

Application of Current Hapten in the Production of Broad Specificity Antibodies Against Organophosphorus Pesticides

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Abstract

Diethylphosphono acetic acid (DPA) was used as a current hapten to generate broad specificity polyclonal antibodies against a group of organophosphorus pesticides. Six New Zealand white rabbits were immunized with immunogens synthesized by the active ester method (AEM) or 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide method (EDC). The titers of antisera reached 25 600 by AEM and 6 400 by EDC, respectively. Polyclonal antibodies raised against DPA were screened and selected for the competitive indirect enzyme-linked immunosorbent assay (CI-ELISA). A CI-ELISA for DPA was developed with a detection limit of 3.536 ng mL⁻¹ and an I₅₀ value of 0.182 μg mL⁻¹. The assay specificity was evaluated by obtaining competitive curves for several structurally related compounds as competitors. The antiserum showed high affinities to chlorpyrifos, diazinon, omethoate, parathion-ethyl and profenofos with I₅₀ of 0.12, 0.15, 0.21, 0.88, 0.97 and 2.5 μg mL⁻¹, respectively. The results indicate that the assay could be a screening tool for quantitation and semi-quantitation determination of the above former five organophosphorus pesticides.

Key words: organophosphorus pesticides, broad specificity antibody, enzyme-linked immunosorbent assay (ELISA)

INTRODUCTION

As an important kind of broad-spectrum and effective insecticide, organophosphorus pesticides (OP) are broadly used in the prevention of pests in farm and sanitation. They currently dominate the market. From 1982, the production of HCB and DDT has stopped after they were forbidden to use by the Chinese government. The application of organophosphorus pesticide is being increased sharply. Although most organophosphorus pesticides are nonpersistent pesticides, the residues would be present in all compartments of the environment and engender acute toxicity for people and livestock, because of the widespread use of pesticides. Moreover, some organophosphorus pesticides could accumulate in animal body and induce

chronic toxicity. Now, the organophosphorus pesticide residue is one of the most serious problems in China, and it is starving for a rapid and effect method for determination of organophosphorus pesticides.

As an alternative to the laborious and lengthy chromatographic procedure for OP analysis, several immunoassay methods have been developed both as semiquantitative screening tools and as quantitative methods. In general, anti-OP antibodies raised using conventional methodology are highly specific toward a single analyte. Although antibody specificity is an advantageous characteristic for low level detection of trace amounts of individual OPs, the broad-specificity antibody's recognition of groups of analyte is desirable in applications, such as in cost-effective screening programs, in immunoaffinity columns, and in automated biosensor techniques.