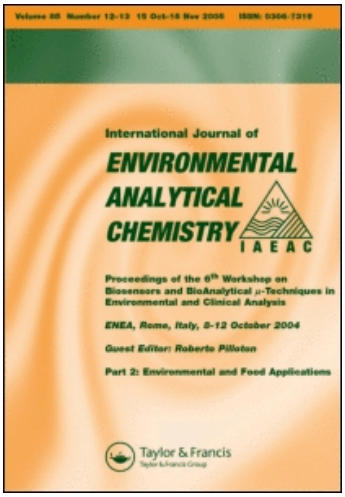

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A Detection Tube for Cholinesterase Inhibiting Compounds

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The enzyme butyrylcholinesterase from horse serum catalyses the hydrolysis of certain esters. The orange-red 2,6-dichloroindophenyl acetate will be converted by the enzyme into a deep blue alcohol. The colour transformation does not occur when the enzyme is inactivated. By making use of this biochemical reaction a cheap and simple, but very sensitive and specific detection tube could be developed. The tube comprises a breakable ampoule with an aqueous buffer solution, a freeze-dried preparation of the chromogenic ester with a filler promoting its dissolution, a freeze-dried preparation of butyrylcholinesterase with a filler promoting its stability, and an indication layer. DDVP can be detected at concentrations as low as 0.4 mg/m^3, when the sampled airvolume is 21.

KEY WORDS: Organophosphorous insecticides, cholinesterase inhibition, detection tube, industrial hygiene, safety.

INTRODUCTION

The increasing use of very toxic organophosphorous insecticides requires a simple analytical tool for a regular check on the environmental contamination at places where these products are produced, processed, transported or used. Recognizing the lack of a cheap and simple but very sensitive and selective detection device which can be applied without having expert knowledge and/or a well-equipped laboratory, a detection tube has been developed. The toxicity of organophosphorous insecticides is mainly based on their ability to inactivate acetylcholinesterase by

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stoichiometric phosphorylation. Cholinesterases act as highly active catalysts for the hydrolysis of certain esters. Upon inactivation by inhibiting compounds the enzyme is not able to catalyse the ester hydrolysis. Hence, the hydrolysis products, alcohol and acid, are not observed. Consequently, the biochemical reaction offers possibilities for a very sensitive and selective detection method of cholinesterase-inhibiting compounds.

Butyrylcholinesterase (BuChE) has been selected as enzyme. It is commercially available in large quantities, is relatively cheap and is stable upon storage as a freeze-dried product.

Selection criteria for the ester involve:

—sufficient solubility in water to ensure reaction with the enzyme;
—rapidly catalysed hydrolysis by the enzyme;
—little spontaneous hydrolysis in aqueous solution;
—formation of hydrolysis products which can be visually detected directly or by converting them into coloured products;
—sufficient stability of the solid ester to guarantee several years of storage.

Esters such as butyrylcholine, acetylthiocholine, α-naphthyl acetate and 2,6-dichloro-indophenyl acetate could be applied. The hydrolysis of butyrylcholine can be followed from the production of butyric acid by using a pH-indicator. However, the biochemical reaction has to be carried out in a buffered aqueous solution because the enzyme activity is pH-dependent. Also, acidic and basic vapours will interfere with the detection reaction. Therefore, sensing of the "alcohol" produced improves selectivity.

With acetylthiocholine the resultant thiocholine can be colorimetrically detected after forming a yellow product with DTNB (5,5′-dithio-bis-[2-nitrobenzene]). This colour is not contrastful enough for visual observation. The ester α-naphthyl acetate is therefore often preferred. α-Naphthol forms a distinctive red-purple colour upon reaction with Fast Blue B-salt (3,3′-dimethoxy-diphenyl-bisdiazonium chloride). Application of this ester in a detection tube is difficult, because too many chemicals are involved. The orange-red coloured 2,6-dichloro-indophenyl acetate (DIPA) on the other hand, immediately forms a distinct blue product upon hydrolysis (Figure 1).

Although DIPA offers many advantages its poor solubility in water presents problems. Since organic solvents have an adverse effect on the activity of BuChE an attempt was made to bring DIPA into such a form that a small but sufficient amount can be dissolved in water upon implementation of the detection reaction. This was achieved by making use of a freeze-dried DIPA/gelatine preparation. In order to obtain a manageable product which is not too elastic or too brittle, and which will

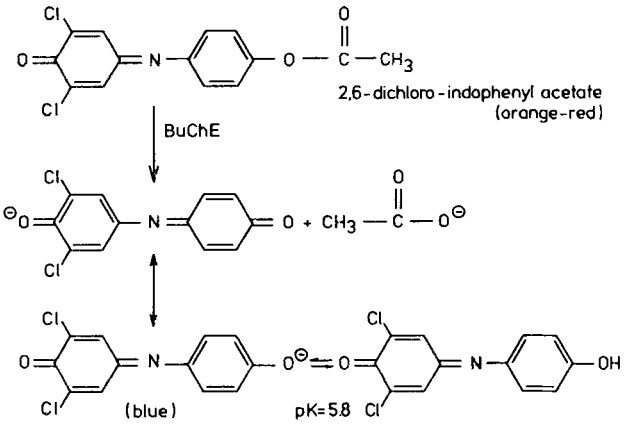

FIGURE 1 The enzymatic catalysed hydrolysis of 2,6-dichloro-indophenyl acetate.

dissolve very quickly in water, partially hydrolised gelatine is preferred among other natural or synthetic pharmaceutical gums[1].

The enzyme is to be stored in a dry solid state, whereas the amount of enzyme must be manageable during the production of the detection tubes. That is why an inert filler which also has a stabilizing influence, is added to the commercially available freeze-dried enzyme product. This filler together with the enzyme must dissolve instantaneously upon administration of the aqueous buffer solution. Again, this was achieved by making use of a freeze-dried BuChE/partially hydrolysed gelatine preparation.

Furthermore, in the test tube a buffered aqueous solution must be present. After capturing the cholinesterase-inhibiting compounds, a medium has to be created in which both the enzyme-inhibitor reaction and the enzyme-ester reaction occur to an optimum extent and in which an optimal colour formation is obtained for visual observation. Application of a tris(hydroxymethyl)aminomethane buffer solution (TRIS) proved to be adequate.

PREPARATION OF THE REAGENTS

The DIPA/gelatine preparation

1 g of gelatine is dissolved in 50 ml of water. To this 160 mg NaOH is added. The solution is heated during 90 minutes at 60°C. The partially hydrolysed gelatine solution is cooled and carefully acidified to pH 3.5 with an HCl-solution. To this solution 7.4 mg DIPA, dissolved in 7.4 ml dioxane, is added. The solution thus obtained is freeze-dried during 24 hours. The product is somewhat pulverized with a spatula and stored in a

desiccator over P_2O_5. Results of accelerated aging tests showed that this product is sufficiently stable, provided it is stored in the dark and free from moisture. Under such conditions its half life at 20°C was estimated to be at least 5 years.

The BuChE/gelatine preparation

2.5 g of gelatine is dissolved in 125 ml of water. To this 0.4 g NaOH is added. This solution is heated during 90 minutes at 60°C. Then the partially hydrolysed gelatine solution is cooled and carefully neutralized to pH 7.5 with an HCl-solution. In this solution 60 mg (about 180 IU†) BuChE is dissolved while the flask is gently shaken. The solution thus obtained is freeze-dried during 24 hours.

The product is somewhat pulverized with a spatula and stored in a desiccator over P_2O_5. No loss of enzyme activity was observed during this process.

Accelerated aging tests showed that this product has very good storage qualities. Based on these tests the half-life at 20°C was estimated to be at least 9 years but possibly 36 years.

CONSTRUCTION AND DEPLOYMENT OF THE DETECTION TUBE

A detection tube for cholinesterase-inhibiting compounds has been constructed with the reagents described (Figure 2).

The detection tube comprises:

—an ampoule with 0.15 ml of 0.1 M TRIS-buffer solution, pH 8;
—5 mg of the DIPA/gelatine preparation;
—10 mg of the BuChE/gelatine preparation;
—85 mg of silica (0.2–0.5 mm).

Each layer is separated from the other by a small inert nylon gauze.

The steps to be performed are:

—the sealed glass ends of the detection tube are broken off;
—an airvolume of 2 l is pumped through the tube in the direction indicated;
—the ampoule with the buffer solution is broken. A small flexible hose surrounding the detection tube prevents a loss of liquid. Both gelatine

† 1 IU = 1 μmol of acetylcholine/min, pH 7.5, 25°C.

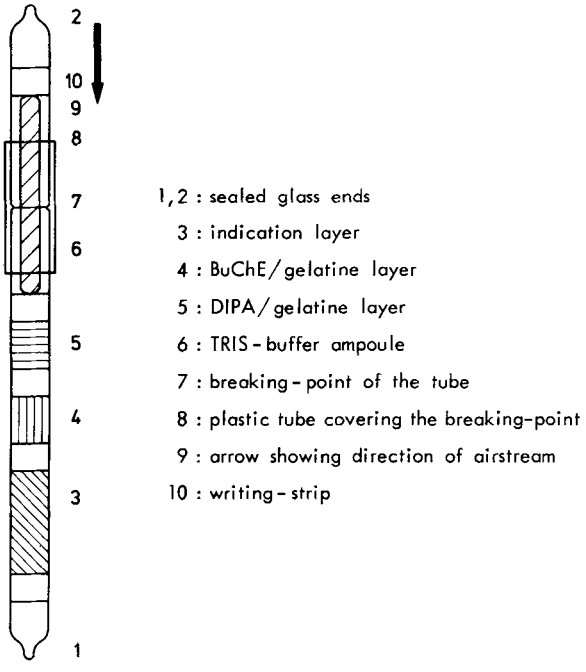

FIGURE 2 Detection tube for cholinesterase-inhibiting compounds.

preparations will almost instantaneously dissolve in the buffer solution which flows downwards onto the indication layer;

—the liquid is tossed down for complete wetting of the indication layer;

—One minute after wetting the colour on the indication layer is observed. In the absence of cholinesterase-inhibiting compounds the orange-red ester will have been converted into a deep blue hydrolysis product. If cholinesterase-inhibiting compounds are present in detectable concentrations the enzyme is inactivated and no blue colour will be visible.

DISCUSSION

When the detection tubes are properly assembled the pressure drop over the various layers and separation gauzes in the tube is about 300 mm H_2O. This allows 5 to 7 pump strokes of 100 ml per minute. Since a total air volume of 21 has to be sampled one test can be performed within about 4 minutes.

The air must be sampled through the reagent layers onto the indication layer. In this case the BuChE is probably already partially inhibited before it arrives at the indication layer. If the air is sampled in the opposite direction, the cholinesterase-inhibiting compounds will be captured at the very bottom of the adsorptive indication layer. Then, a blue hydrolysis product of DIPA will be formed even before the BuChE gets inhibited. The detection will be seriously impaired.

The colour change of the indication layer can be evaluated in two ways. If the enzyme is not completely inactivated, the slight remnant activity will still hydrolyse a small part of the ester, causing a slight blue-colouring of the indication layer. A difference in colour will be clearly visible when compared with a blank reference test. Although very low concentrations of cholinesterase-inhibiting compounds can be detected in this way, blank references tests are not recommended for reasons of simplicity.

A more objective criterion is the complete absence of a blue colour. This requires complete inactivation of the enzyme and hence higher concentrations of cholinesterase-inhibiting compounds. Nevertheless, dimethyl-2,2-dichlorovinyl phosphate (DDVP) can be detected according to the latter criterion at concentrations as low as 0.4 mg/m^3, when an air volume of 2 l is sampled (MAC-value: 1 mg/m^3).

The durability of the detection tubes has been estimated with isothermal accelerated storage tests at 30, 50 and 70°C. At 70°C the tubes proved to perform adequately up to 80 days, at 50°C up to 250 days of storage. It may therefore be concluded that at normal storage temperatures up to 20°C the durability will be at least 5 years.

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