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Immunochromatographic colloidal carbon-based assay for detection of methiocarb in surface water

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ABSTRACT

A simple and rapid immunochromatographic assay for a sensitive and inexpensive monitoring of methiocarb in surface water was developed using a binding inhibition format on a membrane strip. In the assay, detection reagent consisted of anti-methiocarb antibody and colloidal carbon-labelled secondary antibody. Methiocarb-ovalbumin conjugate was immobilized in a test line of the strip as a capture reagent. Colour intensity of the test line in methiocarb-positive assay was visually distinguishable from that of negative sample within 10 min. The optimized semi-quantitative method provided a visual detection limit of 0.5 ng mL^{-1}. Cross-reactions with other carbamate pesticides were not found (<1%). Only a negligible matrix effect of surface water was recognized. In parallel analyses of spiked water samples, the assay results were in a good agreement with those of ELISA. The stability test indicated the strips could be used at least 2 months without change in performance. All characteristics of the visually evaluated assay mentioned above were verified by instrumental quantification of colour intensity in test lines. The developed immunochromatographic assay offers potential as a useful on-site screening tool for environmental analysis.

1. Introduction

Although the use of pesticides has had a range of benefits, these compounds also can cause adverse environmental effects, including degradation of water quality. Monitoring the concentration of pesticides in surface water is important for maintaining aquatic health and eventually ensuring safe drinking water supplies.

Methiocarb [3,5-dimethyl-4-(methylthio) phenyl methylcarbamate] belongs to chemical class of N-methylcarbamate pesticides. As a broad-spectrum insecticide, molluscicide, acaricide, and bird repellent, it is commonly used in agriculture and household practice throughout the world. Therefore, contamination of water and agricultural products becomes imminent, and consequently, adverse health effects are possible in humans and animals. Due to its action as a potent acetylcholinesterase inhibitor and other toxic activity, methiocarb is considered to be highly hazardous according to WHO classification. Nevertheless, a more pressing concern is the toxicity to aquatic organisms. For some kinds of aquatic invertebrates, commonly used as bioindicators of water contamination in environment, values of LC_{50} or EC_{50} (acute) were found extremely low, in the range of 1.6–19 μg L^{-1} (Munn et al., 2006; Péry et al., 2004). U.S. Environmental Protection Agency declared that the methiocarb value of an acute aquatic life benchmark for invertebrates is 3.5 μg L^{-1} (Anonymous, 2007).

The use of methiocarb, as well as all pesticides, has been regulated for a long time in many countries. However, unwanted amounts of certain pesticides still are found in environmental media and residues exceeding regulatory limits still sometimes occur in agricultural produce. Until now, European Union legislation has established maximum residue levels for food (Commission Regulations (EC) No. 149/2008) and drinking water (Council Directive 98/83/EC) but not for surface water. However, the European Commission introduced the Water Framework Directive (2000/60/EC) as an instrument to sustain and improve quality of environmental waters. The directive aims to achieve good chemical and ecological status for all waters by 2015. The backbone of the directive implementation is monitoring of chemical substances and values of LC_{50} or the acute life benchmark for aquatic invertebrates are important reference concentrations for assessing good ecological status of surface water. Although the total volume of methiocarb used is low relative to some other pesticides, it could have major impacts in localized areas if there is concentrated outdoor use. Then, pesticide concentration in runoff may approach or exceed LC_{50} value for aquatic organisms (Primus et al., 2001; Schäfer et al., 2007; Vecchia et al., 2008; Wilson et al., 2005). Despite recommendations for pesticide application in good agriculture practices, contamination of fruit food (Blasco et al., 2005; Schulze et al., 2002) and surface water with methiocarb has been reported in the last years (Anonymous, 2005; Borkovcova et al., 2004; Fytianos et al.,

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2006; Garcia de Llasera and Bernal-Gonzales, 2001; Primus et al., 2001). Therefore, developments of analytical methods that are fast, inexpensive and may be used on-site are needed.

Current methods applied to analyze pesticides in water samples are mainly based on high-performance liquid chromatography or gas chromatography coupled to various selective detectors. Such procedures are also described for methiocarb (Huertas-Pérez and García-Campaña, 2008; Rodrigues et al., 2007; Saraji and Esteki, 2008). Although highly sensitive and reliable, they are time-consuming, involve multiple steps in sample preparation and analysis, and require expensive equipment and skilled analysts.

Nowadays, with increasing development of immunoassays, these methods have been shown to be useful alternative for analysis of pesticides in environmental samples (Anand et al., 2007; Jiang et al., 2008; Krämer et al., 2007; Mauriz et al., 2007). Currently, enzyme-linked immunosorbent assay (ELISA) carried out in a microtitre plate is the most common technique used for immunoassays. This technique has been successfully applied for the analysis of methiocarb (Abad et al., 1999; Mickova et al., 2003, 2005). However, for non-specialized laboratories and for field-use, it can be difficult to perform labour intensive operations including repeated incubation and washing, and enzyme reaction for final signal generation in ELISA. Immunosensors have also become increasingly practical tools in environmental monitoring (Marchesini et al., 2007). With the aim of real-time output, various kinds of immunosensors have been developed for pesticide analysis (Kim et al., 2007; Long et al., 2008; Zacco et al., 2007). In terms of field-use, these methods are often considered to be requiring expensive equipment and specialized personnel. With the demand for overall speed and simplicity, a lateral-flow immunochromatographic assay (ICA) could be a more suitable alternative (Posthuma-Trumpie et al., 2009). It combines several benefits including a user-friendly format, short assay time, and cost-effectiveness. These characteristics make it well suited for on-site screening. In the last years, several research groups have performed an ICA for some of pesticides (Gui et al., 2008; Kaur et al., 2007; Shi et al., 2008; Shim et al., 2006; Wang et al., 2005; Zhou et al., 2004; Zhu et al., 2008) and other environmental contaminants (Li et al., 2009; Zhou et al., 2009). In these works, the detection reagent was typically colloidal gold-labelled antibody. To our best knowledge, any use of ICA principles for methiocarb detection has not been published up to now.

In our previous studies, we described validation of the ELISA for control of methiocarb residues in some food samples (Mickova et al., 2003, 2005). Here we reported a development of ICA test for this pesticide using colloidal carbon nanoparticles as a label. The optimized test has been compared with ELISA results in analysis of spiked surface water samples.

2. Experimental

2.1. Reagents and materials

Standards of methiocarb (98.5%, HPLC/DAD), carbaryl (99%, HPLC/DAD), carbofuran (99.5%, HPLC/DAD), were from Dr. Ehrenstorfer GmbH, Augsburg, Germany. Aldicarb (99.9%, HPLC, Fluka), bendiocarb (99.5%, HPLC, Riedel-de Haën), ethiofencarb (99.0%, HPLC, Riedel-de Haën), fenoxycarb (99.6%, HPLC, Fluka), and methomyl (99.9%, HPLC, Fluka) were supplied by Sigma–Aldrich Inc. (St. Louis, USA). Individual stock standard solutions containing 10 mg mL^{-1} of each compound were prepared by dissolving accurately weighted amounts in ethanol and stored in darkness at 4 °C. Working standard solutions were freshly prepared by serial dilution in deionised water.

The mouse anti-methiocarb monoclonal antibody as well as the methiocarb hapten-ovalbumin conjugate (methiocarb-OVA) was purchased from Centro de Apoyo a la Innovación, la Investigación y la Transferencia de Tecnología (CTT), Universidad Politécnica de Valencia, Spain. Producer indicated these immunoreagents as LIB-MXNB31 and OVA-DPNH, respectively (Abad et al., 1998). Rabbit anti-swine antibody (RASw) as well as the swine anti-mouse antibody (SwAM) was obtained from Nordic Immunological Laboratories, Tilburg, The Netherlands (product codes RASw/IgG(H + L)/7S and SwAM/IgG(H + L)/7S). Both antibodies were supplied as purified IgG fraction of polyclonal antiserum.

Carbon nanoparticles (Spezial Schwartz 4, Degussa AG, Germany) were kindly provided from Agrotechnology & Food Sciences Group (Wageningen University and Research Centre, The Netherlands) as a dry powder (particles of amorphous shape with average size of 120 nm). Bovine serum albumin (BSA), Tween 20, polyethylene glycol (PEG, MW 3350) and *o*-phenylenediamine (OPD) were purchased from Sigma–Aldrich Inc. (St. Louis, USA). Horseradish peroxidase (HRP)-labelled swine anti-mouse IgG (SwAM, HRP/SwIgG = 1.81, concentration 8.9 mg mL^{-1}) was obtained from Seva Pharma, Czech Republic.

Other common chemicals were of the highest purity available and purchased from Sigma–Aldrich. Deionised water for standards and buffer solutions was prepared on apparatus RO-TFM-5SV (Fresh Water Systems, Inc., Greenville, USA).

Whatman GmbH (Dassel, Germany) supplied various types of nitrocellulose membranes (PRIMA 80, PRIMA 125, AE 98 FAST, AE 98, AE 99, AE 100, FT 020, FT 060, Protran BA 79, Protran BA 83, and Protran BA 85) tested in strip assay. Vinyl backing ARcare® 7823 was from Adhesives Research Inc. (USA), and the absorbent pad CFSP from Millipore Corp. (USA). Ninety-six-well ELISA polystyrene microtitre plates Costar (catalogue no. 9018) were obtained from Corning Inc. (USA).

2.2. Water samples

Samples of surface water were collected from streams in lowland agricultural area of central part of Czech Republic. Freshly collected samples were filtered through nylon filter to remove suspended particulate matter and than stored in darkness at 4 °C until analysis.

Water samples containing methiocarb (concentration range of 0.01–1000 ng mL^{-1}) were prepared as follows: methiocarb-free samples, as verified by LC/MS analysis, were spiked with a known amount of methiocarb derived from stock solution and used immediately for analysis.

2.3. Labelling of swine anti-mouse antibody with carbon nanoparticles

Carbon nanoparticles bind proteins non-covalently without changing their bioactivity (Van Amerongen et al., 1993). The swine anti-mouse antibody was labelled with colloidal carbon nanoparticles according to the O'Keeffe et al. (2003) with gentle modification. A colloidal carbon suspension (carbon nanoparticles 2 mg mL^{-1} of 5 mM borate buffer, pH 8.8) was sonicated for 10 min on ice using a Sonic 1 (Polsonic, Poland). Subsequently, with a simultaneous gentle stirring the SwAM was added to give a final protein concentration of 350 μg mL^{-1}. Then, this mixture was stirred gently at 4 °C overnight. In the end, the suspension was washed three times in a 5 mM borate buffer, pH 8.8 (containing 1% BSA and 0.02% NaN_3) using centrifugation (10 000 g, 15 min, 10 °C). Final sediment was resuspended to the initial volume. Prepared stock suspension of the SwAM-carbon conjugate was stored at 4 °C in the dark.

2.4. Preparation of immunochromatographic test strips

Each strip contained methiocarb-ovalbumin conjugate (methiocarb-OVA) and a rabbit anti-swine antibody immobi-