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Agricultural Organophosphate Applicators Cholinesterase Activity and Lipoprotein Metabolism

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The organophosphorus compounds are widely employed in the control of crop and animal insects. The toxicity of these compounds results from their irreversible inhibition of cholinesterase (ChE), resulting in the accumulation of acetylcholine at cholinergic sites. From the end of 1987 onwards, large area in Africa have been treated with pesticides to control the desert locust (Schistosoma gregaria). This increases the use of insecticides, especially organophosphorus (OP) by the Crop Protection Service (CPS). Type of pesticides used is dependent upon crop and field conditions. The most used is fenitrothion (SUMITHION*). In recent years, the synthetic pyrethroid, fenvalerate has been used in association with fenitrothion (SUMICOMBI*). In the same way, interest in non-conventional aerial application methods such as ultra-low-volume (ULV) oil sprays using vegetable oil carriers and water-oil mixtures as pesticide carriers has increased (Cloud et al, 1987).This has resulted in an important exposure of applicators in the field.

The purpose of medical supervision is to protect the health of workers regulary exposed to cholinesterase-inhibiting pesticides. Its primary goal is to prevent the insidious development of profound ChE depression and poisoning (Ames et al,1989). The objectives of the present study were to assess the effects of OP formulations on the whole blood (WChE),

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plasma (PChE) and red blood cells (AChE) cholinesterase activity in CPS pesticide applicators during the 1989 cropping season and to compare the modifications of these activities with some lipid and protein fractions.

MATERIALS AND METHODS

49 base line ChE activity control persons were selected from University Hospital Centre of Dakar-Fann (UHC). 65 CPS persons used were distributed in four regions of Senegal (Thies n=9 ; Saint-Louis n=19 ; Dakar n=30 ; Kaolack n=7). The initial blood collection done prior to exposure to the chemicals will give the ChE reference value (base line). This collection concerned all the 49 control and the 65 CPS persons. During the exposure, blood sampling was repeated according to the formulations and the sprayer materials.

At Thies and Saint-Louis, the formulation used was SUMITHION* (fenitrothion alone). At Thies, it was an emulsion concentrate (EC 500 g/l) and the sprayer was a backpack (japan model MARUYAMA®) which daily delivery was about 30 to 50 L while at Saint-Louis, it was an ULV (500 g/L) and the sprayer model was italian JACTO® mounted on vehicle (german model UNIMOG®) with a 800 L daily delivery. At Dakar, the formulation was SUMICOMBI*, a mixture of fenitrothion (250 g/L) and fenvalerate (50 g/L). It was an EC (300 g/L) and the sprayer was a Backpack (italian model POLYJACTO®) with 32 L daily delivery. At Kaolack, an ULV SUMITHION* was used.The spayer model was a french model berthoud mounted on vehicle UNIMOG® with a daily delivery of600 L.

The daily exposure time was 6 to 8 hr for the applicators. The same standard cotton/polyester protective coveralls were used with spectacles, boots and gloves. During the exposure to the compounds, blood sampling was repeated once or twice. The repeated collection did not concerne all the 65 CPS persons but

some of them. At thies (n=9) and Saint-Louis (n=8), we have two collections, one after the first period of exposure (JULY 23th to AUGUST 10th for Thies ; JULY 17th to AUGUST 10th for Saint-Louis). The second period runs from AUGUST 11th to AUGUST 25th for Thies and AUGUST 11th to SEPTEMBER 1st for Saint-Louis. Only one collection was done after 12 days exposure at Dakar (SEPTEMBER 4th to SEPTEMBER 16th). The persons concerned were repartited in two groups : applicators (n=8) and others CPS workers (n=8) as drivers, store-keepers and pesticides loaders.

Blood samples were taken by venipuncture into heparinized tubes. The ChE activity was measured immediately or not far than 48 hr according to the colorimetric method of Ellman et al (1961). WChE, PChE and AChE were assayed for ChE activity using acetylthiocholine iodide and propionylthiocholine iodide (Sigma chemical Co,La Verpillière, France) as the substrates.

The assay contained 3 ml of a 0.05 M pH 8.0 tribuffer with dithiobisnitrobenzoic acid (DTNB), 0.1 ml of enzyme (after appropriate dilute) and 0.1 ml of 1.25 mM of substrate. The temperature was 37° C. Duplicate measurements were made on each sample. Change of absorbance per min at 405 nm using a perkin-elmer spectrophotometer was calculated to µM substrate hydrolyzed/min/mL. Specific activities were calculated using $1.36.\ 10^{4}.\ cm^{-1}.\ M^{-1}$ as extinction coefficient of DTNB.

In this study,clothing were not analysed for fenitrothion or fenvalerate.

Lipid and lipoprotein substrates as cholesterol (CHL), phospholipids (PLP), triglycerides (TRG), high density lipoprotein (HDL), low density lipoprotein (LDL) were assayed with commercial kits (Biomerieux, France). Gamma Glutamyl transpeptidase (GGT) was also determined with

commercial kits (Biomerieux, France).

The paired t test was used to analyse for differences between enzyme activities before and during exposure.

RESULTS AND DISCUSSION

The 49 persons selected at UHC were supposed to have by their professional activity no contact with pesticides. Their job is variable: administration officials,students teachers and tradespeople. Mean ChE activity(applicators before exposure and ChE base line activity control) is shown in table 1 and in table 2 the percentage of depression during applicators exposure. With the whole blood, the inhibition was about 5 to 28 % when acetylthiocholine (Ace) was used and 14 to 40 if propionylthiocholine (Prop) was assayed. The mean PChE inhibition found was 21 to 32 % with Ace and 26 % to 33 % with Prop. In the same way, the AChE declined from 4 to 27 % and 13 to 41 % respectively. It is observed that CPS workers other than applicators were also declined (table 2).

In addition to ChE activity, lipid and lipoprotein level was followed throughout TRG, CHL, PLP, HDL and LDL. According to previous studies as those of Kutty et al (1977), there has been evidence that ChE is somehow associated with lipid and lipoprotein metabolism. So, the treatment of laboratory animals as rabbits by dichlorvos was found to promote simultaneous decrease in PchE, CHL and LDL and increase in HDL (Ryhanen et al, 1984). On the other hand, OP poisoning was correlated with a decrease of GGT. Variations of lipids and lipoprotein fractions after pesticide exposure may become if confirmed an useful diagnostic and experimental tool.Samples of applicators from Thies, Saint-Louis and Dakar were selected for lipids and lipoprotein metabolism. No difference was observed for samples from Thies. CHL increased for Saint-Louis and Dakar but not significantly. It was in the same way with PLP for Dakar. Nevertheless, TRG increased significantly for the 3

Table 1 : Cholinesterase activity of persons who are not exposed to OP (UHC) and of Crop Protection Service (CPS) before exposure

	Whole blood activity		Red blood activity		Plasma activity	
	Ace [a]	Prop. [b]	Ace	Prop.	Ace	Prop.
Persons from University Hospital Center of Dakar-Fann (UHC) n=49	4.47 (0.25)	3.42 (0.22)	4.73 (0.35)	2.66 (0.22)	1.82 (0.17)	2.84 (0.25)
Persons from CPS n=65	4.94 (0.22)	3.92 (0.23)	5.10 (0.30)	3.16 (0.23)	1.71 (0.12)	2.99 (0.16)

a. activity expressed as µM of Acetylthiocholine hydrolyzed per min and per mL ; Mean (SEM)
b. activity expressed as µM of Propionylthiocholine per min and per mL ; Mean (SEM)

Table 2 : Cholinesterase depression after exposure to SUMICOMBI* or SUMITHION* (percent of pre exposure level)

	Days of exposure	Whole blood activity		Red blood activity		Plasma activity	
		Ace a	Prop. b	Ace	Prop	Ace	Prop
Thies	18	5.1	14.3	4.7	13.2	21.1	26.2
	32	24	40.4	17	30.6	26.8	30.5
Saint-Louis	24	10.7	31.5	27.5	41.3	21.4	26.2
Dakar applicators	12	15.8	20.8	22.1	29.1	12.4	12.6
Dakar other workers	32	28.3	30.8	19.5	24.8	33	33.4

a : Depression of acetylthiocholine b : Depression of propionylthiocholine

Table 3 : Variation of some lipids and lipoprotein fractions

	Cholesterol [a]	Triglyceride [a]	Phospholipid [a]	LDL [a]	HDL [a]	GGT [b]
Thies Before	1.46 (0.18)	0.47 (0.10)	1.72 (0.17)	0.88 (0.10)	0.45 (0.12)	14.76 (2.06)
After 32 days exposure	1.49 (0.17)	0.60 (0.16)	1.76(0.18)	0.91 (0.08)	0.50 (0.10)	14.62 (2.62)
Saint-Louis Before	1.46 (0.24)	0.54 (0.22)	-	1.06(0.18)	0.35 (0.07)	-
After 24 days exposure	1.62(0.23)	0.73(0.32)	-	1.01(0.15)	0.37 (0.03)	-
Dakar applicators Before	1.51(0.14)	0.49(0.11)	1.70(0.25)	1.01(013)	0.46 (0.08)	17.50 (8.98)
After 12 days exposure	1.62(0.15)	0.65(0.21)	1.85 (0.24)	0.99(0.15)	0.44 (0.06)	16.62 (4.97)

a. level expressed in g/l b. activity expressed in U/l

regions (table 3). Samples from Thies and Dakar applicators were assayed for GGT and no-difference was found before and during exposure (table 3).

A number of studies have been reported on the inhibitory effects of OP on the enzyme ChE among agricultural workers. With the increasing use of OP, surveys of blood ChE activity on exposed workers may help to identify workers at greatest risk (Duncan et al, 1986). So, a number of countries require medical supervision of agricultural pesticide applicators with monitoring of blood ChE activity levels, (Ames et al, 1986 ; Brown et al, 1989). Two approachs have been developed. One has been to compare differences in group mean values among OP exposed workers and controls for statistically significant depression among the exposed (Tocci et al, 1969). The second approach has been to compare

individual preexposure values to exposure values and report the findings as a percent of depression experienced by the workers (Wickers et al, 1979). Each approach has its advantage or inconvenience. In the case of studies involving field workers perfoming haversting,trimming and thinning activities, pre exposure blood samples are taken. The ChE values obtained prior to the field work are compared to those obtained during or after the work period. In monitoring studies group means for workers and controls are compared. In California for instance, each applicator acts as his or her own control

In this study, only the base line ChE activity of applicators was compared to values provided by an unexposed control population. The size of the two unexposed and applicators groups is important enough to evaluate intergroup variations before applicators exposure. No significant differences in ChE activity were detected between base line ChE activity controls (UHC) and pesticide applicators prior to the start of the study then, exposure applicators activity was compared as percent of depression of preexposure levels. In a previous study (Abiola et al, 1988), our laboratory has investigated the effect of two fenitrothion formulations and the findings suggested synergic effect of fenitrothion and fenvalerate. This study was then undertaken with more persons, to confirm or not those previous findings.

The comparison of the OP formulations concerned Thies and Dakar applicators. At Thies, SUMITHION* was used and at Dakar, it was SUMICOMBI*. The sprayers were the same. After 18 days application at Thies and only 12 at Dakar; AChE depression was more important at Dakar when acetylthiocholine was used (17.04 % at Thies and 22.17 % at Dakar). There was no difference with propionylthiocholine (Thies, 30.59 % ; Dakar 29.12 %). In despite of a 20 days difference, AChE depression was more important at Dakar where SUMICOMBI* was used. Nevertheless,WChE and PChE were more declined at Thies.

A conclusion is difficult and it is desirable to pursue the study and to compare the results after the same days exposure and the use of the same sprayers for the both formulations.

For the presentation of SUMITHION*,two regions were concerned. Thies were SUMITHION* was used as an EC and Saint-Louis where it was assayed as an ULV. After 32 days application at Thies and 24 at Saint-Louis, AChE depression was more important at Saint-Louis (table 2). This difference can be related to the presentation. ULV using vegetable oil carriers and water-oil mixtures as fenitrothion carriers could penetrate more the red blood cells. The difference can also be related to the sprayers. At Thies backpack was used while at Saint-Louis, the sprayer was jacto mounted on vehicle. The same remark than above could aid to assess this hypothesis. It is necessary to assay the SUMITHION* ULV and EC with the same sprayers in the same periods.

Generally, workers other than applicators as the field pesticide keepers and the loaders were not concerned by ChE monitoring. These persons with the same protective coveralls as the applicators are in the field during the spraying. Their job is to keep or load the pesticides mixture used by the applicators. Our results show that they are exposed as well as the applicators.After 32 days of exposure, ChE depression of this category runs from 19.51 % to 33.44 % (table 2). This depression is as important as the applicators'one.

Lipids and lipoprotein metabolism associated with PChE depression is not found in this study. No significant differences were detected before and during exposure.

This study may appear easy to initiate given the large number of people originally available. Unfortunatly, there were many limitations associated to it. One of the most important limitation was the inconsistency in the presence of agents during the experiment period. Due to this, only the agents consistently present throughout

the experiment were considered in the establishment of exposure value. In some situation, exposures values could not be available. This was the case of Kaolack where only 3 agents were consistently present throughout the study. For the purpose of this experiment, we may in the future need to find some ways to interest more the applicators.

Our laboratory is willing to collaborate with any program whose objectives are to establish the required protective means and to set the maximal exposure time for workers such that their chances to be poisonned are reduced.

REFERENCES

Abiola F A, Sere A, Sawadogo J G, Diatta F, Ly M (1988) Cholinesterase depression among senegalese crop protection workers exposed to organophosphorous pesticides. Bull Environ Contam Toxicol 4 : 483-488

Ames R G, Brown S K, Mengle D C, Kahn E, Stratton J W , Jackson R.J. (1989) Cholinesterase activity depression among California agricultural pesticide applicators. Am J Ind Med 15 : 143-150

Brown T H, Ames R G, Mengle D C, (1989) Occupation illnesses from cholinesterase inhibiting pesticides among agricultural applicators in California 1982-1985 Arch Env Hlth 44 : 34-39

Cloud R M, Zimpfer M L, Yanes J, Buco S M, Harmond C W (1987) Insecticide residues on clothing worn by crop consultants in soybean fields treated with non conventional application technology. Bull Environ Contam Toxicol38 : 277-282

Ducan R C, Griffith J, Konefal J (1986) Comparison of plasma cholinesterase depression among workers occupationally exposed to organophosphorus pesticide as reported by various study. J Toxicol Environ Hlth 18 : 1-11

Ellman L, Diane Courtney K, Andres V, Feather Stone R M (1961) A new and rapid colorimetric determination of acetylcholinesterase. Bioch Pharmacol 7 : 88-95

Kutty K M, Redheendran R, Murphy D (1977) Serum cholinesterase:function in lipoprotein metabolism. Experientia 33 : 420- 422
Ryhanen R, Herranen J, Korhonen K, Penttila I, Polvilampi M, Puhakainen E (1984 b) Relation between serum lipids, lipoprotein and pseudocholinesterase during organophosphate poisoning in rabbits. Int J Biochem 16: 687-690
Tocci P M, Mann J B, Davies J C, Edmundson W F (1969) Biochemical differences found in persons chronically exposed to high levels of pesticides. Ind Med 38:40-47
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