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How to accurately assay the algal toxicity of pesticides with low water solubility

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A new method is described for assaying the toxicity of water insoluble pesticides.

Abstract

A novel method for assaying and calculating the toxicity of water-insoluble pesticides to green algae has been put forward in this work. First, a solvent is selected for use in bioassays; there should be a detailed screening to identify a solvent with inherently low toxicity to the test organism. Second, the EC_{50} is determined for selected pesticides by measuring the toxicity of various concentrations of each of the selected pesticides in a fixed concentration of selected solvent. Third, concentrations of the selected solvent are varied and the EC_{50} of each pesticide tested is assayed at a fixed concentration. Fourth, several suitable groups of solvent concentrations are selected and the corresponding EC_{50} values of tested pesticides are considered to establish the linear regression equation. Letting the solvent concentration be zero, one calculates the corresponding EC_{50} value, which corresponds to the inherent toxicity of the tested pesticide.

Keywords: Organic solvents; Pesticides; Acute toxicity; Assays; Green algae

1. Introduction

Pesticides play an important role in agricultural practices. Increase in the use of pesticides has elicited extensive research into pesticide effects on non-target organisms such as algae. Therefore, their potential effects on the aquatic primary producers are particularly important, and have to be studied in ecotoxicological experiments (Berard, 1996).

In ecotoxicological laboratory bioassays, the use of organic solvents is unavoidable since many organic pollutants have low water solubility and need to be dissolved in organic solvents prior to addition into experimental systems. So, one aspect of concern with laboratory bioassays is the stress imposed on test organisms by organic solvents. Most reports on the comparative toxicity of solvents towards test organisms deal with the effects of solvents on fish and aquatic invertebrates with some data available for blue-green algae and green algae (Stratton and Corke, 1981; Stratton et al., 1982; Hughes and Vilkas, 1983; Le Blanc and Surprenant, 1983; Stratton, 1985; Stratton and Smith, 1988; Tadros et al., 1994). The US Environmental Protection Agency (USEPA) recommends maximum allowable limits of 0.05% solvent for acute tests and 0.01% for chronic tests (Jay, 1996). But, in professional papers, the nature of the solvent and the final concentration used vary among different authors and are often higher than USEPA limits due to problems associated

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with the use of small test volumes and toxicant solubility (Jay, 1996). Organic solvents can cause toxic effects on their own, but it has also been reported that they can interact with pesticides and alter toxicity. To ensure that bioassay data are accurate and not the result of solvent interference, a screening method is available to identify and minimize solvent interactions in pesticide bioassays (Stratton, 1985, 1989; Stratton and Corke, 1981; Stratton and Smith, 1988; Stratton et al., 1982). First, choosing a solvent for use in bioassays should involve a detailed screening to identify solvents with inherently low toxicity to the test organism, followed by an interaction study involving pesticide and solvent interactions to choose the best concentration to use (Stratton and Smith, 1988). However, there are a few problems in using the method concerning minimizing solvent interactions in pesticide bioassays or using the maximum allowable limits of solvents according to the USEPA recommendations. Theoretically, to equate the combined toxicity of mixtures (solvent and pesticide) with the inherent toxicity of the pesticide is incorrect. In practice, the range of using the minimum solvent interaction method is limited because of the difficulty in assaying the toxicity owing to the quite low solubility of the pesticides to be tested. In order to solve these problems, a new assay and computed method – a linear regression method—is advanced in the present study.

2. Materials and methods

2.1. Test chemicals

The solvents employed included acetone, alcohol, methanol, dimethyl sulfoxide (DMSO), *N*,*N*-dimethylformamide (DMF), furanidine and acetidin, which were obtained from Pinghu Chemical Reagent Factory, People's Republic of China. All solvents were analytical reagent (AR). The purity of bispyribac-sodium and fosetyl-aluminum was >98%. All solvent concentrations are given as percent volume. Bispyribac-sodium (CAS-nr: 125401-92-5) and fosetyl-aluminum (CAS-nr: 39148-24-8) were used in solvent–pesticide interaction studies respectively, and were purchased from Jiangsu Institute of Ecomones and Shandong Agricultural Chemical Industry Stock Co., Ltd., People's Republic of China. The concentration of two compounds was given with mg/l of active ingredient. The tested pesticide was dissolved in distilled water.

2.2. Test methods

The green alga *Chlorella pyrenoidosa* was used as the test organism and was obtained from The Institute of Hydrobiology (Wuhan), the Chinese Academy of Science. Cells of *C. pyrenoidosa* were propagated photoautotrophically in a 250 ml Erlenmeyer flask containing 100 ml liquid HB-4 medium (Li, 1959) that is composed of distilled water and the following chemical ingredients (mg/l): $(NH_4)_2SO_4$, 200; $Ca(H_2PO_4)_2\cdot H_2O+(CaSO_4\cdot H_2O)$, 30; $MgSO_4\cdot 7H_2O$, 80; $NaHCO_3$ 100; KCl, 23; $FeCl_3$, 1.5 and 0.5 ml of A_5 liquid (chemical ingredients of A_5 liquid in (mg/l) are H_3BO_3, 2.86; $MnCl_2\cdot 4H_2O$, 1.81; $ZnSO_4\cdot 7H_2O$, 0.222; $Na_2MoO_4\cdot 2H_2O$, 0.391 and $CuSO_4\cdot 5H_2O$, 0.079 (Ma et al., 2001). The flask was kept on a rotary shaker (100 rpm) at 25 °C, and illuminated with cool-white fluorescent light at a continuous light intensity of 5000 lx. The culture medium was sterilized at 121 °C, 1.05 kg/cm^2 for 30 min (Ma et al., 2002a). Aliquots of 15 ml of the HB-4 medium containing green algal cells, about 6×10^5 cells/ml (initial cell concentration), were distributed to sterile 50 ml Erlenmeyer flasks. The medium containing *C. pyrenoidosa* was then treated with various concentrations of solvent and/or pesticides, and incubated for 96 h on a rotary shaker (100 rpm) at a temperature of 25 °C and a continuous light intensity of 5000 lx (Yan et al., 1995; Yan and Pan, 2000). A wide range of concentrations was examined in a previous test in order to find the adequate range of toxicity for each solvent or pesticide. Then, similar concentrations were tested according to the results of the previous test (Moreno-Garrido et al., 2000). Cell counts were correlated with absorbance over time for 96 h on a Shimadzu UV-2401PC spectrophotometer. The most suitable wavelength to use for monitoring culture growth was 680 nm (Ma and Liang, 2001). Each solvent or pesticide concentration was replicated three times. Appropriate control systems containing no solvent and pesticide were included in each experiment. Control and treated cultures were grown under the same conditions of temperature, photoperiod and shaking as the stock cultures. In each experiment, percent inhibition values, relative to growth in control systems, were calculated using spectrophotometric data (Ma, 2002, in press). During solvent bioassay experiments test organisms were cultured in 50 ml Erlenmeyer flasks containing 15 ml medium, in both the presence and absence of solvent, for solvent–pesticide interaction bioassays; bispyribac-sodium or fosetyl-aluminum was also added to test systems.

2.3. Calculation methods

Joint toxicity between the tested toxicant and adjuvant (including solvent and/or emulsifier) will display one, two or three status among synergistic, additive or antagonistic effects. For a specific toxicant and adjuvant, their joint toxicity may be a synergistic effect at the specific adjuvant concentration range, but may be additive or antagonistic at another concentration range. However, we can always find the adequate range of adjuvant concentration when the joint toxicity