Arh hig rada toksikol 1997;48:219-224

SHORT COMMUNICATION

IDENTIFICATION OF THE CONTENTS AND THE SHELF-LIFE OF INDICATOR TUBES FROM FIELD KITS FOR DETECTION OF ORGANOPHOSPHORUS COMPOUNDS IN THE AIR

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Received March 10, 1997

Indicator tubes from field kits for detection of organophosphorus compounds in the air were found on the territory of Croatia, in barracks liberated during the war years of 1991/1992, and were analysed during 1993/95. The detection kit consisted of glass indicator tubes with two sealed vials within each tube, a device for opening the tubes and breaking the vials, and an air sampling pump. The tubes were marked with serial numbers and expiry dates (1974–1992), but the description as to their contents was unavailable. The aim of this study was to identify the contents of the indicator tubes and to establish whether they were still suitable for detection of organophosphorus compounds. One vial was found to contain a cholinesterase (EC 3.1.1.7) preparation, while the other vial contained a non-thiocholine substrate and a pH-sensitive indicator (most probably cresol red). Applying DDVP as an organophosphorus cholinesterase inhibitor, it was found that all sets of indicator tubes were suitable for use regardless of the indicated expiry dates. Furthermore, the same tubes were found suitable for detection of organophosphorus compounds in water.

Key terms:
cholinesterase preparation, cholinesterase inhibitors, DDVP, detection kit, expiry date

During the war years of 1991 and 1992, sets of field-kit for detection of organophosphorus compounds (OP) in the air were found in army barracks liberated on the Croatian territory. They were found without description as to their contents and they did not bear a manufacturer name. The detection kit consisted of glass indicator tubes, a glass breaking device and a hand pump for air suction. Glass indicator tubes contained reagents for OP detection which were marked with serial numbers and expiry dates ranging from 1974 to 1992. Fourteen sets of indicator tubes with different serial numbers were tested.

The aim of this study was to identify the reagents within the glass indicator tubes and to test whether they were still suitable for detection. The indicator tubes were tested in 1993 and 1995. A preliminary account of the results was published earlier (1).

DESCRIPTION OF THE INDICATOR TUBES

The indicator tubes were 9 cm long with the diameter of 3–4 mm. A narrowing located at one third of the distance between the ends divided the tubes in two compartments. The smaller compartment contained a sealed vial (vial 1) with a colourless solution (about 50 μL). The other compartment contained a 2 cm thick absorbent layer and another sealed vial (vial 2) with a yellow solution (about 50 μL).

IDENTIFICATION OF THE REAGENTS

So far, several field methods for detection of OP have been developed, based on inhibition of cholinesterases by these compounds (2, 3). The identification of reagents from the indicator tubes was therefore started from the assumption that the kit for detection of OP in the air contained a cholinesterase preparation and an indicator of its activity.

The following reagents were used to identify the substances within vials:

Substrates: Propionylthiocholine iodide, PTCh (final concentration 1.25 mM) and benzoylcholine chloride, BzCh (final concentration 50 μM).

Cholinesterase inhibitor: DDVP (dichlorvos; O,O'-dimethyl 2,2-dichlorovinyl phosphate); stock solution 5 μg/mL.

Buffer: 0.1 mM phosphate buffer pH=7.4 or pH=8.5.

SH-reagent: DTNB (5,5'-dithiobisnitrobenzoic acid; final concentration 0.33 mM).

pH-indicators: bromthymol blue, cresol red, phenolphthaleine and thymol blue were prepared in water according to the standard procedure (4). Indicators were prepared in 0.2 N HCl or in phosphate buffer pH=8.5.

The pH of the solutions in the vials was determined with a paper indicator because the quantity of the solution was too small for applying a more precise pH-measurement. Solutions in vials 1 and 2 had pH values above 8 and below 7 respectively.

Assuming that vial 1 contained a cholinesterase preparation, the activity of the solution from vial 1 was tested spectrophotometrically with PTCh and BzCh as substrates (5–7). PTCh is a substrate of both acetylcholinesterase (EC 3.1.1.7) and butyrylcholinesterase (EC 3.1.1.8), while BzCh is a specific substrate of butyrylcholinesterase. The following procedure was applied for measuring the activity with PTCh: Vial 1 was removed from the glass tube, broken with a glass rod into a test tube and the content was mixed with 3.0 mL buffer pH=7.4. To 0.5 mL of that solution, we added 2.5 mL buffer pH=7.4, 100 μL DTNB and 100 μL PTCh and recorded the activity at 412 nm for 2 min (5, 7). The reaction mixture for activity measurements with benzoylcholine contained 1.0 mL enzyme solution prepared as described above and BzCh in buffer (2.0 mL). The activity was recorded at 240 nm for 6 min (6, 7).

The activity of the solutions from 17 vials belonging to 14 different sets was measured with propionylthiocholine, while the solution activity of two vials from two sets was measured with benzoylcholine. All solutions were active and the results were taken as a proof that vial 1 contained a butyrylcholinesterase preparation. The activities with PTCh as a substrate are given in Table 1. The average cholinesterase activity in solutions from the vial sets with the expiry date from 1974 to 1982 was 25% lower than in the sets expiring from 1984 to 1992.

Table 1. *Cholinesterase activity of solutions from vial 1 against propionylthiocholine and the time required for colour to change from violet to yellow in indicator tubes.*

Range of declared years of expiry	Enzyme activity (μmol / min / mL)*	Time required for yellow colour to appear (min)	
		Control test (with water)	Inhibitor test (with DDVP)
1974 – 1982	3.3 ± 0.9 (6)	1 – 2	30 – 180
1984 – 1992	4.2 ± 1.0 (11)	0.25 – 1	10 – 35

*Propionylthiocholine activity (±SD) expressed per mL of solution from vial 1.
Fourteen sets of indicator tubes were tested. The number of vials tested for activity is given in parentheses. The control and inhibitor tests for the time of colour appearance were performed once for each set.

The enzyme solution from vial 1 was subjected to inhibition by a cholinesterase inhibitor, DDVP. The reaction mixture containing the enzyme preparation (prepared as described above), the buffer and DTNB was incubated for 15 min with DDVP (100 μL, 5 μg/mL) before adding PTCh. The DDVP concentration was chosen on the basis of the second order rate constants of cholinesterase inhibition determined earlier (8). Control samples contained water instead of DDVP. The activity was measured with PTCh as described above. Seven vials from different indicator sets were tested and displayed an inhibition of about 70%.

The solution from vial 2 evidently contained a cholinesterase substrate and an indicator of cholinesterase activity. No reaction was recorded with the SH-reagent DTNB (5), which excluded the presence of thiocholine substrates. The hydrolysis of cholinesterase substrates leads to formation of acids. Hence, there are several methods for measuring cholinesterase activity based on the change of colour in pH-indicators. The contents of vial 2 were therefore tested for the presence of a pH-indicator. We measured the absorption spectra between 300 and 600 nm of the solution from vial 2 in the buffer pH=8.5 and in HCl solution. The absorption spectra of several common pH-indicators such as bromthymol blue, cresol red, phenolphthalein and thymol blue solutions were also measured for comparison. The solution from vial 2 had a pronounced absorption peak at 560 nm in the buffer pH=8.5, while the peak in acid solution was at 502 nm. The most similar spectra were those of the cresol red solutions showing peaks at 572 nm in the buffer pH=8.5 and at 511 nm in acid solution.

SHELF-LIFE OF INDICATOR TUBES

The rationale of OP detection in the air is the following: When the cholinesterase preparation from vial 1 (pH>8) is mixed with the substrate solution from vial 2, the yellow colour of the indicator changes immediately to violet as the pH of the solution becomes alkaline. If the enzyme is active, the solution will soon turn acid and the indicator colour will change back to yellow. Since cholinesterase substrates are esters, their hydrolysis makes the reaction media acid. If the air containing an organophosphorus compound is sucked through the indicator tube, the cholinesterase preparation from vial 1 will become inhibited and, when mixed with the content of vial 2, it will not hydrolyse the substrate as fast as an uninhibited enzyme. The time required for the violet colour to turn yellow depends on the OP concentration in the air.

Fourteen sets of indicator tubes were tested applying this rationale. Instead of sucking contaminated air through the tubes, the procedure was modified by adding water (control test) or a solution of DDVP (inhibitor test) into the tubes. The procedure was the following:

Inhibitor test: The tip of the tube on the opposite end of the absorbent layer was cut off. Vial 1 containing a colourless solution was broken and 100 mL of DDVP (5 mg/mL) were pipetted into the test tube through the opening. The inhibitor was mixed with the content of vial 1 by gentle shakes, and left for 15 min to enable cholinesterase preparation interact with DDVP. Subsequently, the other tip of the tube was cut off and vial 2 containing a yellow solution was broken. In order to mix the solutions, the air was carefully sucked through the tube. The colour of the mixture on the absorbent layer became violet. The time when the colour turned to yellow was recorded.

Control test: The procedure was the same as described for the inhibitor test except that DDVP was replaced by 100 mL of water.

The results shown in Table 1 confirm that all tested sets were still suitable for use. Control tests required less than 2 min and inhibitor tests more than 35 min to change colour from violet to yellow. The tubes labelled to expire between 1974 and 1982 took more time to change the colour than tubes labelled to expire between 1984 and 1992. The shelf-life of all sets of tubes was longer than declared.

The modification in testing the tubes with an aqueous OP solution instead of OP contaminated air applied in our study showed that the same indicator tubes could be used for both contaminated air and contaminated water. The tested tubes were sensitive to 5 μg/mL of DDVP. A field-test developed for detecting contaminated water, based on inhibition of human serum cholinesterase, had a detection limit of 0.05 μg/mL DDVP (3), while another field kit described elsewhere had a detection limit of 0.5 μg/mL (9). Although all three field tests are based on inhibition of the same enzyme, the difference in sensitivity is probably due to different sources of butyrylcholinesterase. The comparison of human, horse and rat serum cholinesterase showed that the human serum enzyme is the most sensitive to DDVP (8).

CONCLUSION

Indicator tubes in the field kit for detection of OP in the air contain a butyrylcholinesterase preparation, a non-thiocholine ester as a substrate and a pH-indicator, most probably cresol red. The cholinesterase preparations, substrates and pH-indicators from the tubes marked with expiry dates between 1974 and 1992 were still suitable for OP detection in 1993 and 1995 when the tests took place. Moreover, the same field kits were found suitable for detection of OP compounds in water.

Acknowledgement The authors wish to thank Mrs. Anđelka Buntić for her skilful technical assistance.

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Sažetak

SASTAV I VALJANOST INDIKATORSKIH CJEVČICA IZ TEST-PAKETA ZA DETEKCIJU ORGANOFOSFORNIH SPOJEVA U ZRAKU

U tijeku 1991/92. pronađeni su na teritoriju Hrvatske u oslobođenim vojarnicama test-paketi za detekciju organofosfornih spojeva u zraku. Test-paketi nađeni su bez oznake podrijetla i bili su pohranjeni u vojarnicama u nepoznatim okolnostima. Test-paketi sadrže indikatorske cjevčice, alat za otvaranje cjevčice i lomljenje zataljenih staklenih ampula unutar cjevčica, te crpku za zrak. Unutar indikatorske cjevčice nalaze se dvije zataljene ampulice

I sloj apsorbensa. U toku 1993.–1995. testirano je 14 setova indikatorskih cjevčica na kojima su bili označeni serijski brojevi i godina uporabljivosti (1974. do 1992.). Utvrđeno je da se u jednoj ampulici nalazi preparat butirilkolinesteraze (EC 3.1.1.8), a da druga sadrži indikator koji reagira na promjenu pH (vjerojatno krezolno crvenilo) i supstrat, koji nije ester tiokolina. U svim je setovima zabilježena aktivnost enzimskog preparata prema propioniltiokolinu kao supstratu, a u dva seta izmjerena je i aktivnost prema benzoilkolinu, koji je specifični inhibitor butirilkolinesteraze. Uporabljivost indikatorskih cjevčica iskušana je s dimetilnim diklorovinilfosfatom (DDVP), koji je organofosforni inhibitor butirilkolinesteraze. Kada se, bez prisutnosti inhibitora, sadržaj ampulice s preparatom butirilkolinesteraze pomiješa sa sadržajem druge ampulice, pojavljuje se ljubičasta boja, koja nakon 1–2 min prelazi u žutu. U prisutnosti DDVP-a svim je setovima cjevčica bilo potrebno mnogo dulje vrijeme za promjenu boje nego kada je test izveden bez prisutnosti inhibitora kolinesteraze. Provedeno testiranje pokazalo je da su reagencije unutar cjevčica ostale stabilne tijekom pohranjivanja, te da su bile uporabljive dulje nego što je označen njihov rok valjanosti. Pokazano je također da se indikatorske cjevčice mogu upotrijebiti i za dokazivanje organofosfornih spojeva i drugih direktnih inhibitora kolinesteraze u vodi.

Ključne riječi:
DDVP, inhibitori kolinesterazâ, preparat butirilkolinesteraze, rok valjanosti, test-paketi

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