

Microplate absorbance-based Toxicity Bioassay by analysis. Vitro.-hibition. Acetylcholinesterase

Shasha Liu

College. Plant Science & technology huazhong agricultural University;

Abstract: Chemical pollutants assessment requires a broader-spectrum.-Throughput analysis. guarantee compliance. established standards. nowadays, increasing number. pollutants,. important effects lead. development. general toxicity bioassays. based. Vitro inhibition. acetylcholinesterase (AChE) this work established a microplate absorbance technology. measure. toxicity. chemicals.. AChE concentration acetylthiocholine iodide (ATCI) concentration, 5,5 '-dithiobis-2-nitrobenzoic acid (DTNB) con-Centre were optimized, and their effects on absorb, absorb change rate and chemical test toxicity were investigated. The optimized conditions for AChE microplate toxicity analysis were DTNB $0.2\text{g} \cdot \text{L}^{-1}$, ATCI $0.2\text{g} \cdot \text{L}^{-1}$, AChE $0.04\text{U} \cdot \text{ml}^{-1}$, PH 6.8, reaction temperature 29 and exposure time 15 min. the results showold that the concentrations of ache, DTNB, and ATCI created the absorbance. the concentration of AChE was positively related with the absorption change rate, the concentrations of DTNB was unrelated with the absorption change rate, and the concentrations of ATCI was bibasic-curved relationship with the absorb change rate. the dose-response curve (DRC) of methomyl moved to the right with innovation ATCI concentration. by comparison with the national standard method, the reagent injection order had the significant impact on the toxicity measurement. the microplate award-based Toxicity bioassay was further validated to show a good S-type DRC with methodyl toxicity. this DRC can be well chartered by the Weibull function, and the fitting COEFFicient. determination $R^2 > 0.97$, blank variation. $\pm 10\%$. this method can be used. high-throughput toxicity assays. chemical pollutants.

Keywords: Methomyl; Acetylcholinesterase; dose-response curve; microplate absorbance method; biphasic relation-ship

1. Ache(AChE)

The most commonly used enzyme inhibition method, the basic principle isAcheCatalytic substrate acetylcholine(Ach)Hydrolysis of choline, choline and chromogenic two agent dithitwo dinitrobenzoic Acid(DT-NB)React to produce yellow substances5-Thio-2-Nitro-Benzoic acid, through412 nmThe change of absorbance of the yellow substance was detected to reflect the change of enzyme catalytic reaction.^[2].Organophosphorus or carbamate pesticides.AcheThe inhibition rate was positively correlated with the concentration of pesticides. It can be determined by inhibiting toxicity.

The presence and residue of Organophosphorus or carbamate pesticides in the samples were determined.^[3-4].AcheInhibition method is widely used and effective.^[5], For mature detection methods Available for variousAcheInhibitor^[6-8], ^[9].

Screening, and detection of some pesticide residues, suchAcheInhibition Method for vegetables,Fruit,Organic Phosphorus and carbamate in tea Influence of Determination.

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The colorimetric spectrophotometry is usually used to determine the inhibition method, which to some extent restricts its repeated, and is not conducive to the realization of High-throughput operation, and requires a lot of reagent drugs. Pesticides in environmental media generally exist in low-dose and mixed residues.^[16-17], In order to predict and evaluate the toxicity of mixtures from a single pollutant, it is necessary to accurately measure the low dose effect and global dose of a single pollutant. -Effect curve(DRC)Therefore, it is necessary to increase the number and repeatability of experiments, and the colorimetric tube method can not meet the requirements..

Microplate with high throughput operation, Easy to repeat, Due to the small sample size, it is increasingly used for pollutant toxicity, ^[1], [18] [19]

Microplate can be used as a carrier for the exposure of luminescent bacteria, even nematodes. As a carbamate pesticide, methomyl residues in the environment after application form pollutants.^[20]Take this research as an example

Effect of the concentration on the determination of methomyl toxicity, and finally (Said national standard method Variation1), Plate(38)Of Dtnb atci AchE (Said National Standard Method Variation2) 3Of and-like order; 3Of material the first both 37

Temperature bath 15 min Plus the first 3Of Material color and in 37Test; Corresponding to exposure time said for temperature bath time (15 min) With actual color time but national standard method Variation 2Of temperature bath time not in exposure time in.

2. Results and discussion (REsults, discussion)

2.1 Acetylcholinesterase system components concentration on color absorbance of influence 1For different AChE Concentration under of absorbance curve can see with AChE Concentration (0.02~1 U in mL⁻¹) Of absorption increase Photometric corresponding increase from 0.173 To 2.212. Similar to more than-Ying [23] ~ In -1 And reports AChE Concentration in 0.0667 U mL The range maintained a good linear relationship with absorbance, and it was recommended that the final concentration of enzyme in the reaction system

Degrees should be controlled in 0.0667 U. ml⁻¹ No no. Figure 1 (B) For different DTNB The absorbance curve of DTNB Concentration

2.2 Effects of component concentration of AChE system on the change rate of chromogenic Absorbance

Map 1. China 15 min Determined A Value minus separately 0 min

A Value, the difference divided 15 min The change rate of Absorbance (A. Min⁻¹) Can be seen Ache, DTNB With ATCI The change rate of concentration and absorbance showed completely different types of curves. (Figure

2). With Ache The change rate of absorbance increased with the increase of concentration. (Figure 2a). With DTNB With the increase of the concentration, the absorbance change rate showed a basic level curve. (Figure 2b), Every minute A The change in value is about 0.004. And ATCI With the increase of the concentration, the absorbance change rate showed a biphasic curve which increased first and then decreased. (Figure 2c), Most

Large values appear in ATCI Concentration is 0.315 G. l⁻¹ (Namely 1.09 mmol ·

L⁻¹) And this reflects Ache Excessive substrate inhibition effect; The ascending stage curve is recommended, ATCI The concentration is less 0.315 G. l⁻¹ Appropriate. Ellman Wait.^[2] Report of acetylcholine water catalyzed by cholinesterase from bovine Erythrocytes

Solution rate also first increase after reduction of Duplex curve its maximum value appeared in Acetyl choline concentration about 0.5 mmol in L⁻¹. Dante of choline esterase (BChE) No excess substrate suppression effect such as chicken serum BChE^[9] This is also distinguish AChE And BChE Of characteristics one^[25].

2.3 Pollutant Control Acetylcholinesterase of Influence Factors Research

3 (C) For different exposure time under methomyl DRCCan see with the exposure time of increase methomyl DRCTo left mobile methomyl of toxicity with increase table 1 In EC_{50} Decreases, 5, 15, 30 min An arcane respectively 5.08×10^{-6} , 2.41×10^{-6} , 1.60×10^{-6} Mol

In L^{-1} . Can see 15 min After toxicity change has little consider try

Test efficiency and factors and reference practice select 15 min As a toxicity test default of exposure time.

3 (B) For AChE Concentration 0.004, 0.01, 0.04, 0.16 U

In mL^{-1} When methomyl DRCThis 4 Article DRC Basic coincidence table

One range in AChE Concentration change of climate will not pollutants of toxicity to the effect on the testing. Levi^[9] Also found different than enzyme activity of chicken serum BChE The pesticide of inhibition rate basic same. Figure 3 (c) For DT-NB Concentration 0.02, 0.2, 2 G in L^{-1} When methomyl DRCThis 3 DRC Basic coincidence show that certain range in DT-NB Concentration change also not the pollutants of toxicity to the effect on the testing.

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