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Original Research Article

Effect of using pesticide, on erythrocyte acetylcholinesterase level and risk factors of poisoning among cotton workers in Benin

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To evaluate the acetylcholinesterase level and risk factors of poisoning in pesticide users in cotton production areas in Benin. Using a cross sectional study design, 454 individuals who have been working as pesticide sprayers for at least 5 years were recruited. They completed a questionnaire and underwent AChE test before and after pesticide spraying using the Test Mate® model 400 devices (EQM Research Inc) with a photometric sensor. Empty pesticide containers/packagings were either burnt (32.82%) or re-used for domestic purposes (15.20%). After spraying, 47.93% of the participants consume milk believing that it is a good precaution against poisoning. Only 21.13% systematically use Personal Protective Equipment (PPE) while spraying pesticide. There was a significant inhibition between pre-exposure (AChE 2.92 ± 1.56 UI / ml) and post-exposure (AChE 2.73 ± 0.61 IU / ml): p = 0.01) AChE levels in the participants. AChE inhibition was higher in farmers who do not use PPE than in others (*p*=0.04).Pesticide poisoning is a reality therefore training and more preventive precaution should be taken and AChE monitoring is urgently needed for farm worker surveillance.

Key words: Acetylcholinesterase, cholinesterase inhibitor, pesticide poisoning, Benin.

INTRODUCTION

Nowadays, the use of phytosanitary substances has become a necessity as well as a danger. Indeed, the number of diseases and deaths linked to the use of pesticides increases every year. The fraction of crops in the world that would be destroyed annually by pest species if chemical pesticides were not used, is estimated to be close to 1/3 (Fournier, 1988). Also, pesticides save millions of lives by destroying insects which are vectors of disease. However, by protecting crops and contributing to improved agricultural yields, these pesticides are accountable for harmful effects on human health and the environment (Deguine and Ferron, 2006; Mawussi, 2008). The public health and environmental problems posed far outweighs the benefits that can be derived. For human health, the problem is one of individual and even collective poisoning. The risk assessment of chemical substances can be found in food, considering the results of toxicological studies carried out in animals in order to establish a daily or weekly intake acceptable or tolerable for humans. Residual levels of pesticides in plant and animal products remain little studied.

In Benin Republic, conventional cotton farming consumes the majority of chemical pesticides used in agriculture. The increase in cotton production observed in recent years also reflects the level of pesticide consumption. Thus, at the national level, official consumption of chemical pesticides has risen from 2.314.127 liters in 2006 to 4.917.136 liters in 2014: an increase of 47.06% in nine years (Société Nationale pour la Promotion Agricole, 2014). This growing increase in the volume of insecticides used has resulted in an increase in food poisoning frequency in cotton producing areas (Société Nationale pour la Promotion Agricole, 2014). An average of about 100 cases of pesticide poisoning is recorded each year, with at least 10 deaths (Badarou and Coppieters, 2009). From 2006 to 2013, three hundred and twenty-three cases of food poisoning were recorded in Banikoara (Orou, 2014). These tragedies are just the expression of incidents that occurred for years but there is unfortunately no documentation on the subject. In Benin Republic, and especially in cotton-producing townships such as Banikoara, Kandi, Savè and Glazoué where people use large quantities of inputs, health risks remain poorly controlled not only by the producers themselves but also by health workers. To contribute to cotton producer's health protection this study was initiated by analyzing acetylcholinesterase level before and after pesticide use to document pesticide poisoning beside clinical symptoms.

MATERIALS AND METHODS

This cross-sectional study was conducted from April 16 2016 to October 31 2016. During this study, questionnaires were administered to elicit data and some erythrocyte acetylcholinesterase (AChE) tests were carried out before and after 5 sets of pesticides spraying.

Ethical consideration

This study received the agreement of the Minister 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 did not understand French. Prior to any inclusion, participants provided informed consent to participate in the study. Throughout the study, the participants were allowed to voluntarily withdraw if they wished. Data were treated in a confidential manner with access only to the investigators.

Population of study

It is composed of pesticides users from the four great cotton producing areas in Benin (two each in the North: Banikoara, Kandi and Center: Glazoué and Savè) as four of great cotton producer's area. For inclusion in the study, pesticide users met the following criteria: could complete the questionnaire and undergo AChE test, resides in the study area, gave consent, owned a farm or worked at a farm where pesticides have been used for the past 5 years, did not have jaundice or evolutionary hepatitis which can influence the AChE levels (Hinson et al., 2016), and underwent biological tests both at pre- and post-exposure. Those who answered the questionnaire or underwent only pre-exposure dosage were excluded.

Sampling and sample size

The study used convenience sampling, through a systematic recruitment process of individuals fulfilling all the inclusion criteria and available to participate in the study. A total of 717 farmers participated in the first stage (questionnaire + AChE pre-exposure dosage) however, only 454 farmers met the inclusion criteria.

Data collection techniques and tools

The questionnaire contained 87 questions which were at first translated into local language. Acetylcholinesterase pre-exposure dosage was obtained by automated colorimetric technique according to the method of Ellman (1961). After 4 months of pesticide spraying, (5 sets of pesticides spraying in average) a dosage of post-exposure was carried out again to quantify the inhibition. The AChE dosage was obtained using the Test Mate® model 400 devices (EQM Research Inc., Ohio, and USA, 2003) with Model 460 AChE Assay Kit (EQM Research Inc., 2003). 10 μL were collected for each blood test using a fingerstick sample. The entire assay was completed in less than 4 min. The main component of the device is a photometric sensor (wave length 450 nm) powered by a battery of 9.0 volts. It permits the dosage of the erythrocyte cholinesterase. The referential values especially those of the AChE and 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). With the usual ascendancy of anaemia in the populations, the coefficient Q which is the AChE adjusted with regard to the rate of haemoglobin was preferred. The procedures for AChE dosage were carried out at ambient temperature (25 to 28°C) in the laboratory. The percentage enzyme inhibition was calculated as follows:

[(AChE pre-exposure – AchE post-exposure) / AChE pre-exposure] ×100 (Mohammad et al., 2007; Ahmed and Mohammad, 2005).

AChE inhibition rate of between 20-30% indicate that the person is exposed to an AChE inhibitor (WHO et al., 1986); Lotti (1995) in the absence of clinical signs. There is a toxic risk when AChE is less than 50% (EQM Research Inc, 2003; Lotti, 1995; Wilson and Henderson, 1992; Wilson, 1999;

Table 1. Socio demographic characteristics of the population

Characteristics		Number	%
Age (year)	<15	2	0.44
	15-24	41	9.03
	25-34	145	31.94
	35-44	154	33.92
	45-55	70	15.42
	>55	42	9.25
	Total	**454**	**100**
Gender	Male	438	96.48
	Female	16	3.52
	Total	**454**	**100**
Education level	None	264	58.15
	Primary school	109	24.01
	Secondary school	81	17.84
	Total	**454**	**100**
Years spraying	≤5	28	06.17
	]5-10]	77	16.96
	>10	349	76.87
	Total	**454**	**100**

Aygun et al., 2002).

Data analysis

The double capturing of data was performed and validated using the Epi Data software version 3.1 (Odense, Denmark). Statistical analysis was conducted using STATA 12.0 software (Texas, USA). 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. The Fisher and Pearson's tests were used to determine possible association between the nominal variables of the study. Finally, logistic regression was carried out to determine the explanatory factors of AChE inhibition. The level of significance for all tests used was $p \leq 0.05$.

RESULTS

Socio-demographic characteristics of the population

The studied population is essentially young: 75.33% were under 45 years old while 58.15% of the population possessed no education. The sex ratio was 27.38 in favour of men and 76.87% of the farmers possessed more than 10 years of spraying experience (Table 1).

Poisoning risks behaviors

Empty packings are 43.61% rejected, 32.82% were burnt and 15.20% re-used for domestic purpose. About 97.36% of all the surveyed persons declared that they took a bath systematically after pulverization of which 91.26% did when they get home in the evening. 54.21% indicated that they could read the pictograms on the packings. We noted that 2.61% of the farmers still used domestic containers to prepare pesticides. As precaution after spraying, 47.93% of the participants consumed milk. About 31.81% had good level of understanding of the instructions written on the packings while 60.78% did not. Only 21.13% declared to systematically use Personal Protective Equipment (PPE) at the time of pulverization. Gloves and masks were the only PPE used in 13.73% and 5.66%, respectively of the cases (Table 2).

Cholinesterase activity

There was a significant inhibition between pre-exposure (AChE 2.92 ± 1.56 UI/ml) and post-exposure (AChE 2.73 ± 0.61 IU/ml) ($p = 0.01$). This AChE inhibition was confirmed when its value was compared to that of hemoglobin. Post-exposure (AChE /Hb: Q level 23.35 ± 3.87U/ g) was significantly lower than pre-exposure (24.6 ± 4.58 U / g): $p<0.001$ (Table 4). The inhibition was observed in 57.71% of which 82 farmers showed more than 20% inhibition.
Inhibition was higher in farmers who did not use/wear PPE during spraying ($p=0.04$). The other factors did not significantly influence the variation in AChE rate. We noticed however the highest rates of inhibition in respondents who had more than ten years of pulverization practice and those who could not read the instructions on the packing (Table 5).

Table 2. Identification of risk factors in pesticide poisoning

Risk behaviors		Number	%
Management of the empty packages	Re-use in domestic purpose	69	15.20
	Relinquishment in the nature	198	43.61
	Singeing	149	32.82
	Burying in the ground	97	21.37
Preparation of pesticides in domestic packages	Yes	12	2.61
Bad precautions taken after spraying	None	165	35.95
	Drinking some milk	220	47.93
	Drinking some oil	113	24.62
	Drinking some alcohol	54	11.76
	Other precautions	74	16.12
Smoke, drink or eat during the manipulations	Yes	159	34.64
Taking of systematic shower after the spraying	Yes	442	97.36
	No	12	2.64
Place of taking shower	In the field	27	6.11
	At the house at the end of the day	415	91.26
Knowledge of the meaning of the pictograms	Yes	143	31.50
	No	311	68.50
Level of understanding of the instructions on packages	Yes, averagely	146	31.81
	Not very well	34	7.41
	No, I do not understand them	279	60.78
Systematic use of PPE during products preparation	Yes	82	17.86
Systematic use of PPE during the pesticides spraying	Yes	97	21.13
PPE used	None	372	81.05
	Gloves	63	13.73
	Mask	26	5.66
	Hat	2	0.44
	Boots	2	0.44

Effect of decreased cholinesterase activity

Logistic regression analysis showed that age, level of education and seniority had no effect on AChE inhibition ($p>0.05$). Nevertheless, the secondary-educated reduced their risk by 57% to AChE inhibition while the primary-educated increased their risk by 1.30% (Table 6).

DISCUSSION

Socio-demographic characteristics of the population

This study showed that 75.33% of the farmers were below 45 years of age thus it is an essentially young population. The same conclusion was made by Hinson et al. (2015) who finds that 88.4% of farmers are below 50 years of age in Benin. Therefore, the young in the study area were therefore highly involved in farming activities which are energy-requiring activities, as agriculture is not well mechanized yet.

A male: female sex ratio of 27.38 in this study shows that men work more in the farms than women. This situation is due to the sociocultural and rural realities of the study area which imposes that in the households, only men do the farming whereas women are devoted to household chores.

More than 58.15% of the respondents are illiterate compared to 41.85% who are schooled, among which 24.01 % completed primary school education and 17.84%, secondary school. Illiteracy is a factor that limits the farmers' understanding of instructions written on the labels of pesticides or their assimilation during trainings they receive. We can then understand the wrong uses of pesticides as is noticed in cotton producers, despite the trainings they receive. Indeed, even in developed countries there exists the problem of low educational level in farmers. In a 2014 cohort study in the United States, among 215 farmers in a bio-surveillance program of the State of

Table 3. Variation in AChE activity

Parameters		Mean	SD	p-value
AChE	Pre-exposure	2.92	1.56	
(UI/ml)	Post-exposure	2.73	0.61	0.01
Q(AChE/Hb)	Pre-exposure	24.6	4.58	<0.001
(U/g)	Post-exposure	23.35	3.87	
	Pre- exposition	11.57	1.8	
Hb (g/dl)	Post-exposition	11.7	1.44	0.13

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

AChE		Number	%
Inhibition of AChE	Yes	262	57.71
	No	192	42.29
	Total	**454**	**100**
Level of AChE inhibition (%)	<20	180	68.7
	[20-30[	45	17.18
	[30-50[	29	11.07
	≥50	8	3.05
	Total	**262**	**100**

Washington, Strelitz finds that 63.72% of the 215 participants could not read in English (Strelitz et al., 2014).

In this study, 93.83% of cotton farmers had more than 5 years of practice and 76.87% had 10 years. This study agrees with Ouédraogo's (2009) study in Burkina – Faso where more than 90% of cotton farmers had more than 5 years of practice.

Poisoning risks behaviors

In our study, we noted that 15.20% of the farmers re-use empty pesticide containers in their households. This percentage is slightly higher than that the 12% reported by Ouédraogo (2009) but lower than the value (42.10%) reported by Hinson et al. (2007) in Benin. The difference between our results and those of Hinson et al. (2007) can be explained by the delivery of more and more pesticides in small-sized containers (1/2, ¾ and 1 L) which are not very practicable for farmers compared with larger (5 in 10 L) containers. 43.61% of the farmers in our study area abandoned the empty containers to nature therefore leaving them within the reach of the general population, especially children. This practice is often a source of poisoning for both humans and the environment. In July 2000, in the North of Benin (Hinson et al., 2007), the death of an 8 years-old-child who, on the way back from the farm used an empty bottle of callisulfan (endosulfan) to drink water was reported.

54.19% of the farmers in the study area get rid of empty pesticide containers through burning and burying. However, in Brazil (Pasiani et al., 2012) reports that 16.1% of farmers bury and/or burn empty pesticide packaging; 82.1% of the farmers declared that they return the empty pesticide packaging to the governmental program on pesticides elimination. About 2.61% of this study's population continues to prepare pesticides in household containers instead of directly in the sprayers' containers which are graduated for that purpose. By so doing, they expose themselves as well as their immediate neighborhood to the risk of collective accidental poisoning because of the possibility of confusion between the recycled packaging and those which are contaminated.

Concerning precautions taken after pulverization, only 6.11% of the cotton farmers systematically take a bath on the farm as is advisable compared to 91.26% who do it at home at the end of the day. However, Ouédraogo (2007) finds that 35% of pesticides users in Burkina Faso take a bath immediately on the farm after spraying. In Benin, Hinson et al. (2015) reports that all the producers take a bath when they get home at the end of day. This therefore confirms our results. The habit of taking a bath long after spraying favours pesticides penetration with prolonged contact with the skin, since the farmers do not have appropriate PPE. In their attempts to detoxify themselves

Table 5. Influence of risk behaviors on AChE inhibition

AChE		Year spraying			
		<5	**5-10**	**>10**	**p-value**
Inhibition	Yes	13	44	205	0.44
	No	15	33	144	
% of inhibition	<20	9	31	140	
	[20-30[	2	5	38	0.3
	[30-50[	2	4	23	
	≥50	0	4	4	

AChE		Education level			
		Illiterate	**Primary**	**Secondary**	**p-value**
Inhibition	Yes	**164**	54	44	0.07
	No	100	55	37	
% of inhibition	<20	111	36	33	
	[20-30[	31	8	**6**	
	[30-50[	19	5	5	0.24*
	≥50	**3**	5	0	

AChE		Knowledge of the pictograms meanings		
		Yes	**No**	**p-value**
Inhibition	Yes	83	179	
	No	60	132	0.92
% of inhibition	<20	59	121	
	[20-30[	13	32	
	[30-50[	10	19	0.71*
	≥50	1	7	

AChE		Gender		
		Female	**Male**	**p-value**
Inhibition	Yes	9	253	
	No	7	185	1*
% of inhibition	<20	8	172	
	[20-30[	0	45	
	[30-50[	0	29	0.16*
	≥50	1	7	

AChE		Protection		
		With protection	**Without protection**	**p-value**
% of inhibition	<20	28	152	
	[20-30[	15	30	0.04
	[30-50[	8	21	
	≥50	1	7	

***=**Fisher's test.

after spraying, 47.93% of the farmers drink milk; 24.62% of them drink palm oil and 11.76% drink alcohol. Pasiani (2012) finds that 56.2% of farmers drink alcohol in Brazil. Some farmers say that they sometimes have a skin massage with palm oil. All these products are likely to favour the absorption of pesticides which are known to be very

Table 6. Multivariate analysis of AChE inhibition

Risks factors	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

lipophilic. Contrary to their intention to detoxify themselves, the farmers expose themselves more to pesticides.

In this study, about 34.64% of the farmers eat, drink and smoke during spraying. This percentage is considerably higher than that (12.8%) reported by Foulhoux (1998) in France, 15% by Jors et al. (2006) in Bolivia, 12% by Fayomi et al. (1998) in Benin and 22.36% by Hinson et al. (2007) in Dèkpo in the township of Aplahoué. It seems like this behaviour is increasing among pesticide users in Benin.

About 68.50% of the study's populations ignore the significance of danger pictograms. This percentage is higher than that reported by Magauzi (2011) in Zimbabwe who indicated that 58.5% of the population did not know the significance of any pictogram. In the same way 60.78% of the farmers in this study do not understand any of the instructions written on the packaging of pesticides. This can be explained by the level of illiteracy (58.15%) in this study which is roughly equal to the non-understanding of instructions written on the packaging.

Only 17.86% of the surveyed farmers protect themselves during preparations compared to 21.13% during treatment. The difference can be explained by the fact that for some farmers there is no need for protection during pesticide preparation. None of those who protect themselves wear all the personal protection equipment (PPE) normally recommended. Contrarily, Neupane et al. (2014) reports that only 13% of farmers did not use any PPE while spraying pesticides in the field. Most farmers use only gloves (13.73%), temporary mufflers (5.66%), hats (0.44%) and boots (0.44%) in addition to their usual farm clothes. The gloves are therefore the most widely used personal protection equipment. Neupane et al. (2014) found that about 46% of farmers used dust mask to protect against pesticide while spraying in the field and 33% farmers used long legged trousers or pants in Nepal. The percentage of respondents who used glass, gowns, boots and gloves was less than 10% (Neupane and Jørs, 2014). However, Hinson et al. (2016) found that mufflers (29.9%) and gloves (12.3%) were the most widely used personal protection equipment in Benin. World Bank (2006) reports that in Bangladesh, 87% of farmers use incomplete protection measures during the handling of pesticides which with this study. The same reports were made in Burkina - Faso by Toe et al. (2013) who asserts that very few farmers (0.93%) possess complete protection equipment. In Zimbabwe, even though Magauzi (2011) confirms that the PPE used were never complete, 71% of the surveyed farmers, nevertheless, said they received at least a PPE (overalls, boots, gloves, masks, hats or glasses) from local technical support structures. This insufficient PPE supply was also reported in studies conducted in the Philippines (Lu, 2005; Prado and Lu, 2007).

Acetylcholinesterase levels

During pre-exposure, the average percentage of acetylcholinesterase is 2.92 U/ml vs. 2.72 U/ml post - exposure at a significant difference (p=0.01). This considerable reduction in the percentage of acetylcholinesterase is confirmed when the Q quotient which is the percentage of acetylcholinesterase adjusted with the percentage of hemoglobin is taken into account. Indeed, Q is 24.6 U/g in pre-exposure compared to 23.35 U/g in post-exposure at p <0.001 significance level. Exposure to pesticides, notably organo-phosphate pesticides, influences the percentage of acetylcholinesterase, as a better means to follow-up occupational exposure to organo-phosphate pesticides according to the WHO (1986).

This study's results are lower than those reported by Mamadou in Niger (Mamadou, 2008) which is 3.63 U/ml in pre - exposure against 3.21 U/ml in post - exposure, but they are however close to those by Hinson et al. (2016) in Benin which is 2.91 U/ml in pre-exposure as against 2.93 U/ml in post-exposure. It must be noticed in this case that the reduction in acetylcholinesterase percentage is neither in favour of post-exposure nor significant enough.

After post-exposure, a percentage inhibition of 57.71% in acetylcholinesterase was observed in this study while cases of more than 30% AChE inhibition were reported in 14.12%. This percentage is lower than 16.50% reported by Mamadou et al. (2013) in Niger, 24.1% reported by Magauzi et al. (2011) in Zimbabwe, 26% by Ahmed and Mohammed (2005) in Iraq and 26.82% by Hinson et al. (2016) in Benin. The difference between these studies and our results can be explained by the study population size.

Among all the factors of risk studied, only the lack of PPE usage considerably influenced acetylcholinesterase percentage at p = 0.04. Our results are confirmed by those by Magauzi et al. (2011) who finds that not being provided with personal protection equipment was associated with an abnormal cholinesterase activity, with the risk increasing from 1.07 to 3.68. Similarly, Khan et al. (2010) reports that the absence of personal protection equipment influenced a decrease in cholinesterase activity even though the authors worked on seric acetylcholinesterase.

From the results of our study, it is important to stress that even in the absence of a significant difference, the highest percentage inhibition of acetylcholinesterase were observed in persons with more than 10 years of work experience or illiterate.

Conclusion

From this study, it is evident that risk factors of occupational pesticide poisoning were common among farmers: after spraying, 47.93% of the farmers drink milk still believing in twists that it is a way of decontamination. Only 21.13% systematically use Personal Protective Equipment (PPE) during spraying. acetylcholinerase inhibition occurred in 57.71% farmers of which 82 farmers showed more than 20% inhibition. There was more inhibition in farmers who did not wear PPE during spraying than otherwise (p=0.04). There is still an urgent need for educating farmers on improved pesticide handling techniques to protect their health. Routine monitoring of AChE may allow for early recognition of frequent and continuous low-level exposure to OPs.

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Conflicts of Interest

The authors have declared none.

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