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ABSTRACT

OBJECTIVE: To assess the degree of poisoning in farmers using the erythrocyte acetylcholinesterase (AChE) test before and after the exposure to pesticides in townships in central Benin (Glazoué and Savè) and to identify the associated risk factors.

METHODS: Using a cross-sectional study design, we recruited 264 farm pesticide sprayers, who have been working for at least 5 years. They completed a questionnaire and underwent the AChE test using the Test-mate Model 400 device (EQM Research Inc.) with a photometric sensor, based on the works of Ellman.

RESULTS: Organophosphate/pyrethroids were the most common pesticides used by at least 72.96% of the farmworkers. We observed an inhibition of AChE between pre-exposure and post-exposure ($P = .002$) for 60.61% of the farmworkers. Among them, 11.88% displayed more than 20% AChE inhibition.

CONCLUSIONS: Pesticide poisoning is a reality, and AChE monitoring is urgently needed for farmworker surveillance.

KEYWORDS: Acetylcholinesterase, cholinesterase inhibitor, poisoning, Glazoué, Savè, Benin

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Background

The concern for preserving plants and other agricultural products against the harmful actions of some devastating organisms has caused farmers to consider substances capable of destroying or preventing those organisms' actions.[1] The use of those substances, called pesticides, has increased considerably worldwide because of their spectacular efficiency. The necessity to increase agricultural output to meet the demands of a constantly increasing population corresponds well to the use of pesticides in many African countries. Unfortunately, the extensive use of pesticides can also lead to serious health and environmental consequences.

According to the World Health Organization (WHO), 2 to 5 million cases of poisonings per year by pesticides worldwide are recorded,[1] among which 300 000 cases have resulted in death.[2] Developing countries, such as Benin, which use no more than 25% of the pesticides produced in the world, suffer from this situation and have recorded 99% of the deaths related to this type of poisoning.[3] In Chad, in the region of Forcha (West of Ndjamena), 10 persons from the same family were poisoned after having eaten salad contaminated by pesticides, and 4 deaths followed.[4] In Morocco, the anti-poison centre recorded more than 2609 cases of poisoning by pesticides between January 1992 and December 2007.[5] In Benin, nearly 37 persons died after endosulfan poisoning in the department of Borgou during the 1999/2000 season.[6] In addition, 9 of 105 deaths due to endosulfan were recorded between May 2007 and July 2008 in the same department.[7] Although the symptoms of severe poisoning by pesticides are nearly immediately detectable, those of chronic poisonings are rather pernicious. Attacks on the nervous and reproductive systems (hypo-fertility, barrenness, and malformations) and genetic heritage and cancer-genesis are often reported.[8] However, the relevant proportions of morbidity or mortality due to pesticides in terms of chronic poisonings has not been determined yet,[9] although they abound in our region. The most commonly used pesticides in our region are organophosphates (OPs). The symptoms related to the toxicity of OPs are due to the inhibition of the acetylcholinesterase (AChE) enzyme that can be easily measured for surveillance. The inhibition of the erythrocyte AChE leads to the accumulation of acetylcholine in synapses, leading to various clinical symptoms.[10]

Single or repeated exposure to AChE inhibitors leads to the accumulation of acetylcholine in the synaptic cleft, possibly causing excessive stimulation of muscarinic and nicotinic receptors throughout the body and producing toxic effects, such as nausea, bronchoconstriction, sialorrhoea, hypertension, and tremor, as well as affecting the central nervous system.[11,12]

The infra-clinical exposure effects of chronic OP, such as the inhibition of AChE, can be detected early through biological tests. Intra-individual modifications of active AChE exist and range from 2% to 35%, necessitating a referential dosage during the period of non-exposure for every individual. When a person presents a decrease larger than 20% to 35% in relation to his or her reference value, he or she must be removed from his or her work until the rate returns to normal. The work absence must span 2 to 3 months; the necessary period for enzyme regeneration and re-exposure must not be allowed before a return to 80% of the base value.[13] An inhibition rate that reaches 50% imposes momentary withdrawal from spraying activities. Within the framework of the surveillance of chronic poisoning by OPs, to confirm chronic poisoning, it is advised, as far as possible, to determine the dosage of AChE before and after exposure to quantify the level of inhibition for treatment and/or prevention decisions. Very few scientific studies, especially in Africa, have been conducted with the perspective of pre- and post-exposure dosages of AChE. Chakraborty et al[14] in India demonstrated an AChE inhibition of approximately 34.2% among Indian farmers. Pathak et al,[15] also in India, obtained an inhibition of 55% between pre-exposure and post-exposure. In Nigeria, Sosan et al[16] observed that 8 of 76 farmers showed 30% to 50% AChE inhibition between pre-exposure and post-exposure. This study was aimed to comply with the recommendations of the WHO, where the individual is expected to self-monitor his or her dosage of AChE. It represents a rare case study in Africa in general and in Benin concerning the dosage of AChE and especially pre-exposure and post-exposure of farmers.

The main objective of this study is to assess the degree of poisoning by testing AChE activity before and after the exposure of farmworkers to pesticides in the townships of central Benin (Glazoué and Savè) and to identify the risks factors associated with exposure.

Methods and Materials

This cross-sectional study was conducted from April 16, 2016 to October 31, 2016. During this study, we conducted an investigation through a questionnaire and performed AChE tests before and after an average of 5 instances of pesticide spraying.

Study population

It comprised pesticide users from 2 townships in central Benin, representing 2 of the largest cotton production areas (Glazoué and Savè). The pesticide users meeting the following criteria could complete the questionnaire and undergo the AChE test: resided in the study area (Glazoué and Savè), 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 dosage of AChE,[17] and underwent biological tests both at pre-exposure and at post-exposure. Those who only answered the questionnaire or only did the pre-exposure dosage were excluded.

Sampling and size

The study used convenience sampling (non-probability sampling) through a systematic recruitment process of all persons fulfilling all the inclusion criteria and available to participate in the study. A total of 392 farmers participated in the first stage (questionnaire + AChE pre-exposure dosage). However, finally, only 264 farmers participated in all the stages (first stage + AChE post-exposure dosage) of the study.

Data collection techniques and tools

The data gathered during the questionnaire included 87 questions, first translated into the local language, and the AChE dosages of the pesticide users. The questionnaire items included general information, type of pesticides used, knowledge-attitude-practice on the use of pesticides, and neuropsychic signs, and the questionnaire ended with obtaining the AChE pre-exposure dosage using an automated colorimetric technique based on the method of Ellman et al.[18] After 4 months of pesticide spraying (5 instances of pesticide spraying on average), the post-exposure dosage was determined anew to quantify the inhibition. The AChE dosage was determined using the Test-mate Model 400 device (EQM Research Inc., Cincinnati, OH, USA) with the Model 460 AChE Assay Kit.[19] We collected only 10 μL for each blood test using a fingerstick sample. The entire assay was completed in less than 4 minutes, including the result. The main component of the device is a photometric sensor (wavelength: 450 nm) powered by a 9.0-volt battery; the principle is based on the work of Ellman et al.[18] It measures the dosage of the erythrocyte cholinesterase. The referential values, especially those of AChE and haemoglobin (Hb), were recorded as follows: 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). Considering the usual increase in anaemia in our populations, we preferred the coefficient Q, which is the AChE

Table 1. Characteristics of the population.

CHARACTERISTICS		NUMBER	%
Age category, y	<15	2	0.76
	15–24	20	7.57
	25–34	68	25.76
	35–44	94	35.61
	45–55	41	15.53
	>55	39	14.77
	Total	264	100
Sex	Male	249	94.32
	Female	15	5.68
	Total	264	100
Education level	None	126	47.73
	Primary school	91	34.47
	Secondary school	47	17.80
	Total	264	100
Experience with spraying, y	<5	15	5.68
	5–10	34	12.88
	>10	215	81.44
	Total	264	100

adjusted regarding the level of Hb. The procedures regarding the dosage of AChE were performed under the ambient temperature of the laboratory (25°C-28°C). The percent enzyme inhibition was calculated as follows:

$$\left[\frac{(\text{AChE pre-exposure} - \text{AChE post-exposure})}{\text{AChE pre-exposure}}\right] \times 100 [20,21]$$

A rate of inhibition of AChE between 20% and 30% indicated that the person was exposed to a cholinesterase inhibitor[20,21] in the absence of clinical signs. For AChE, there is a toxic risk when there is reduction by less than 50% of the normal.[19,21-24]

Data analysis

The double capturing of data was performed and validated using the EpiData software version 3.1. Statistical analysis was conducted using version 12.0 of the Stata software. After a general description of the sample, the results of the quantitative variables are presented using the parameters of positioning and scattering; those of the qualitative variables are presented as percentages. The Fisher test and Pearson test of χ^2 were used to determine the possible association between the nominal variables of the study. At the end, logistic regression was performed to identify explanatory factors of AChE inhibition. For all tests used, the threshold of significance was 5%.

Results

Socio-demographic characteristics of the population

The population was essentially young: 84.46% of the questioned persons were aged less than 45 years, including 2 children. The sex ratio (male/female) was equal to 16.6. In addition, 47.73% of the population were illiterate, and 81.44% of the population had more than 10 years of exposure (Table 1).

Inventory of pesticides used for the 2015 to 2016 cotton season

Organophosphate/pyrethroids were the most common pesticides used by 72.96% of the farmworkers, and 1.78% of the farmworkers were found to have used endosulfan, although its use is forbidden in Benin (Table 2).

Poisoning risk factors

The main risks factors we identified were as follows: 44.7% of farmworkers discarded the empty pesticide packaging in nature, and 22.73% of farmworkers used the packaging for

Table 2. Nature of the most used pesticides in the study townships (for the nature of pesticides, we considered the number interviewed for the first stage: 392 farmers).

PESTICIDES	WHO CLASS	CHEMICAL GROUP	NUMBER	%
Kd Plus (chlorpyrifos/cypermethrin)	II	Organophosphorus/pyrethroid	286	72.96
Caiman B (emamectin)	II	Avermectins	273	69.64
Acer (acephate)	III	Organophosphorus	232	59.18
Kalach	III	Organophosphorus	193	49.23
Moacatarine (acetamiprid/λ cyhalothrin)	II	Pyrethroid/neonicotinoid	184	46.94
Calfos (profenofos)	II	Organophosphorus	177	45.15
Ema star (emamectin)	II	Avermectin	163	41.58
Califor G	III	Permethrin/fluometuron/glyphosate	152	38.77
Napeco Metafos (acetamiprid/emamectin)	II	Neonicotinoid/avermectine	144	36.73
Lambdacal (profenofos/λ cyhalothrin)	II	Organophosphorus/pyrethroid	136	34.69
Glyphogan, Glycel Glyphostar (glyphosate)	III	Organophosphorus/pyrethroid	128	32.65
Cottonet	II	Metolachlor/terbutryn	117	29.85
Cotton plus	II	Herbicide	115	29.34
Sharp	III	Glyphosate	33	8.42
Endrine	Obsolete	Organochloride	12	3.06
Endosulfan	Ia	Organochloride	07	1.78
Thian	III	Spirotetramat/flubendiamide	03	0.76

Abbreviation: WHO, World Health Organization.

domestic purposes. As a precaution, 75.47% farmworkers drank some milk after spraying, and 41.89% drank oil. Concerning the pictograms, 84.85% did not know their meanings, 92.83% of the farmworkers did not wear the personal protective equipment (PPE), and only 4.25% took a shower on the farm before returning home (Table 3).

Level of cholinesterase activity

We did not note any significant difference between AChE at pre-exposure (2.8 ± 0.62 UI/mL) and post-exposure (2.8 ± 0.67 UI/mL) (P = .87). However, when we considered Q (AChE/Hb), we noted a significant inhibition (P = .002) of AChE adjusted at the post-exposure (Table 4).

In total, 60.61% of the farmworkers had a decrease in their AChE rate. Among them, 11.88% had a more than 20% AChE inhibition (Table 5).

Risk factors of pesticide poisoning

Women are more likely to show inhibition of AChE than men (P = .03 and P = .04 for regression, respectively). Except for this risk factor, no other factor influenced significantly the increase in AChE inhibition. However, we noted that 61.86% of those with more than 10 years of exposure showed a higher reduction in AChE with a strong rate of reduction level. In addition, 62.05% of those who did not know the meaning of the pictograms were more likely to demonstrate reduction in AChE. Moreover, 59.52% of the illiterate individuals were more likely to show a reduction in AChE. Regarding the wearing of PPE, those who did not wear it showed more inhibition of AChE (Tables 6 and 7).

Discussion

One of the strengths of this study was determining the dosage of the erythrocyte cholinesterase activity before and after the exposure of the farmers to organophosphate pesticides, considering each individual's level. Most of the studies that have been conducted thus far in our country, as an under-developed country, used instant dosages of AChE and a limited number of samples. We have impacted through exhaustive sampling all the populations of the 2 large regions of pesticide users based on our inclusion criteria.

Our study population comprised 94.32% men and 5.68% women. This situation reveals our sociocultural and rural reality, where only men in rural families have to engage in farmwork, whereas women are responsible for housework. The same situation was noticed in Brazil by Pasiani et al[25] in 2012,

Table 3. Identification of poisoning risk factors.

RISKS FACTORS	VARIABLES	NUMBER	%
Management of the empty packaging (n = 264)	Reuse for domestic purposes	60	22.73
	Relinquishment in nature	118	44.70
	Burning	69	26.14
	Burying in the ground	73	27.65
Preparation of pesticides in domestic packaging (n = 264)	Yes	8	3.03
Precautions taken after spraying (n = 264)	None	30	11.36
	Drinking some milk	200	75.75
	Drinking some oil	111	42.04
	Drinking some alcohol	51	19.32
	Other precautions	36	13.63
Smoke, drink, or eat during the manipulation (n = 264)	Yes	159	(60.22)
Taking of a systematic shower after the spraying (n = 264)	Yes	259	98.11
	No	5	1.89
Location of shower (n = 259)	On the farm	11	4.25
	Once at home at the end of the day	248	95.75
Knowledge of the pictogram meanings (n = 264)	Yes	40	15.15
	No	224	84.85
Level of understanding of the instructions on the packaging (n = 264)	Yes, on average	59	22.35
	Not very well	10	3.79
	No, I do not understand them	196	74.24
Systematic use of PPE during the preparation of products (n = 264)	Yes	18	6.82
Systematic use of PPE during the pesticide spraying (n = 264)	Yes	30	11.36
PPE used (n = 264)	None	246	93.18
	Gloves	11	4.17
	Mask	9	3.41
	Hat	2	0.76
	Boots	2	0.76

Abbreviation: PPE, personal protective equipment.

where they found 99.1% men doing farmwork. Considering Toe et al[26] in Burkina Faso found 84.7% of the sampled population aged under 50 years, our population is relatively young: 84.46% of the farmers were under 45 years. Therefore, the younger active population was devoted to cotton production; in particular, 7.57% of children aged less than 15 years and still fragile in their development were involved. The early exposure of farmers to pesticides possesses problems. We demonstrated that 94.32% of the farmers had more than 5 years of exposure. This rate of farmers more than 5 years of exposure agrees with that reported by Ouédraogo et al[27] in Burkina Faso, who found that more than 90% of the farmers had more 5 years of exposure.

Factors of pesticide poisoning risk

Less than half (47.73%) of the population were illiterate, and only approximately 30% among the schooled population went beyond the elementary study level. These rates were close to those in the results of Fayomi et al[28] in 1998, who found that among the educated persons, only 1 in 3 went beyond primary school. Nevertheless, our rate was low compared with that

Table 4. Variation in AChE activity.

		MEAN	SD	*P* VALUE
AChE, UI/mL	Pre-exposure	2.8	0.62	.87
	Post-exposure	2.8	0.67	
Q (AChE/Hb), U/g	Pre-exposure	24.39	4.33	.002
	Post-exposure	23.67	3.98	
Hb, g/L	Pre-exposure	11.41	1.76	.006
	Post-exposure	11.81	1.53	

Abbreviation: AChE, erythrocyte acetylcholinesterase.

Table 5. Inhibition of AChE.

	ACHE	NUMBER	%
Inhibition of AChE	Yes	160	60.61
	No	104	39.39
	Total	264	100
Level of AChE inhibition, %	<20	141	88.13
	20–29.9	12	7.50
	30–49.9	5	3.12
	⩾50	2	1.25
	Total	160	100

Abbreviation: AChE, erythrocyte acetylcholinesterase.

obtained by Hinson et al in 2015, with 80.3% illiterate.[17] Illiteracy or a very low level of education is a factor that limits the understanding of the instructions displayed on the labels of the pesticides. This could explain the improper uses of pesticides found among the farmers despite the training they received. In addition, even in developed countries, the problem of farmers having a low level of education arises. In a study conducted in the United States in 2014 on 215 farmers in the biosurveillance programme of the state of Washington, Strelitz found that 63.72% of the 215 participants could not read English.[29] This rate of illiteracy is the cause of the ignorance of the meaning of the warning pictograms of the pesticides in 84.85% of the users in our study. This rate is largely beyond that of Magauzi et al[30] in 2011 in Zimbabwe, who found that 58.5% of the farmers did not know the meaning of any pictogram.

In our study, 22.73% of the farmers reused the empty containers for household needs. This rate was higher than that by Ouédraogo in Burkina Faso[22] at 12% but lower than that found by Hinson et al[31] in Benin in 2007 of 42.10%. The difference between our results and those of Hinson et al could be explained by the increasing delivery of pesticides in smaller containers (1/2, 3/4, and 1 L), which are less practical for the farmers than the previously larger containers (5-10 L), which are very often more practical for different household needs.

Concerning empty container management, 44.70% of the farmers abandoned them in nature accessible to anyone, particularly children. This rate is a little below the 48% observed by Ouédraogo in Burkina Faso.[27] Our rate is much lower than the 72% found by Jors et al in Bolivia.[32] This practice often also causes poisoning of the environment and humans. This was, for instance, the case in Northern Benin in July 2000,[31] with the death of an 8-year-old child who on the way back from the farm, drank water in an empty small bottle of callisulfan (endosulfan).

Regarding the empty containers, 53.79% of the farmers discarded them by burning or burying. In Brazil,[25] 16.1% of the farmers buried and/or burned empty packaging; the most of the farmers (82.1%) declared that they returned the empty packaging of pesticides to the governmental programme for the elimination of pesticides.

Another practice as dangerous as the previous one is the use of household containers for the preparation of pesticides. Approximately, 3.02% of our study population prepared the pesticides in household containers instead of performing this activity directly in the containers of atomisers devoted specifically for that use. Thus, they exposed themselves as well as the rest of their household to the risk of collective accidental poisoning because of the possibility of confusion between the recycled packaging and contaminated ones.

Regarding the precautions taken after spraying, of 259 persons, only 4.25% systematically took a shower on the farm as advised, compared with 95.75% who showered at home at the end of the day. Hinson et al[31] found that all of the farmers took a bath when they returned home at the end of the day. The practice of taking a bath long after spraying facilitates the intra-skin penetration of the pesticides via prolonged contact with the skin because the farmers do not have any convenient PPE on them; it is also a risk factor of poisoning for the family at home. To detoxify themselves after spraying, 75.47%, corresponding to 3 farmers out of 4, drank milk, 41.89% drank palm oil, and 19.25% drank alcohol. Some farmers specify that they sometimes undergo skin massage with palm oil. Pasiani et al[25] in Brazil found that 56.2% of the farmers drank alcohol. All these products are more likely to facilitate the absorption of the

Table 6. Identification of the risk factors of poisoning in the study population.

AChE		EXPERIENCE WITH SPRAYING			*P* VALUE
		<5	5-10	>10	
Inhibition	Yes	4	14	107	.17
	No	11	20	108	
% of inhibition	<20	3	10	83	.02
	20–29.9	1	0	10	
	30–49.9	0	0	11	
	⩾50	0	4	3	
AChE		EDUCATION LEVEL			*P* VALUE
		ILLITERATE	PRIMARY	SECONDARY	
Inhibition	Yes	75	54	31	
	No	51	37	16	.71
% of inhibition	<20	67	44	30	
	20–29.9	5	6	1	
	30–49.9	2	3	0	.63[a]
	⩾50	1	1	0	
AChE		KNOWLEDGE OF THE PICTOGRAM MEANINGS			*P* VALUE
		YES	NO		
Inhibition	Yes	21	139		
	No	19	85		.26
% of inhibition	<20	19	122		
	20–29.9	2	10		.88[a]
	30–49.9	0	5		
	⩾50	0	2		
AChE		SEX			*P* VALUE
		FEMALE	MALE		
Inhibition	Yes	13	147		
	No	2	102		.03
% of inhibition	< 20	12	129		
	20–29.9	0	12		.21[a]
	30–49.9	0	5		
	⩾50	1	1		
AChE		PROTECTION			*P* VALUE
		WITH PROTECTION	WITHOUT PROTECTION		
Inhibition	Yes	21	139		
	No	9	95		.26
% of inhibition	<20	18	123		
	20–29.9	2	10		.87
	30–49.9	1	4		
	⩾50	0	2		

Abbreviation: AChE, erythrocyte acetylcholinesterase.
[a]The Fisher test.

Table 7. Multivariate analysis by logistic regression to identify explanatory factors of AChE inhibition.

ACHE	OR [95% CI]	*P* VALUE
Age, y		
<15		
⩾15	1.44 [0.47–4.39]	.52
Sex		
Female		
Male	0.19 [0.04–0.92]	.04
Education level		
None		
Primary school	1.06 [0.61–1.87]	.83
Secondary school	1.64 [0.78–3.42]	.19
Years spraying		
<5		
5-10	1.88 [0.50–7.03]	.35
>10	2.37 [0.69–8.20]	.17

Abbreviations: AChE, erythrocyte acetylcholinesterase; CI, confidence interval.

pesticides because they are all very lipophilic. Worse, during spraying, 60% of pesticides users in our study consumed foods. This rate is largely beyond the 12.8% found by Foulhoux and Nguyen[33] in France in 1998 and also beyond the 15% found by Jors et al in Bolivia in 2002.[32] In Benin, in 1998, this percentage was 12% in Banikoara[28]; in 2005, at Dèkpo, in the township of Aplahoué, it was 22.36%.[31] It seems that now more and more pesticides users adopt this practice in Benin. In addition, 92.83% of the farmers did not use any individual protection equipment during spraying. This rate was largely beyond that found by Pasiani et al[25] in Brazil, who found that only 7.2% of the farmers did not protect themselves. The large difference with our results could be explained by Brazil's level of development and by the country being one of the largest pesticide users in the world.[34] In addition, only 6.79% of the questioned farmers protected themselves during the preparation compared with 11.32% during the treatment. The difference could be explained by some farmers believing that there was no need for protection during the preparation of pesticides.

No farmers in this study wore any individual protection devices as advised. Most of those who protected themselves used only boots, gloves, hats, and a muffler made of a handkerchief in addition to their usual farm clothes. The same was noticed in Burkina Faso where Toe et al[26] affirmed that very few farmers (0.93%) have complete protection. The World Bank in Bangladesh found that 87% of the farmers in that country used insufficient protective measures while handling pesticides.[35]

In this study, the most commonly used individual protection equipment were gloves (4.15%) and masks (3.40%). However, Toe et al[26] in Burkina Faso found that the most commonly used individual protection equipment were masks (40%) and boots (28.8%).

In our study, the pesticides used by more than 72.96% of the farmers were OPs and pyrethroids. Jors et al[32] in Bolivia found that OPs constituted the group of pesticides more frequently used by 88% of the farmers. In addition, it is necessary to note that among the mentioned pesticides, the presence of endrine and endosulfan was found, which are prohibited organochloride pesticides. Their presence reveals the existence of sources for the informal provision of pesticides, opening the door to increased risks of poisoning. The predominance of the OPs in this study showed the importance of monitoring the activity of cholinesterase, as the main biomarker of poisoning with OPs.

Variation in the activity of cholinesterase within the study population

The measurement of blood AChE activity in individuals is a non-invasive biomarker method to monitor poisoning or exposure to organophosphate and carbamate insecticides.[21,23,24,36,37] This measurement can be performed anywhere, even on farms.

At the end of study, we concluded that the rate of AChE at pre-exposure (2.8 ± 0.62 UI/mL) was practically the same as that at post-exposure (2.8 ± 0.67 UI/mL): $P = .87$. However, considering the preponderance of anaemia in the populations in general in under-developed countries (68.3% of anaemia in the population),[38] it is important to adjust this rate of AChE in relation to Hb through *Q* values, which are provided by the EQM device. Considering the *Q* values (AChE/Hb), in 60.61% of the population, we observed a significant decrease ($P = .002$) between the post-exposure (23.67 ± 3.98 U/g) and pre-exposure of AChE (24.39 ± 4.33 U/g). It was the same average obtained by Loko et al 24.05 U/g, in a study conducted among farmers in cotton production areas.[39] In our study, 60.61% of the population had an inhibition of more than 20% of AChE. Our rate was largely higher than those obtained by Mamadou et al[40] in Niger (16.50%), Magauzi et al[30] in Zimbabwe (24.1%), Ahmed and Mohammad[41] in Iraq (26%), and Hinson et al[17] in Benin (26.82%).

Women were more likely to show inhibition of AChE than men ($P < .05$). Does this mean that women are more sensitive to OPs? We are tempted to speculate that the women in our study seemed more sensitive to OPs and carbamates than men. However, we must consider the relatively low number of women in our study (15 vs 249). Moreover, this finding seems to have been confirmed after logistical regression: odds ratio = 0.19; 95% confidence interval = [0.04-0.92]. This report was not the same as that by El-Kettani et al[42] in Morocco, who found no significant difference between the sexes ($P = .14$), although they had used a gender-balanced sample (168 women and 157 men). Except for this factor of sex risk in our study, no other factor could significantly influence the occurrence of a decrease in AChE. However, we noted that 61.86% of the individuals who had more than 10 years of exposure showed a high level of decrease in AChE. In addition, 62.05% of the persons who could not read the pictograms showed nearly all high levels of AChE decrease. Moreover, 59.52% of the illiterate individuals showed an AChE decrease, but there were high levels of decrease in individuals with an elementary level of education. Regarding the use of PPE, those who did not wear any PPE displayed more inhibition of AChE.

During pre-exposure, we obtained an AChE average of 2.8 ± 0.62 UI/mL and an AChE/Hb average of 24.39 ± 4.33 U/g. These values were lower than the 2.91 U/mL obtained at pre-exposure by Hinson et al[17] in the northern part of the country and lower than the 3.63 U/mL obtained by Mamadou in Niger but higher than those found by Mohammad et al[43] in Mosul (Iraq), who found average levels of erythrocyte cholinesterase of 1.39 and 1.22 for men and women, respectively. Mamadou et al[40] asserted that the rate of AChE is not influenced by age, and our study does not demonstrate the opposite. However, Loko et al[39] in Benin asserted the opposite while proving that the rate of AChE varies according to age because they obtained significant differences in the averages of AChE between children and adults.

Paradoxically, the use of PPE by the farmers here did influence the rate of AChE (P = .27); nevertheless, we noticed that 86.87% of those who had AChE inhibition did not wear PPE. In fact, only 11.32% of the population used PPE, and the protection that PPE would provide was surely not efficient. Magauzi et al[30] in Zimbabwe found that not being provided with any PPE was significantly associated with abnormal cholinesterase activity, with the risk increasing from 1.07 to 3.68. This result was confirmed in 2009 by Khan et al[44] in Pakistan, who found that the absence of the use of PPE influences the decrease in the cholinesterase activity, although they monitored the serum AChE only.

Conclusions

Thus, this study revealed many risk factors of pesticide poisoning. In addition, this study allowed us to collect objective biological data on pesticide poisoning risks and analyse these data according to WHO recommendations. Thus, we demonstrated an inhibition of AChE between pre-exposure and post-exposure in 60.61% of the farmworkers, a very high proportion. It is important that farmers and agricultural workers who are routinely using OPs establish their baseline AChE activity and have access to regular AChE activity checks to compare with baseline. Routine monitoring of AChE may allow for the early recognition of frequent and continuous low-level exposure to OPs.

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Authors' Contributions

HAV and DF initiated of the research, conceived and designed the research protocol. HAV, DF, YPE, HH, and KA performed and implemented the protocol at the workplace and analysed the data. HP analysed the statistical data. HAV, DF, HH, and KA wrote the first draft of the manuscript. HAV and HH contributed to the writing of the manuscript and jointly developed the structure and arguments for the paper, with the supervision of HAV. LH, and FB made critical revisions and approved the final version. AB, LW, GF, YPE, HAV, and FB agreed with the manuscript results and conclusions. FB chaired the research. HAV, DF, YPE, HH, LH, HP, KA, AB, GF, and FB reviewed and approved the final manuscript.

Ethical Approval

This study received the agreement of the Minister of Health (reference 3040/MS/DC/SGM/DNSP/SA on April 20, 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.

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