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Blood Cholinesterase Activities of Flower Garden Workers After Exposure to Organophosphates

R. Rhyänen,[1] J. Liesivuori,[2] M. Närhi,[1] E. Puhakainen,[3] and O. Hänninen[1]

Dept. of Physiology,[1] University of Kuopio, P.O.B. 6, 70211 Kuopio 21, Kuopio Regional Institute of Occupational Health,[2] P.O.B. 261, 70101 Kuopio 10 and Dept. of Clinical Chemistry,[3] Kuopio University Central Hospital, 70210 Kuopio 21, Finland

Organophosphate insecticides, toxic also to man, are extensively used for pest control in agriculture and gardens (Kilgore and Akesson 1980). Garden workers may thus have a high pesticide exposure and an increased incidence of occupational related health problem. Unfortunately only limited amount of data is, however, available on the actual chemical loading of the garden workers. Blood cholinesterase activities can serve as useful indicators in organophosphate pesticide exposures (Vandekar 1980). The measurement of erythrocyte acetylcholinesterase (EC 3.1.1.7, AcChE) and plasma cholinesterase (EC 3.1.1.8, PCE) at the same time is favorable, because these enzymes may be inhibited to different degrees and for differing time periods after an exposure to organophosphates (OP). However, the collection and storage of blood samples for activity measurements is a problem under field conditions. The separation of erythrocytes and plasma before transport is time-consuming and centrifugation is needed. The unknown amount of hemolysis during preparation and transport is a potential disadvantage when whole blood samples are used. The reactivation process of the phosphorylated or carbamoylated cholinesterases is one considerable source of error (Eriksson and Faijersson 1980). A technique of applying samples of whole blood on filter paper has earlier been reported and applied under tropical conditions to avoid these difficulties (Augustinsson and Holmstedt 1965). The aim of the present study was to test the suitability of this method with slight modifications in the follow-up of occupational exposure of garden workers.

MATERIALS AND METHODS

Garden workers (n=7) exposed to organophosphates were used as test persons. The workers operating under conditions without organophosphates were used as controls. The garden workers were in different tasks during spraying season. Workers A,C and D worked as OP spray-

ers, workers A,B,C and D as flower assorters. Worker E was a foreman moving daily in OP treated areas. Workers F and G were flower salesmen. The workers used protective clothing only when they were spraying (respiration mask with filter, cotton clothes, hat and plastic gloves).

The following organophosphates were used by the flower garden workers: ethyl pyrophosphate (Bladan®), 2,2-dichloroethenyl dimethyl phosphoate (Dedevap®), diethyl p-nitrophenyl phosphorothionate (E-605®), dithio-6-methyl-2,3-quinoxalinedithiol carbonate (Morestan®), dimethyl 1-methoxycarbonyl-1-propen-2-yl phosphate (Phosdrin®) and O,O-diethyl 2-ethylthioethyl phosphorothioate (Systox®). The spraying scheme is depicted in Figure 1.

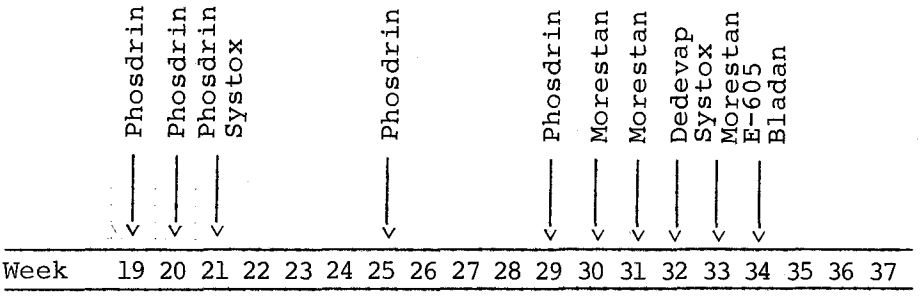

Figure 1. Organophosphates and their spraying times in the flower garden studied. Organophosphates were sprayed three times a week (2 hrs per time).

Blood samples were taken by venepuncture in garden. The garden was a greenhouse. First samples were taken before spraying season and then in the middle of each OP spraying weeks (two hours per day; three times a week). For determing whole blood cholinesterases 50 µl of blood were applied to filter papers (Munktell No 3). Each filter paper contained three blood spots (two papers for one person). The paper discs were allowed to dry for one hour. After drying, the papers were properly closed into plastic bags (Minigrip, Finland) and sent by express mail to the laboratory for analysis. It took about 24 hrs for blood samples arrive at analysis laboratory. If activity measurements were not done immediately the samples were preserved in vacuum desiccator at +4 ^{o}C.

Cholinesterase measurements in the flower garden were made before the spreading of insecticides and every four weeks these compounds were used. The cholinester-

ase activities in whole blood were determined by using the technique of Eriksson and Faijersson (1980) with minor modifications.

Blood spots of filter papers were cut out, placed separately in pieces in glass tubes. To one sample 2.95 ml of deionized water and to another 2.95 ml 1 % Triton X-100 (in water) was added. The tubes were shaken twice during one hour at room temperature. After eluting with water, 300 µl of the sample was added to a cuvette filled with 2.65 ml of 4,4´-dithiopyridine (Sigma Chemical Co., St. Louis, MO, U.S.A.) solution in 50 mmol/l Na-phosphate buffer (pH 7.7). The cholinesterase activity was then recorded at 37 °C after the addition 50 µl of 60 mmol/l of propionylthiocholine iodide (Sigma Chemical Co., St. Louis, MO, U.S.A.) solution at 324 nm. Cholinesterase hydrolyzes propionylthiocholine and and the thiocholine produced reacts with 4,4´-dithiopyridine. The reaction product 4-thiopyridone has an absorption maximum at 324 nm (Augustinsson et al. 1978). The measurement of cholinesterase was repeated once in presence of 0.1 mmol/l Astra 1397 or 10-(α-diethylaminopropionyl)-phenothiazine-HCl (Astra, Södertälje, Sweden). Astra 1397 is a specific PCE inhibitor (Augustinsson et al. 1978). After addition of Astra 1397 one must wait for one min before substrate could be added. The difference in activity was the plasma cholinesterase activity. The enzyme activity of the sample eluted with Triton X-100 was recorded in an analogous way. The activity in the presence of 0.1 mmol/l Astra 1397 was the erythrocyte acetylcholinesterase activity (Eriksson and Faijersson 1980).

The results were expressed in units-per-liter (U/l) according to the formula:

$$\text{Enzyme activity (U/l)} = \frac{\text{Total volume (µl)}}{\text{Sample volume (µl)}} \times \frac{1000}{E_m} \times \Delta A,$$

where ΔA = change in absorbance per min. E_m = molar absorptivity of 4-thiopyridone at 324 nm, pH 7.7; = 19.8.

RESULTS AND DISCUSSION

Proper care must be taken to ensure the stability of the blood enzymes. Therefore the stability of the two cholinesterases was followed for fourteen days when the samples were stored on filter papers at different conditions at room temperature (Figure 2 A). The activities, especially AcChE, declined sharply already after one day without protection against the effect of the air. The preservation times were similar in normal paper envelopes (data not presented) as in air. The activities remained, however, stable for one week when

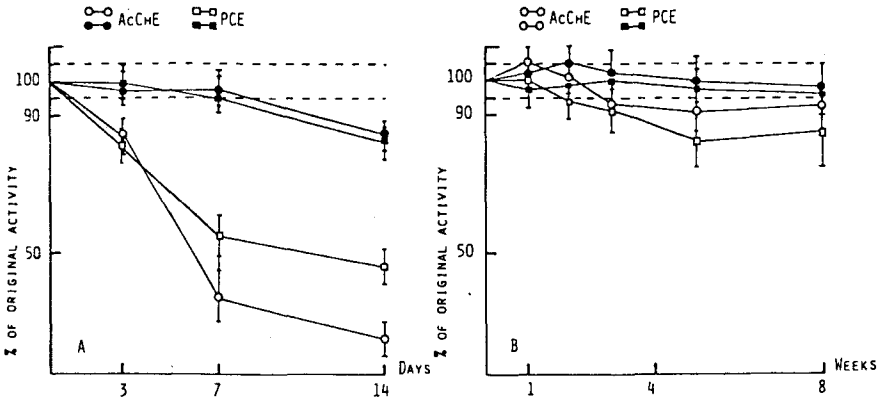

Figure 2. The preservation of blood cholinesterases on paper at room temperature (A) and +4 °C (B). At room temperature (A) the blood spots on paper were kept uncovered (○—○) on a test tube stand or closed into plastic bags (●—●). At +4 °C (B) papers in plastic bags were kept with (●—●) and without (○—○) vacuum. The bars indicate the standard deviations of the enzyme activities.

paper discs were closed into plastic bags under the same conditions (Figure 2 A). The stability of the enzymes on paper when stored in plastic bags at +4 °C using with and without vacuum desiccator was also measured. Without vacuum the enzymes remained stable in plastic bags for two weeks and in vacuum at least eight weeks. PCE seemed to be more unstable than AcChE without vacuum (Figure 2 B).

The filter paper method was used in the follow-up of blood specimens of garden workers exposed to organophosphates. The cholinesterase values of control workers varied ±12.5 % (Table 1). The exposure of the garden workers to organophosphates varied depending on the task. Most workers in the flower garden showed some degree of blood cholinesterase inhibition when compared to their values before OP spreading (Table 2). The foreman (worker E) exhibited the most pronounced AcChE depression (40 %) and worker G (one salesman) the PCE depression (27 %). Both blood enzymes were mostly inhibited in worker F (another salesman). Only one worker (C) had normal values through the whole spraying period. Mostly OP exposure inhibited the red cell AcChE more than plasma PCE (Table 2). Worker A (sprayer and flower assorter) had clinical symptoms at the spraying time (headache and irritation of eyes), although her red cell acetylcholinesterase values were decreased only 25 % and her plasma PCE values remained unchanged.

Table 1. The blood cholinesterase activities (kU/l±S.D.) of the control workers. The blood samples were taken from every worker twice a week (monday and friday).

Week no		1		2		3		4	
Worker	Weekday	AcChE	PCE	AcChE	PCE	AcChE	PCE	AcChE	PCE
1	Monday	3.36± .20	2.61± .08	3.58± .23	2.90± .08	3.20± .32	2.80± .10	3.41± .04	2.95± .00
	Friday	3.36± .04	2.88± .17	3.38± .13	3.18± .11	3.47± .04	2.88± .07	3.42± .12	2.94± .02
2	Monday	3.00± .04	2.25± .42	3.05± .29	2.26± .08	2.83± .16	2.32± .10	3.44± .04	2.20± .07
	Friday	2.83± .18	2.03± .25	3.25± .04	2.42± .13	3.11± .09	2.25± .20	3.09± .08	2.37± .02
3	Monday	2.90± .24	2.63± .11	2.95± .04	2.60± .15	3.10± .13	2.37± .29	3.20± .05	2.50± .07
	Friday	2.66± .01	2.65± .05	2.98± .09	2.74± .08	2.96± .18	2.54± .04	3.14± .04	2.53± .04

Table 2. The blood cholinesterase activities (kU/l±S.D.) of the flower garden workers during spraying season. The blood samples were taken from every worker in the middle of each week.

Week no	19		23		27		31		35	
Worker	AcChE	PCE	AcChE	PCE	AcChE	PCE	AcChE	PCE	AcChE	PCE
A	3.11± .20	3.51± .02	3.05± .08	3.11± .25	3.22± .15	3.21± .04	2.94± .16	3.19± .30	2.36± .27 **	3.50± .19
B	4.01± .24	3.21± .32	3.19± .39 *	2.66± .31	3.51± .33	2.87± .18	3.46± .29	2.77± .37		
C	2.91± .30	3.65± .12	2.60± .20	3.33± .08 *	2.66± .16	3.41± .23	2.58± .04	3.43± .19	2.77± .08	3.38± .18
D	3.55± .58	1.88± .04	2.93± .12	2.15± .10	3.36± .15	2.27± .10	2.42± .16 *	1.84± .21	2.77± .39	1.91± .22
E	3.90± .18	3.99± .14	3.30± .09 **	3.73± .23			3.72± .29	3.89± .28	2.34± .23 ***	3.40± .40
F	3.31± .36	2.30± .09	3.04± .39	2.05± .17	2.79± .09	2.15± .24	2.73± .31	1.91± .02 **	2.29± .13 **	1.71± .15 **
G	3.20± .33	4.37± .52	3.26± .57	3.43± .34	3.13± .46	3.95± .33	3.69± .24	3.97± .27	3.76± .35	3.18± .30 *

$p<0.05^{*}$; $p<0.01^{**}$; $p<0.001^{***}$; Student t-test.

The present results indicate that with plastic bags the blood samples collected under field conditions can be used in the follow-up of blood cholinesterases in biological monitoring. According to Augustinsson and Holmstedt (1965) no difference in cholinesterase activities could be found, if the samples are closed into envelopes or not. Our results indicate, however, that the paper envelopes are not suitable, since as in air a considerable loss of enzyme activity occurred with time. On the other hand plastic bag blood samples, kept dry on paper, retained their cholinesterase activities well for two months at +4 °C and one week at room temperature. Variations of enzyme activities in the nonexposure period were small enough to not interfere with the observation of possible decreased values during organophosphate loading. The blood cholinesterases recover from organophosphate inhibition when the sample is stored in a tube at +4 °C or room temperature. The cholinesterases stored on paper do not show reactivation during a three week period (Eriksson and Faijersson 1980).

There is little information available concerning poisonings in garden workers (Kilgore and Akesson 1980). In the present study a defective protective clothing during cultivation and even selling of flowers seemed to result in a depression of cholinesterase values in blood. None of the workers had their blood cholinesterases inhibited 50 % or more which usually causes cholinergic symptoms in humans (Wilhelm and Bradamante 1980). However, in one person who showed inhibition of only 25 % of her red cell acetylcholinesterase clear clinical symptoms were discernible. Thus the sensitivity of the workers may vary. It cannot, however, be excluded that other conditions in the garden during spraying season may have caused the symptoms of the particular worker. Air conditioning is quite difficult to arrange properly in greenhouses.

In conclusion the paper method is simple and practical for use in gardens and greenhouses, and for monitoring workers exposed to organophosphates and carbamates under field conditions. The method is especiallly suitable when samples must be mailed to laboratories making the analysis. There is no need for preliminary preparations of blood samples (centrifugation or freezing).

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