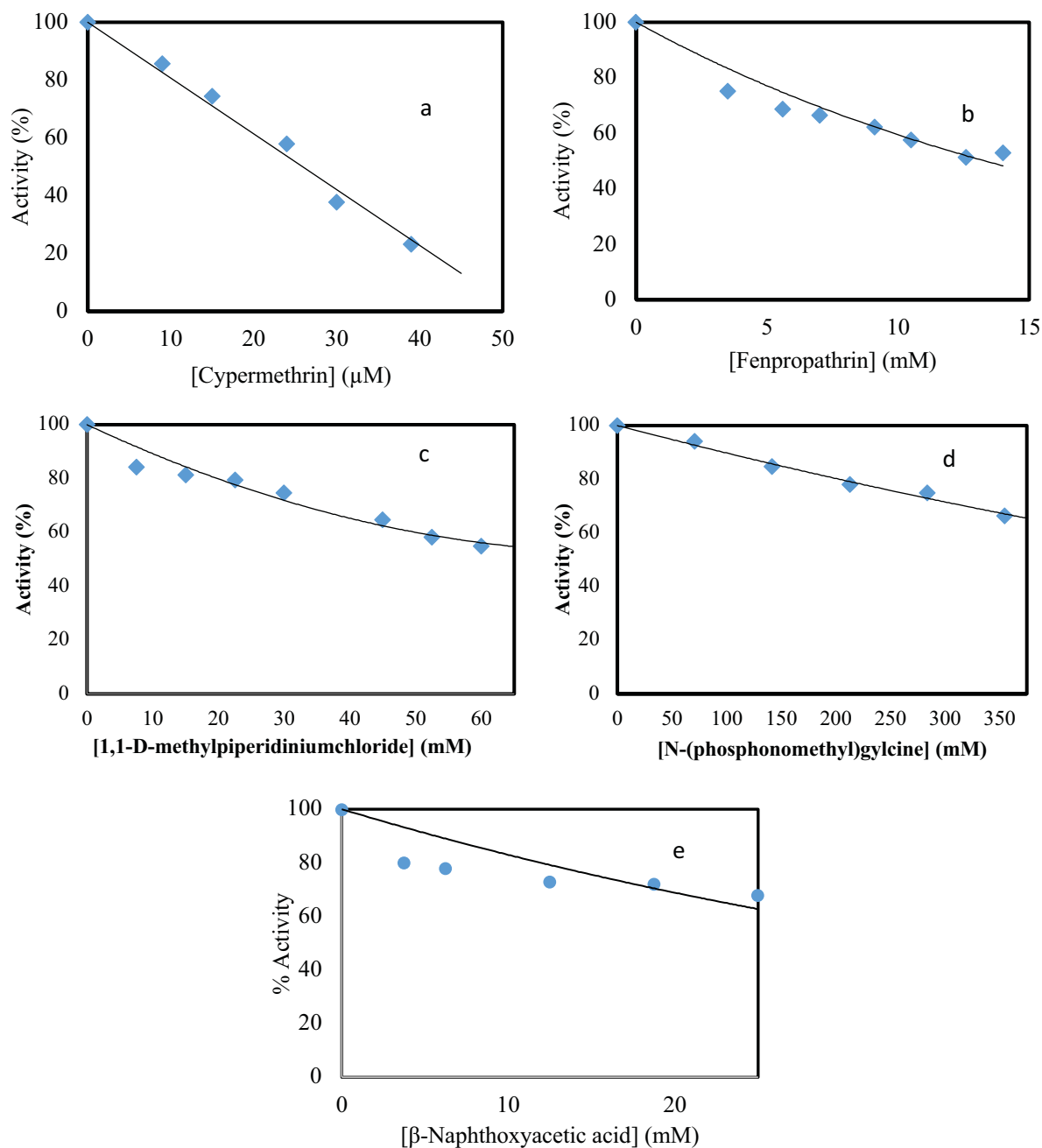
The curve of  $1/V$  against  $1/[S]$  for AChE enzyme

their action on the parasympathetic nervous system, causing slow heart rate, low blood pressure, bronchial hypersecretion/constriction, gastrointestinal hypermobility, and decreased intraocular pressure [2]. The pesticides used have different functional groups such as cyanide, quaternary ammonium salt,

carboxyl group and organophosphate group. The effects of these functional groups on AChE were different from each other. Pesticides such as cypermethrin, fenpropathrin, 1,1-D-methylpiperidinium chloride, N-(phosphonomethyl)glycine, and  $\beta$ -naphthoxyacetic acid inhibited AChE activity while lambda-cyhalothrin and deltamethrin increased

AChE activity. 2,4-dichlorophenoxy acetic acid pesticide did not alter the AChE activity. When the results are examined, it is seen that the molecular structure is more effective in inhibition than functional group. For example, cypermethrin and fenprothrin having a cyanide functional group inhibited the AChE activity, while lambda-cyhalothrin and deltamethrin activated the AChE activity. Also,  $\beta$ -naphthoxyacetic acid with carboxyl functional group inhibited AChE activity, while 2,4-dichlorophenoxy acetic acid did not alter AChE activity.

Figures 3 and 4 show the effects of concentrations of different pesticides used in the study on AChE activity. The effects of pesticides showing inhibition effect (Figure 3) on AChE activity are in a concentration dependent manner. The calculated  $IC_{50}$  values from % activity-inhibitory concentration graphs have been given in Table 2. When the values in Table 2 are examined, it can be seen that the  $IC_{50}$  values of the pesticides showing the inhibitory effect were different from each other. The fact that these values is different may be the result of the interaction between the enzyme and the inhibitor.



**FIGURE 3**

The curves of % activity against [pesticides] for AChE enzyme:

a. Cypermethrin, b. Fenprothrin, c. 1,1-D-methylpiperidiniumchloride, d. N-(phosphonomethyl)glycine, and e.  $\beta$ -naphthoxyacetic acid

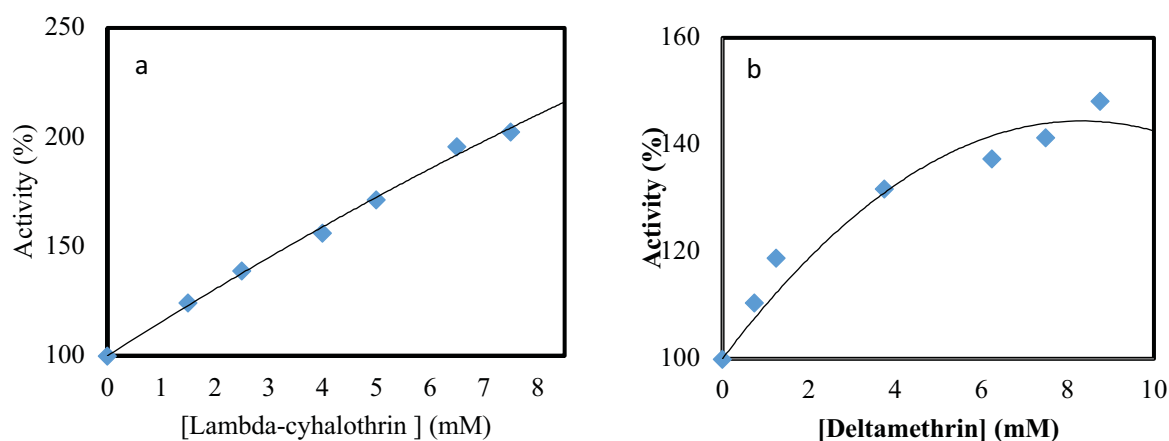


FIGURE 4

The curves of % activity against [pesticides] for AChE enzyme:  
a. Lambda-cyhalothrin and b. deltamethrin

TABLE 2

**IC<sub>50</sub> values calculated from % activity-inhibitor concentration curves for AChE activity**

Pesticides	Effect	IC <sub>50</sub> (mM)
Cypermethrin	inhibitor	24.45
Fenpropathrin	inhibitor	16.95
Lambda-cyhalothrin	activator	---
1,1-D-methylpiperidinium chloride	inhibitor	69.09
2,4-dichlorophenoxy acetic acid	inactive	---
N-(phosphonomethyl)glycine	inhibitor	585
β-naphthoxyacetic acid	inhibitor	16.34
Deltamethrin	activator	---

IC<sub>50</sub> values for cypermethrin, fenpropathrin, 1,1-D-methylpiperidinium chloride, N-(phosphonomethyl)glycine, and β-naphthoxyacetic acid pesticides showing inhibitory activity were calculated as 24.45, 16.95, 69.09, 585 and 16.34 mM, respectively. When these values are examined, it can be said that the pesticide that inhibits the most AChE activity is β-naphthoxyacetic acid, followed by fenpropathrin, cypermethrin, 1,1-D-methylpiperidinium chloride, and N-(phosphonomethyl)glycine pesticides. Again, it can also be said that the inhibition power of β-naphthoxyacetic acid and fenpropathrin pesticides are approximately equal to each other. These results indicate that the amount of acetylcholine will increase by inhibiting AChE activity in the bovine brain. Except for N-(phosphonomethyl)glycine, the IC<sub>50</sub> values of the other inhibitors used in this study were higher than the IC<sub>50</sub> of ACT (IC<sub>50</sub>=75.2 mM and 85.4 mM) [22]. Comparing these data it can be concluded, that pesticides used in this study can represent a substantial environmental risk primary to pollinator organisms. It has been postulated that inhibiting AChE activity by 50%–60% may result to weakness, headache, dizziness, nausea and salivation that commonly resolve within 1–3 days. At a 60%–90% inhibition level, moderate-intensity symptoms are seen including diaphoresis, vomiting, diarrhea, tremors, ambulatory

disturbance, chest pain, and cyanosis that may reverse within a few weeks. However, at a 90%–100% inhibition level, respiratory or cardiac failure occurs resulting to death [2,3]. When a decrease in AChE activity occurs, acetylcholine accumulates in synapses and this event causes physiological impairment in many functions including feeding, swimming and behavior [26]. The occurrence of AChE inhibition may be related to lower AChE expression [27, 28] and causes neurotoxic alterations in the nervous system [29]. Previous studies indicated that pesticides may cause AChE inhibition in fish tissues [27, 28, 30].

When the results in Figure 3 are examined, it can be said that the lambda-cyhalothrin and deltamethrin pesticides increase AChE activity and exhibit activator behavior. In this case, these pesticides increase the AChE activity, accelerating the conversion of acetylcholine into choline and acetate and preventing the increase of acetylcholine in the brain. The results in Table 2 have shown that the 2,4-dichlorophenoxy acetic acid having carboxylic acid functional group does not alter the AChE activity. This may be due to the absence of an interaction between the active site of the enzyme and the functional group of 2,4-dichlorophenoxy acetic acid. Similar results were found for BDCEE pesticide by Bukowska et al. (2018) [31].

From the above results it was determined that i. pesticides having the same functional group showed different effects on AChE enzyme, ii. pesticides such as cypermethrin, fenpropathrin, 1,1-D-methylpiperidinium chloride, N-(phosphonomethyl)glycine, and  $\beta$ -naphthoxyacetic acid, inhibited AChE activity, iii. lambda-cyhalothrin and deltamethrin pesticides increased AChE activity, iv. 2,4-dichlorophenoxy acetic acid did not have an effect on the AChE activity as an inhibitor and/or activator, v. the most effective inhibitors were  $\beta$ -naphthoxyacetic acid and fenpropathrin pesticides, and vi. the amount of acetylcholine in the brain would increase in the inhibition case and the amount of acetylcholine would decrease in the activator case. In conclusion, the obtained results indicated that pesticides such as cypermethrin, fenpropathrin, 1,1-D-methylpiperidinium chloride, N-(phosphonomethyl)glycine, and  $\beta$ -naphthoxyacetic acid could cause neurotoxic changes by affecting physiological and biochemical functions in the bovine brain.

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# ANALYSIS OF THE MAIN FACTORS AFFECTING 4D CONVERTED-WAVE SEISMIC FEASIBILITY IN CO<sub>2</sub> GEOLOGICAL STORAGE

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## ABSTRACT

The use of 4D converted-wave seismic exploration technology to monitor the safety of CO<sub>2</sub> geological storage can fully exploit the characteristic insensitivity to fluids of shear waves to determine individual changes in reservoir pore pressure and fluid saturation. The suitability of 4D converted-wave technology for CO<sub>2</sub> geological storage has yet to be assessed; therefore, it is necessary to analyze the feasibility of using 4D converted waves and the factors influencing their use. Based on actual geological data acquired from the Weyburn oilfield, this paper constructs a 4D converted-wave forward model based on well-logging analysis data and numerical simulation data. Using two-layer and wedge-shaped fluid substitution models, we analyze the variation in reservoir elastic parameters and fluid parameters with CO<sub>2</sub> injection and study the effects of reservoir pore pressure, fluid saturation, and reservoir porosity on 4D converted waves. Using the results, the feasibility of 4D converted waves is studied. The results show that an increase in reservoir thickness is beneficial for 4D converted waves. Changes in reservoir fluid saturation and reservoir porosity affect the results of 4D converted waves, while reservoir pore pressure plays a significant role in changes seen in 4D converted waves. This study lays a good foundation for subsequent interpretations of 4D converted-wave data.

## KEYWORDS:

Converted-wave, 4D seismic, Feasibility study, CO<sub>2</sub> geological storage

## INTRODUCTION

CCUS technology (Carbon Capture, Utilization and Storage) is currently the most effective way to mitigate greenhouse gas emissions and CO<sub>2</sub> geological storage is the core technology of CCUS [1, 2, 3]. The 4D seismic technique is the most effective means to confirm the security of CO<sub>2</sub> geological storage [4, 5]. Currently, 4D seismic technology is focused on traditional 4D PP wave data in the CCUS field [6, 7]; since changes in pore pressure and fluid saturation in reservoirs both have obvious effects on 4D PP wave data, it is difficult to separate effects of pore pressure and CO<sub>2</sub> saturation in the reservoir. There also remains the problem of determining the potential migration path of CO<sub>2</sub> and possible CO<sub>2</sub> leakage points in the reservoir [8, 9]. PS waves make full use of the insensitivity of shear waves to fluid and show good performance in identifying gas cloud in the gas reservoir area [10, 11, 12]; however, there are few studies that confirm the security of reservoirs using 4D converted waves in CCUS. At the same time, there is plenty of research showing that 4D seismic techniques are not suitable for all reservoirs [13, 14].

The conflicting evidence makes it necessary to carry out a feasibility study on the use of 4D converted waves in CCUS. It is of great significance for the safety monitoring of CO<sub>2</sub> geological sequestration to build 4D converted-wave forward models in CO<sub>2</sub> geological sequestration. We will build the 4D converted-wave forward model based on actual data, including rock physics test data, well-logging data and reservoir simulation analysis data, to study the effects of changes reservoir pore pressure, fluid saturation, reservoir porosity, etc., on 4D converted waves. Finally, we determine the factors influencing feasibility in the use of 4D converted waves.