Chapter 3

Pesticide Poisoning Surveillance at the North Part of Benin (West Africa)

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First Published **September 18, 2017**

Abstract

Objective: Assessing the level of poisoning with the AChE test due to cotton producer's pesticide exposure in the biggest cotton production parts of northern Benin, and to identifying the risk factors.

Method: By a cross sectional study, we recruited in two cotton production townships,190 pesticides sprayers for at least 5 years. They were submitted to a questionnaire and to acetylcholinesterase (AChE) test using the Test Mate® model 400 devices (EQM Research Inc) with main component of the device: photometric sensor and principle based on the works by Ellman. By a non-probabilistic sampling, we recruited 190 farmers from the two townships fulfilling all the inclusion criteria and available to participate to all stages of the study.

Results: The studied population is essentially young: 83.16% were under 45 years old, with 75% illiterates. 70.3% of the farmers have more than 10 years of spraying experience. We noted that 2.06 % of the farmers still used domestic containers to prepare the pesticides. As precautions to prevent poisoning after spraying, 10.31% of the surveyed farmers drink milk. There was a significant AChE decrease between pre-exposure (AChE 3.08 ± 2.3UI / ml) and post-exposure (AChE 2.65 ± 0.52 IU / ml); $p = 0.009$. 73.1% of the famers were concerned by that inhibition. Those who could read the pictograms faced less inhibition of AChE ($p < 0.05$). The age variables, level of education and experience of pulverization do not have any influence on AChE inhibition.

Conclusion: AChE monitoring is needed for the surveillance of farmers.

Keywords

Acetylcholinesterase; Pesticides; Poisoning; Benin.

Introduction

Agriculture remains the world's largest pesticide industry, with about 2.9 million tons of pesticides used every year; out of which developed countries alone take up 75%, though increasing quantities of these substances are used in emerging and developing countries [1]. In most developing countries, agriculture contributes for a significant proportion in the gross domestic product [2]. For cash crops and for food crops, the control of pests during production or storage, is done essentially with chemical pesticides. The number of crops worldwide which could be destroyed annually by harmful species if chemical pesticides were not used is estimated to be close to 1/3 of the overall production [3].

The use of pesticides also saved millions of lives by killing the insects which are known to be potential vectors of disease, by protecting the crops and by contributing to improve agricultural yields. However, those pesticides have harmful effects on human health and on the environment [4;5]. Public health and environmental problems caused by the use of pesticides outweigh the benefits.

In Benin, the conventional cotton production consumes the most important quantity of the chemical pesticides used in agriculture. The increase in cotton production as noted recently reflects the level of pesticide consumption. Thus, the overall quantity of chemical pesticides consumed at the national level rose from 2.314.127 litres in 2006 to 4.917.136 litres in 2014: corresponding to an increase of 47.06% in nine years [6]. This growing increase in the volume of the insecticides used causes frequent food poisoning in cotton production areas; and this is due to the presence of pesticide residues in foods [7]. In average, about 100 cases of pesticide poisoning are reported each year, with at least 10 cases of death [8]. From 2006 to 2013, three hundred and twenty-three cases of food poisoning were reported in Banikoara [9]. These tragedies are just the expression of the incidents which occurred for many years; unfortunately, there is no documentation available on the subject. Banikoara is the top cotton production township in Benin [10]. Many other foodstuffs such as corn, yams, millet, sorghum, etc are also produced in that township.

Whereas the symptoms of sever pesticide poisoning are nearly immediately detectable, those of chronic poisonings are rather pernicious.

The most commonly used pesticides in our region are the OP pesticides irreversibly linked with the inhibition of the acetyl-cholinesterase (AChE). The symptoms related to the toxicity of the OP are due to the inhibition of the acetyl cholinesterase enzyme (AChE) that can possibly be measured out for a better surveillance. The inhibition of the AChE, therefore, leads to ulterior accumulation of acetylcholine at the level of the synapses as sources of the various clinical symptoms [11]. The infra-clinical exposure effects of chronic OP like inhibition of AChE can be detected early through biological tests. Intra - individual modifications of the active acetylcholinesterase exist ranging from 2% to 35% whereby the necessity of a referential dosage during the period of non-exposure for every individual. When a person presents a decrease superior to 20-35% in relation to his/her rate of reference, he/she must be removed from his/her work until the rate gets back to normal.

Very few scientific studies, especially in Africa, have been carried out in this perspective of pre-and-post-exposure dosage of AChE: Chakraborty et al. in India objectified an AChE inhibition at about 34.2% among Indian farmers [12]. Pathak et al still in India got an inhibition of 55% between pre-exposure and post-exposure [13]. In Nigeria, Sosan et al. objectified that 8/76 farmers have between 30 to 50% of AChE inhibition between pre-exposure and post-exposure [14]. This study has been initiated to comply with recommendations of the WHO according to which the individual should be his/her own witness in the dosage of AChE. It constitutes one rare case study in Africa in general and in Benin concerning the dosage of AChE and especially of pre-exposure and post-exposure with so important number of farmers.

The main objective of this study was to assess the degree of poisoning by AChE activity test before and after farmworkers exposure to pesticides in the townships of the first cotton township in the north of Benin (Banikoara and Kandi) and to identify the risk factors.

Patients and Methods

It was a cross-sectional study carried out from May 16th, 2016 to November 30th, 2016. During this study, we did an investigation through a questionnaire and carried out some AChE tests before and after average 7 sets of pesticides spraying.

Population of Study

It is composed of pesticides users from two townships of northern Benin as two of great cotton producer's regions: (Banikoara and Kandi). The pesticides users meeting the following criteria were submitted to the questionnaire and the AChE dosages: Living in study area (Banikoara and Kandi), having given his consent, having a farm or working in a farm where pesticides are used for the last five years, having done biological tests both at pre-exposure and at post-exposure.

Sampling and Size

We did a non-probabilistic sampling with exhaustive recruitment of all persons fulfilling all the inclusion criteria and available to participate in the study. At all we recruited 190 farmers for the two townships.

Data Collection Techniques and Tools

Data collection consisted to an investigation through questionnaire and acetylcholinesterase dosage with the pesticides users. The questionnaire appreciates after general information, knowledge on the use of pesticides, attitudes and practice of pesticides uses.

Blood Dosage

We did firstly the acetylcholinesterase pre-exposure dosage by automated colorimetric technique based on the method of Ellman [15]. After 6 months of pesticide spraying, (7 sets of pesticides spraying in average) a dosage of post-exposure was done anew to quantify the inhibition. In this study, the acetylcholinesterase dosage (AChE) was done using the Test Mate® model 400 devices (EQM Research Inc., Ohio, USA 2003) [16]. The main component of the device is a photometric sensor (wave length 450 nanometres) powered by a battery of 9.0 volts, the principle is based on the works by Ellman [15]. It permits the dosage of the erythrocyte cholinesterase. The referential values especially those of the erythrocyte acetylcholinesterase (AChE) and of haemoglobin (Hb) recorded are: AChE = 4.71 U/ml (N: 2.77 in 5.57 U/ml); Hb = 15.0 g/

dl; Q (AChE/Hb) = 31.4 U/GS (N: 21.9 in 37.3 U/g). Q is the AChE adjusted with regard to the rate of haemoglobin. The procedures of the AChE dosages were done in ambient temperature of laboratory (25° to 28°). The % of enzyme inhibition was calculated as follows:

[(AChE pre-exposure – AchE post-exposure) / AChE pre-exposure] ×100 [*17*]

Data Analysis

The double capturing of data was done and validated using the Epi Data software version 3.1. The statistical analysis was conducted using the version 12.0 of the Stata software. After a general description of the sample, the results of the quantitative variables were presented using the parameters of positioning and scattering; those of the qualitative variables were presented in percentage. We therefore did a bi-varied analysis where the demographic and pathological data were crossed. Concerning the association between the qualitative variables, we used Pearson's test of Chi square. At the end, the logistic regression has been done to search explanatory factors of the AChE inhibition. For all tests used the threshold of significance was 5%.

Results

Socio-Demographic Characteristics

The studied population is essentially young: 83.16% were under 45 years old, 75% of the population are illiterates. The sex ratio H / F is equal to 18.9 and 70.3% of the farmers have more than 10 years of spraying experience (Table 1).

Table 1: Socio demographic characteristics of the population.

Characteristics		Number	%
Ages category (year)	<15	0	0
	15-24	21	11.05
	25-34	77	40.53
	35-44	60	31.58
	45-55	29	15.26
	>55	3	01.58
	Total	**190**	**100**
Gender	Male	189	99.47
	Female	1	0.53
	Total	**190**	**100**
Education level	None	138	72.63
	Primary school	18	9.47
	Secondary school	34	17.89
	Total	**190**	**100**
Years spraying	≤5	13	06.84
	]5-10]	43	22.63
	≥10	134	70.53
	Total	**190**	**100**

Poisoning Risk Factors

The empty packings were rejected as much in the nature as they were burnt 42.11% in each of the cases. Nearly all the surveyed persons 96.32% declared that they took bath systematically after pulverization but when only when they get home in the evening 91.26%. A bit more than half: 54.21% declared they could read the pictograms. We noted that 2.06 % of the farmers still used domestic containers to prepare the pesticides. As precaution after spraying, 10.31% of the surveyed drink milk. Less than half: 44.85% had good level of understanding of the instructions written on the packings, and nearly as many were unable to understand those instructions 42.78 %. About 1/3 declared they systematically use the Personal Protective Equipment (PPE) at the time of pulverization. Gloves and masks were the only PPE used respectively in 26.8% and 8.76% of the cases (Table 2).

Table 2: Identification of the poisoning risk factors.

Risk factors		**Number**	**%**
Management of the empty packages	Re-use in domestic purpose	9	4.74
	Relinquishment in the nature	80	42.11
	Singeing	80	42.11
	Burying in the ground	24	12.63
Preparation of pesticides in domestic packages	Yes	4	2.06
Precaution after spraying	None	135	69.59
	Drinking some milk	20	10.31
	Drinking some oil	2	1.03
	Drinking some alcohol	3	1.55
	Other precautions	38	19.59
Smoke, drink or eat during the manipulations	Yes	0	0.00
Taking of systematic shower after the spraying	Yes	183	96.32
	No	7	3.68
Place of taking shower	On the farm	16	8.74
	At the house at the end of the day	167	91.26
Knowledge of the meaning of the pictograms	Yes	103	54.21
	No	87	45.79
Level of understanding of the instructions on packages	Yes, averagely	87	44.85
	Not very well	24	12.37
	No, I do not understand them	83	42.78
Systematic use of PPE during products preparation	Yes	64	32.99
Systematic use of PPE during the pesticides spraying	Yes	67	34.54
PPE used	None	126	64.95
	Gloves	52	26.80
	Mask	17	8.76
	Hat	0	0.00
	Boots	0	0.00

Cholinesterase Activity Decrease

There was a significant difference between pre-exposure AChE 3.08 ± 2.3UI / ml and post-exposure AChE 2.65 ± 0.52 IU / ml; P = 0.009. This inhibition of AChE is confirmed when we report its value to that of haemoglobin. Post-exposure AChE / Hb level 22.92 ± 3.69 U / g was significantly lower than pre-exposure 24.88 ± 4.9 U / g; P <0.001 (Table 3).

Table 3: Variation of AChE activity.

Mean		**SD**	**p-value**	
AChE (UI/ml)	Pre-exposure	3.08	2.3	0.009
	Post-exposure	2.65	0.52	
Q(AChE/Hb) (U/g)	Pre-exposure	24.88	4.9	<0.001
	Post-exposure	22.92	3.69	

We obtained, in a little more than half of the cases (57.84%), a decrease in cholinesterase activity. 31.3% of these decreases had a fall of more than 20% and 3.05% of them a decrease greater or equal to 50% (Table 4).

Table 4: Evaluation of the exposure: Inhibition of AChE and inhibition level.

AChE		**Number**	**%**
Inhibition of AChE	Yes	139	73.16
	No	51	26.84
	Total	190	100
Level of AChE inhibition (%)	≤20	112	80.58
	[20; 30]	14	10.07
	[30; 50]	12	8.63
	≥50	1	0.72
	Total	139	100

Table 5: Risk factors influence on AChE inhibition.

AChE		**Year spraying**			
		<5	**5-10**	**>10**	**p-value**
Inhibition	Yes	9	30	**98**	0.89
	No	4	13	36	
% of inhibition	<20	6	21	57	0.64*
	[20; 30]	1	5	**28**	
	[30; 50]	2	4	**12**	
	≥50	0	0	**1**	
AChE		**Education level**			
		Illiterate	**Primary**	**Secondary**	**p-value**
Inhibition	Yes	**103**	13	21	0.33
	No	35	5	13	
% of inhibition	<20	64	7	13	
	[20; 30]	26	4	**44**	
	[30; 50]	12	2	4	0.85*
	≥50	**1**	0	0	
AChE		**Knowledge of the pictograms meanings**			
		Yes	**No**		**p-value**
Inhibition	Yes	67	**70**		
	No	36	17		0.02
% of inhibition	<20	46	38		
	[20; 30]	12	**22**		
	[30; 50]	9	9		0.17*
	≥50	0	**1**		
AChE		**Gender**			
		Female	**Male**		**p-value**
Inhibition	Yes	1	**136**		
	No	0	53		1*
% of inhibition	<20	1	83		
	[20; 30]	0	**34**		
	[30; 50]	0	**18**		1*
	≥50	0	**1**		
AChE		**Protection**			
		With protection	**Without protection**		**p-value**
% of inhibition	<20	21	63		
	[20; 30]	13	**21**		0.33
	[30; 50]	7	**11**		
	≥50	0	**1**		

*= Fisher test

Those who knew the meaning of the pictograms were less subjected to inhibition of AChE than the 64.28% who didn't have any understanding at all (p <0.05). The other factors didn't significantly influence the variation of the rate of AChE. We noticed, however, the highest rates of inhibition with those who had more than ten years of pulverization practice and with those who were doing the pulverization without any protection (Table 5).

Impact Factor of Cholinesterase Activity Decreased

The variables of age, level of education and experience of pulverization doesn't influence the AChE inhibition: (p>0.05) Nevertheless, the secondary educated reduced their risk of 57% to AChE inhibition, whereas the primary increased their risk of 1.30 (Table 6).

Table 6: Multivariate analysis by logistic regression searching of the explanatory factors of the AChE inhibition.

		OR	CI 95%	p-value
Ages (years)				
	< 15			
	≥ 15	1.35	[0.37; 4.92]	0.65
Level of instruction				
	None			
	Primary	1.30	[0.39; 4.29]	0.67
	Secondary	0.57	[0.24; 1.34]	0.20
Seniority (years)				
	< 5			
	5 - 10	0.27	[0.05; 1.58]	0.15
	> 10	0.31	[0.05; 2.02]	0.22

Discussion

This study is one rare in this region to come up with cholinesterase data on persons investigated before and after exposure to organophosphorus pesticides. That gives us the opportunity to eliminate the influence of the interpersonal variations making of every person involved in pulverization his/her own witness. The other advantage of this study is that it was about a population the majority of which has many years (more than 10) of exposure to pesticides by massive pulverization of insecticides and pesticides at every cotton season which covers at least six months in a year.

Socio-Demographic and Personal Features

Our sample is essentially male 99.47%. This is to reflect the work organization in Benin farming communities where women are confined to household work while men must take care of farming activities. Passiani et al. [18] got similar results in 2012 with 99.1%. The population of study was relatively young with 83.16% of surveyed persons aged less than 45years. This result is very close to the one obtained by Toe et al. [19] in Burkina which publishes a proportion of 84.7% of users aged less than 50 years. Most the surveyed persons 93.16% had more than five years of pulverization of pesticides, as it has been found out by Ouédraogo et al. [20] who found a proportion of more than 90% in Burkina. The majority 72.63% were illiterate and only 17.89% had reached secondary school level. We can now understand the bad practices of pesticides use as noted among the producers in spite of the training they received. Indeed, even in developed countries the problem of low level of education of the farmers arises; in a cohort carried out in the United States in 2014 on 215 farmers from the bio - surveillance programme in the state of Washington, Strelitz finds that 63.72% of the 215 participants could not read in English [21].

Risk Factors of Poisoning

Most empty packings were thrown away in the nature, 42.11% of the cases, increasing the risk of pollution of the environment. This proportion is lower than the 48% obtained in Burkina-Faso by Ouédraogo [20] and much more than the 72% by Jors et al. [22] in Bolivia. The cases of burning, as observed 42.11% are much higher than the 16.1% observed in Brazil [18].

Another practice as hazardous as the previous ones is the use of household containers for the preparation of pesticides. A fringe of the population of study: 2.06% continue to prepare the pesticides in household containers instead of doing this directly in the sprayers containers which are rather graduated for that purpose. And by so doing, they expose themselves, and their immediate neighbours to accidental and collective risks of poisoning. In fact, children and even the adults could use this container inappropriately or unwashed either by ignorance or by mistake to fetch drinking water or to carry food.

None of the surveyed persons actually eat foods during the time of pulverization contrarily to the 12.8% reported by Foulhoux in France in 1998 [23] and to the 12% and 22.36% reported in Benin in 1998 respectively at Banikoara [26] and at Aplahoué [25]. But this good habit is compromised by the consumption of alcohol, oil or milk after pulverization by 30.41% of them. The consumption of milk is the most widely spread practice with more than 10% of the surveyed persons. Concerning the consumption of alcohol, Pasiani in Brazil [18] finds that 56.2% of the farmers actually drink alcohol. All those products are very likely to favour the absorption of pesticides which in fact are very lipophilic.

If the big majority of the investigated persons 96.32% took bath after the pulverizations, nearly 8.74% among them did it on the workplace as recommended, but the great majority (92.26% of the cases) took bath rather at home at the end of day; this corresponded to the results of Hinson and al. in 2007 in Benin [25]. This delay as to taking bath could favour poisonings through prolonged contact with the teguments and the mucous membranes.

In a bit, more than half of the cases 54.21% the surveyed persons couldn't understand the meaning of the pictograms. And in nearly the same proportions 54.15%, they couldn't either understand well or understand anything at all of the instructions written on the packings. This bad understanding of the security messages could be the result of the high level of illiteracy or the low level of education of the surveyed persons; and this could explain the bad habits observed in our study population. This rate is quite close to that of Magauzi et al. in 2011 in Zimbabwe [26] who found that 58.5% of the farmers couldn't understand any of the pictograms.

The same situation can be observed as far as the use of Personal Protective Equipment (PPE) is concerned where only 64.95 % of the surveyed persons actually used them at the time of preparation and pulverization of the pesticides. Still in Benin, Hinson et al. in 2015 [27] found a result quite close to ours where 69.3% of the farmers didn't use any protection equipment while handling pesticides. The result of our study is on the other hand widely higher than that of Pasiani in Brazil [18] who found that only 7.2% of the farmers don't protect themselves. This great difference with our results could be explained not only by the level of development of Brazil, but also by the fact that Brazil is one of the biggest users of pesticides in the world [21]. Among the few PPE used by the farmers in this study the most widely used ones are the gloves. But for Pasiani et al. [18] the most widely used PPE are the boots.

Variation of the Cholinesterase Activity

The average value of AChE at pre-exposure is significantly lower than the one observed at post-exposure ($p < 0.05$). This tendency is confirmed by the average of AChE compared to the rate of Hb (Q) ($p < 0.05$). Exposure to pesticides in the setting of our study, therefore, influences on the rate of acetyl cholinesterase.

The inhibition of the cholinesterase activity is higher than 20% in 19.42% of the cases. This rate is higher than the one obtained by Mamadou et al. [28] in Niger 16.50% on the one hand, and lower than the 24.1% obtained by Magauzi et al. [26] in Zimbabwé, than the 26% obtained in Iraq by Ahmed [17], and then the 26.82% obtained in Benin by Hinson et al [29].

The persons having no understanding of the meaning of the pictograms suffered more significantly from inhibition of the cholinesterase activity than those who understood the meaning of the pictograms. It has also been observed among them, the greatest number of persons having levels of inhibitions of cholinesterase activities >20%. About 40% of the persons presented a level of cholinesterase inhibition higher than 20%. The surveyed persons who did not wear any IPE had more important levels of inhibition >20%. In a logistical regression model, there seems not to be any significant dependence relation between variables of age, level of education and experience of pulverization (p>0.05). However, high experience of pulverization multiplies by 1.16 the risk of inhibition of the cholinesterase activity: OR=1.16 with IC95% = [0.26; 5.23] between 5 and 10 years of pulverization.

Conclusion

OP poisonings is common in developing countries and is of great concern, since it affects the most productive age group of the society. The population of agriculture work is relatively young: less than 45years old in this study and have to face the OP exposure with a heavy public health burden with the bad practices and attitudes. The decrease in cholinesterase activity was obtained with 73.16% of farmers which is a lot as rate with 18 % with AChE decrease more than 20%. Routine monitoring of AChE may allow for early recognition of frequent and continuous low-level exposure to OPs.

Acknowledgments

We thank all the participants for their participation. We thank also the farmworkers managers without whom we shall not have been able to obtain that participation. Special thanks to Angela Russ, Head of International Program at TRAID (*Textile Recycling for Aid and International Development*) for having accepted to take care of the marginalized population without any way which establish these farmers.

Author Contributions

HAV; DF: Initiators of the research conceived and designed the research protocol;

HAV; DF; YPE; AA: Performed and implemented the protocol at the work place and analysed the data

HAV, DF, YPE, AA: Wrote the draft of the manuscript

HAV; FB: Made critical revisions and approved final version

AA; YPE; HAV; FB: Agree with manuscript results and conclusions

FB: Chaired the research HAV; DF; YPE: Reviewed and approved of the final manuscript.

Ethical Considerations

This study received the agreement of the Ministry of Health (reference 3040/MS/DC/SGM/DNSP/SA on April 20th, 2016. The study was explained to every person and in local languages to the people who would not understand French. Prior to any inclusion, participants gave informed consents to participate in the study. Throughout the study participants could voluntarily withdraw if they wished.

Funding

This study was funding by *Textile Recycling for Aid and International Development* (TRAID) UK organisation.

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