Bull. Environ. Contam. Toxicol. (1992) 49:37–43
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Environmental Contamination and Toxicology

Biological Monitoring of Workers Exposed to Mevinphos in Greenhouses

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Mevinphos (trade name Phosdrin®) is an organophosphorus insecticide used to control mites, beetles, caterpillars and slugs in greenhouses and in fruit and berry plantations. It is a compound of high toxicity not only orally but dermally as well; its LD_{50} value is 3-12 mg/kg orally in rat (Worthing and Walker, 1983), and 4-5 mg/kg dermally, in rat as well (Gaines, 1960). Mevinphos – like the other organophosphorus pesticides – absorbs quickly into the body through the respiratory organs, digestive system, skin and the conjunctiva of the eyes. The compound is fat-soluble and vaporizes easily (Gallo and Lawryk, 1991). Further, the great relative humidity and temperature in greenhouses increase the absorption of pesticides through the skin. Mevinphos is metabolized quickly in the body and the metabolites are secreted into urine and feces. The main metabolite in urine is dimethyl phosphate, DMP (Davies et al., 1979). Mevinphos does not accumulate in the body. The toxic effects of mevinphos are due to it's inhibitory effect on cholinesterase (ChE) activity. If the exposure to mevinphos is continuous, ChE can be inhibited permanently (Gallo and Lawryk, 1991). The restoration of ChE activity at the initial level requires the synthesis of new enzyme, which at least in the case of acetylcholinesterase (AChE) can take several weeks (Grob et al., 1947).

In greenhouses, the workers can be exposed to pesticides during mixing and loading of pesticide mixtures, during spraying operations and during cleaning and repairing the spraying equipment. Other workers can be exposed when doing plant handlings, harvesting or packaging of the flowers.

The purpose of this study was to evaluate the biological effects of the exposure to mevinphos during spraying operations and during plant handling and harvesting of the flowers in greenhouses. The biological effects and the exposure were estimated by using the inhibition of cholinesterase activities and by determining the metabolite of mevinphos, DMP, in urine samples.

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MATERIALS AND METHODS

Experiments were carried out in 1990 in eight greenhouses in Finland (Table 1). Phosdrin® was diluted with water before spraying and applicated in the afternoon with manual sprayers (high-pressure, knapsack and jug sprayers) and in the evening with non-thermal foggers. The foggers functioned automatically and the workers only occasionally visited the greenhouse during the application of the pesticide mixture. The workers entered the sprayed greenhouses the next day for plant handling and harvesting of the flowers.

Blood samples for cholinesterase determinations were taken from the sprayers on the day after the spraying operation (greenhouses no. 1-4). From the plant handlers and harvesters the blood samples were taken on the 2nd working day after the spraying (greenhouses no. 5-8).

Control blood samples to determine the initial cholinesterase activities were collected from the eight studied persons before the spraying operations with Phosdrin®.

The samples were taken from an antecubital vein into heparinized tubes. The blood was gently mixed and immediately pipetted on Munktell filter papers (Stora Filter Products, Grycksbo, Sweden). The dryed filter papers were placed in Minigrip plastic bags and stored in a desiccator in a refrigerator before the cholinesterase analysis. Red blood cell acetylcholinesterase (AChE) and plasma pseudocholinesterase (PChE) activities were then determined according to a spectrophotometric method (Eriksson and Faijersson, 1980).

The cholinesterase activities after the exposure to mevinphos were compared to the personal initial activities in the control samples and the decrease in AChE and PChE activities was calculated as percent (%) from the initial value. All the cholinesterase measurements in the laboratory were done in triplicate.

Urine samples of the workers were collected in clean plastic bottles, each sample in a different bottle. The samples were stored in a refrigerator before they came to the laboratory and thereafter at -70 °C until the analysis of DMP.

Control samples were taken before the spraying operations. The sprayers collected all their urine during one day after the Phosdrin® spraying and also on the morning three days after the spraying. The plant handlers and harvesters collected their urine samples during one day when they started their work in the mevinphos-sprayed greenhouses. Further, they collected the morning urine after one week of the spraying.

The metabolite of mevinphos, DMP, was analyzed in the urine samples by a modification of the methods of Fenske and Leffingwell (1989) and Muan and Skåre (1989).

Urine sample (500 µL) was diluted to 1 mL with deionized water. The sample was eluted through a Bond Elut SAX anion exchange column (Analytichem International, Harbor City, CA, USA). The SAX column was conditioned before the samples by rinsing the cartridge with one volume of methanol and with three volumes of deionized water. After the sample was run through the column, it was washed with 2 mL of deionized water and then it was absorbed to dryness for 10 min. DMP was eluted from the column with 2 x 0.5 mL of 4M HCl in methanol into the Kimax tube. 200 µL of that solution was pipetted into another Kimax tube and 800 µL of acetonitrile was added. The tube was mixed well with a tube mixer and left stand stopped for 2 hr in a room temperature.

After that it was evaporated to dryness in a fume cupboard in N_2 stream. 30 mg of anhydrous potassium carbonate (K_2CO_3) and 1 mL of acetonitrile was added, the solution was mixed with a tube mixer and left stand at room temperature overnight.

20 µL of pentafluorobenzylbromide (PFBBr) was used as a derivatizing reagent, the sample was mixed well and DMP was derivatized at 90 °C for 3 hr in a heated cupboard. After derivatization, the tube was cooled in a fume cupboard, after which it was evaporated to dryness in N_2 stream. The residue was dissolved by shaking in 1 mL of acetonitrile. 1-3 µL portions were injected into a gas chromatograph equipped with a flame photometric detector (FPD) in phosphorus mode. The standards were prepared in deionized water, mixed in control urine taken from non-exposed persons, and analyzed in the same way as the samples. The peak area in the samples was compared to the peak areas of the DMP-standards.

The recovery of the gas chromatographic determination of DMP from the urine samples was 86 %. DMP activity in the urine samples was found to be unchanged for at least 1/2 yr at -70 °C and two weeks in a refrigerator.

RESULTS AND DISCUSSION

Table 1 shows that the concentration of Phosdrin® sprayed was 100 to 500 times greater with the automatic foggers than with the ordinary manual sprayers. This emphasizes the importance of proper personal protection not only during spraying but also during plant handling, harvesting and packaging of the flowers.

The decrease (%) in red blood cell AChE and plasma PChE activities after the exposure to mevinphos is shown in Table 2. The exposure to mevinphos was greatest in the Phosdrin® sprayers in greenhouses 2 and 4, whose AChE was decreased 26 % and 18 % and PChE 29 % and 28 % from their initial values, respectively. The plant handler in the greenhouse 5 was also exposed to the pesticide as indicated by a 24 % and 15 % decrease in AChE and PChE, respectively.

Table 1. Greenhouses in the study, their sizes, flowers, concentration of Phosdrin® sprayed and the sprayer type used.

Greenhouse No.	flowers	size (m^2)	Concentration of Phosdrin® sprayed (%)	Sprayer type
1.	summer flowers	250	0.03	high pressure sprayer
2.	rose	960	0.05	knapsack sprayer
3.	white lily	80	0.01	jug sprayer
4.	rose	1700	0.05	high pressure sprayer
5.	chrysan-themum	1050	5	non-thermal fogger
6.	chrysan-themum	360	5	non-thermal fogger
7.	rose	2000	5	non-thermal fogger
8.	chrysan-themum	2500	5	non-thermal fogger

Table 2. Decrease in blood acetylcholinesterase (AChE) and pseudocholinesterase (PChE) activities (%) after exposure to mevinphos in greenhouses when compared to the personal initial values[a].

	Greenhouse no.							
	1[b]	2[b]	3[b]	4[b]	5[c]	6[c]	7[c]	8[c]
	% decrease							
AChE	0	-26	-6	-18	-24	-8	-11	0
PChE	-11	-29	-2	-28	-15	-15	0	0

[a]Values are mean of 3 determinations.

[b]Sprayer.

[c]Plant handler.

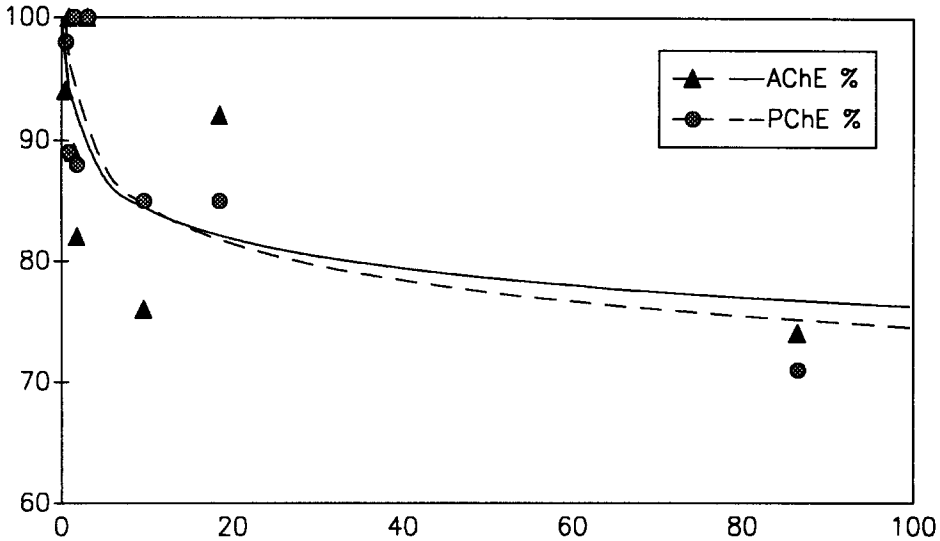

Figure 1.Decrease in greenhouse workers' red blood cell acetylcholinesterase (AChE) and plasma pseudocholinesterase (PChE) activities (% from the initial activity) compared to the total exposure to mevinphos through the workers' skin (µg/hr). The correlation coefficients calculated for the enzymes in the curves are r=0.65 (AChE) and r=0.83 (PChE).

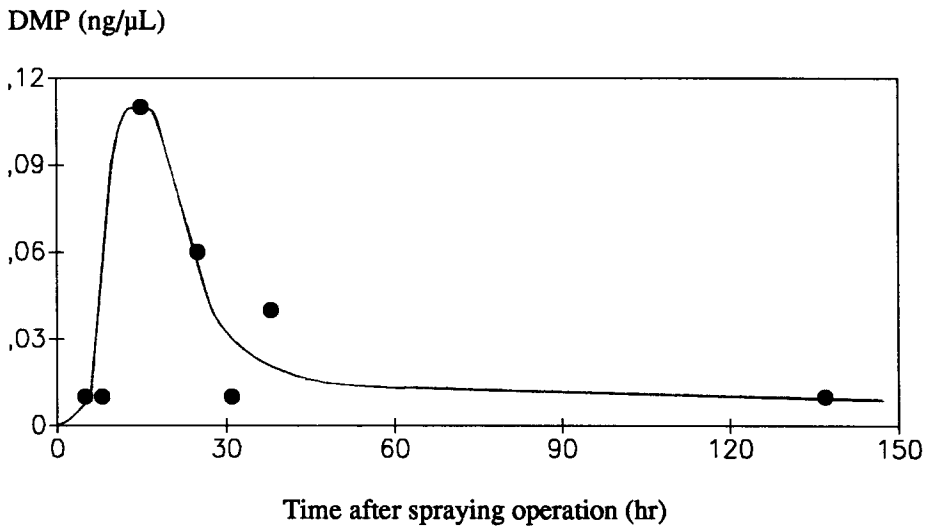

Figure 2. Secretion of the metabolite of mevinphos, DMP, (ng/µL) into urine after the exposure to mevinphos as a function of time (hr).

There seemed to be a high correlation between the decrease in AChE or PChE activities and the total exposure to mevinphos through the greenhouse workers' skin (Figure 1). In this study AChE and PChE did respond nearly in the same way to the exposure. The total exposure through the skin was calculated from the patch samples and hand washing samples taken from the workers during the exposure (*Kangas et al. , ubpublished work*). The Phosdrin® sprayer in greenhouse 2, whose cholinesterase activities were greatly decreased, had also the greatest total exposure to mevinphos (about 90 µg/hr) through his skin.

The metabolite of mevinphos, DMP, was detected in the urine of the sprayer in the greenhouse 2 (Figure 2). The maximum concentration of DMP was seen about 18 hr after the spraying of Phosdrin®. After the peak, the concentration of DMP started to decrease, and after 2 days from spraying operation the concentration of DMP was below its gas chromatographic detection limit (<0.02 ng/µL). The half life of urinary elimination of the metabolite of mevinphos was about 5 hr. The reason for the delayed maximum of the urinary elimination of DMP was most likely due to the dermal penetration of the pesticide. DMP was completely eliminated within 3 days. This is consistent with an earlier study made with DMP (Davies et al., 1979).

Both AChE and PChE activities should be simultaneously determined when biologically monitoring the exposure to mevinphos or to other organophosphorus pesticides, because the organophosphorus pesticides differ somewhat in their ability to inhibit these two cholinesterases (Hayes, 1982). The personal control sample (initial value) should be taken always before the exposure and the samples taken during the exposure should be compared with that value. If the exposure is continuous or if there are symptoms of poisoning, one should take samples more frequently.

The exposure to mevinphos can also be succesfully monitored by measuring DMP in urine samples. The most suitable time for taking a urine sample is about 15-20 hr after the exposure. A 24 hr urine sample can also be used to estimate the exposure.

Acknowledgments. The financial support from the Finnish Work Environment Fund is gratefully acknowledged. The authors also thank Ms. Sari Seppäläinen and Mrs. Leena Korhonen for excellent technical assistance.

REFERENCES

Davies JE, Enos HF, Barquet A, Morgade C, Danauskas JX (1979) Pesticide monitoring studies. The epidemiologic and toxicologic potential of urinary metabolites. Dev Toxicol Environ Sci 4:369-378.

Eriksson H, Faijersson Y (1980) A reliable way of estimating cholinesterases from whole blood in the presence of anti-cholinesterases. Clin Chim Acta 100:165-171.

Fenske RA, Leffingwell JT (1989) Method for the determination of dialkyl phosphate metabolites in urine for studies of human exposure to malathion. J Agric Food Chem 37:995-998.

Gaines TB (1960) The acute toxicity of pesticides to rats. Toxicol Appl Pharmacol 2:88-99.

Gallo MA, Lawryk NJ (1991) Organic phosphorus pesticides. In: Hayes WJ, Laws ER (eds) Handbook of Pesticide Toxicology, vol. 2, Academic Press, San Diego, pp 917-1123.

Grob D, Lilienthal JL, Harvey AM, Jones BF (1947) The administration of di-isopropyl fluorophosphate (DFP) to man. I. Effect on plasma and erythrocyte cholinesterase; general systemic effects; use in study of hepatic function and erythropoiesis; and some properties of plasma cholinesterase. Bull John Hopkins Hosp 81:217-244.

Muan B, Skåre JV (1989) A method for the determination of the main metabolites of malathion in biological samples. J Agric Food Chem 37:1081-1085.

Worthing CR, Walker SB (1983) The Pesticide Manual, a world compendium, 7th edition, Lavenham Press Limited, Lavenham, Suffolk, p 381.

Received August 29, 1991; accepted February 10, 1992.