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An Evaluation of Occupational Exposures to Pesticides in Brazil

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Abstract

All over the world, pesticides are frequently used in large quantities. Even though these substances are potentially toxic and may affect the human health, depending on the degree of exposure, contamination, and toxicity, they are nevertheless constantly released into the environment. Currently, predominantly organophosphorus (OP) and carbamate (CBM) pesticides, which are considered neurotoxic due to their potential to inhibit cholinesterase (ChE) activity, are used frequently in developing countries. Accordingly, many intoxication symptoms regarding these chemicals are diagnosed via the ChE analysis and its degree of inhibition. This is therefore the method of choice for biomonitoring exposed individuals, and has been described in the evaluation of thirteen Brazilian studies, which we retrieved from different online databases and present in this review. However, conflicting results on the effects on ChE as a result of repeated and prolonged exposure to low doses still prevail, mainly because farmers are usually exposed to mixtures of pesticides simultaneously. Reviews based exclusively on the laboratory analyses of ChE activity render the clinical interpretation difficult, as it is not a very sensitive method and does not efficiently address the reality of chronic intoxications in the absence of clinical signs. Many diseases among farmers are related to genetic damage, which includes carcinogenic and neurotoxic processes, reproductive and developmental defects, hormonal changes, as well as immune and neurodegenerative diseases. In this article, we therefore discuss the use of different biomarkers for the occupational evaluation, as clinical interpretations still require improvement and widely available laboratory tests still remain unattainable, especially with respect to individual conditions arising from mild to moderate exposures, which might have no immediate, but can have significant detrimental long-term health effects on the health of exposed individuals.

Keywords: Pesticides; Cholinesterase; Biomonitoring; Farmers.

Introduction

Pesticides are used frequently all over the world, and many sectors, such as agriculture, livestock, and public health are directly concerned. The main purposes of pesticide uses are directed towards the control of disease vectors between humans and animals, in addition to controlling insects, weeds, and plant diseases which affect crops and food production [1,2]. In the process, large quantities of potentially toxic pesticides are released into the environment every year, and can thus affect the human health, depending on the degree of exposure and contamination, as well as on the individual toxicity of the chemical compounds [3-6].

Economic globalization and the need to increase food production have created a distinct dependence on the use of pesticides, especially in the agricultural sector of developing countries [4,7]. In the context of a globalized economy, Brazil is considered a major producer and exporter of agricultural commodities, but inadequacies are observed with respect to the practical use of pesticides, especially when being applied without appropriate technical guidance [8].

Carbamate (CBM) and Organophosphate (OP) Pesticides

Even though organophosphorus (OP) and carbamate (CBM) pesticides are considered neurotoxic to humans, they are still frequently used in various parts of the world [4]. According to He et al. [1], these chemicals are predominantly used in developing countries. The OP pesticide class consists of the esters, amides, and thiol derivatives of phosphoric, phosphonic, or phosphinic acid [9]. These compounds are mainly used as insecticides, since they act on the nervous system of insects, but they can also be part of some herbicides and fungicides [10]. CBM are structurally less complex, derived from the esters of carbamic acid (NH_3CO_2), and generally used as fungicides, herbicides, or insecticides [4].

OP and CBM are known to act as neurotoxic agents, as they can inhibit the activity of cholinesterases (ChE) such as acetylcholinesterase (AChE) and butyrylcholinesterase (BChE). The interference with the activity of the neurotransmitter acetylcholine (ACh) may cause both short- and long-term effects [9,11-13]. ChE inhibitors were first synthesized in the 1930s, and until the end of World War II, approximately 2000 compounds were isolated, especially by the German government, and used as chemical warfare agents. Currently, most commercially available OP and CBM should not interfere directly with ChE anymore, as they are tested in several models prior to production [4].

Regarding a classification of OP, the common denominator is the presence of a phosphorus atom, either in a phosphoryl (P=O) or thiophosphoryl (P=S) group [4,14]. These chemical compounds act via different mechanisms: while some may interfere directly (without metabolic or biotransformation) with ChE, others act through indirect inhibition, i.e. they need metabolic transformations in order to exert

inhibitory activity towards these enzymes [4,11,14]. In the latter case, especially OP with a P=S moiety are exposed to various biotransformations after absorption, which may significantly change their toxicity characteristics, resulting in the formation of metabolites which are sometimes more toxic than the original substance [12]. Especially so, when the metabolic activation proceeds via enzymes of the cytochrome P450 complex, which results in the formation of oxygen analogues of pesticides (oxons), which in turn inhibit AChE and BchE [4,14]. Furthermore, reversible and irreversible complexes can be formed. OP usually form more stable complexes – sometimes even irreversible - with AChE and BChE, whereas CBM tend to form less stable and reversible complexes [11,15,16]. Evidently, the individual effects of OP or CBM poisoning are determined by the metabolism, and accordingly related to the individual chemical structure [12].

Cholinesterases: AChE and BChE

Based on their substrate and inhibitor specificity, ChE are classified as AChE or BChE. As OP and CBM inhibit ChE reversibly or irreversibly, the assessment of exposure is usually based on the enzyme activity in the blood. This can be accomplished via two ways: (a) by the examination of erythrocytic AChE (erythrocytic separation of the whole blood) and serum BChE activity in the plasma, or (b) by specific inhibitors of BChE, which can be used to enable the analysis of AChE in the whole blood, even though this alternative may cause false positive results [17].

AChE and BChE are responsible for the hydrolysis of acetylcholine (ACh), which is ubiquitous in the nervous system of vertebrates [4,15,18]. AChE is a very important neurotransmitter, and after every synaptic transmission, it must be rapidly hydrolyzed to acetic acid and choline, in order to reconstitute an appropriate state for a new nerve transmission. When the inhibition of AChE and BChE by OP and CBM occurs, AChE remains in the synaptic cleft, resulting in overstimulation. Depending on the blocked receptor present on the surface of the nerve cells, various responses of the central nervous system (CNS) at the neuromuscular junctions are observed [9]. Therefore, the repeated analysis of cholinesterase (AChE and BChE) activity in patients poisoned with pesticides can be a valuable monitoring tool, and help to optimize therapeutic measures. Furthermore, they are useful analyses to confirm exposure, since the pathophysiological data available should allow a correlation between established symptoms and the degree of AChE and BChE inhibition [9,4].

Many methods to measure ChE activities have been developed, and usually involve esters of choline and thiocholine, as they are considered good substrates for AChE and BChE. Normally, ACh is the physiological substrate used to verify the activity of AChE and benzoilcolina is the substrate of choice to analyse the activity of BChE [19-24]. In order to apply these methods in the field, commercially available portable kits can be used [25]. With respect to the method of choice, measuring AChE activity is generally considered to be more specific compared to that of BChE. However, this topic remains controversial, as the BChE activity is more specific for some compounds, e.g. malathion and chlorpyrifos (both OP) [4]. But the analysis of ChE activity is a quick and inexpensive clinical toxicology method that facilitates the diagnosis, monitoring, and choice of treatment for acute cases of poisoning, and it clearly supports clinical and laboratory evidence. Nevertheless, the effects on AChE and BChE activity of frequent, repeated, and prolonged exposures to what are perceived to be low pesticide doses still produce controversial results [9,26]. In addition, several studies of OP and CBM poisoning vary between countries, according to the pesticide purpose and frequency of use, which also results in different types of poisoning [4,27,28]. An estimated 50% of workers are employed in the agricultural sector worldwide, and due to the inherent toxicity of pesticides, usually highly specific and complex legislation prescribes in most countries detailed procedures for the risk assessment of exposure, mostly based on the health monitoring of individuals [2].

In Brazil, the corresponding laws (NR7 and NR31) state that all agricultural workers are supposed to conduct periodic medical examinations, in order to assess occupational exposure. Specifically for AChE and BChE, laboratory evaluations are required in the periods of use to determine a possible exposure to OP and CBM. As a result, exposure threshold values could be established, which are considered 50% for BChE activity, 30% for AChE acitivity, and 25% for the whole blood. The reduction of these values is associated with poisoning that could be defined as mild, moderate, and severe. However, when values drop below 75%, a second control test should be performed [29].

Materials and Methods

To address this issue, we performed a systematic literature review of scientific publications by searching different online databases (LILACS, Scielo, Scopus, and MEDLINE). Our goal was to find reviews regarding laboratory studies published in English or Portuguese since 2000, in order to identify poisoning cases among Brazilian agricultural workers, which were determined on the basis of AChE or BchE activity. The following words were part of the search: pesticides; Brazil; acetylcholinesterase and/or butyrylcholinesterase.

Our optimized search strategies took Brazilian studies into consideration, which involved populations exposed to pesticides and biomonitored by analytical methods regarding the ChE activity (AChE and/or BChE). In addition, the following inclusion criteria were considered: (1) populations exposed to pesticides in Brazil; (2) exposure assessment to OP and/or CBM, or to a complex mixture of these and other chemicals, used in agricultural activities; (3) adequate and correct analysis of pesticide exposure by evaluating the activity of ChE (AChE and/or BChE); (4) outline of the presented study and display of results via the frequency of individuals with variations of the ChE (AChE and BChE) activity, or through the mean values with standard deviations, found between exposed and unexposed groups. Moreover, we only considered studies with a clear methodological design, involving individuals exposed to pesticides in agricultural activities in Brazil. Publications, which involved individuals exposed to pesticides in other countries, or which did not demonstrate clear results were excluded.

Results and Discussion

The biomonitoring of populations in Brazil exposed to pesticides through the evaluation of AChE and BChE activity are shows in Table 1 with presenting the studies used in this review. According to selection parameters, we were able to find 21 studies, of which only 13 met the inclusion criteria. It should be recognized that, even though the verification tests for the activity of AChE and BChE are useful for the initial screening of a possible poisoning, they should not be considered as a sensitive and specific method [9]. Among the groups, no significant differences were found in relation to the sample numbers on the activity of AChE and BChE, with the mean value

being within acceptable levels of exposure, as required by Brazilian law (BChE 50%, AChE 30%, 25% whole blood). When the number of individuals with reduced enzyme activity is observed, it may be concluded that in relation to the number of samples, these findings remain rare. Although most of the observed studies use the same methods, the presented results vary, mainly because the diversity of interpretations between laboratories may reflect important causalities, e.g. time elapsed between exposure and sample collection (possible recovery of the enzyme activity), adopted reference values, or restricted access to methodologies and advanced equipment.

Studies	Populations exposed to pesticides	Results		Methodological design employed for analyses of AchE and/or BChE
		Activity tested of AChE and/or BChE: Frequency of reduced activity[a]	Frequency of individuals with values reduced of activities AChE and/or BChE	
Oliveira-Silva et al. [30]	55 Horticulturist farmers working	AChE: 0% and BChE: 0 %	AChE: 3,6% and BChE: 41,6%	Ellman et al. [20]
Moreira et al. [31]	101 Horticulturist farmers working	AChE: 0% and BChE: 0 %	AChE: 11% and BChE: 12%	Ellman et al. [20]
Soares et al. [32]	442 Horticulturist farmers working	BChE: 21 %	BChE: 1,3%	Edson [19]
De Araújo et al. [33]	102 Horticulturist farmers working	AChE: 39% and BChE: 30%	AChE: 7% and BChE: 20%	Ellman et al. [20]
De Figueiredo et al. [34]	370 Horticulturist farmers working	NI[b]	AChE: 22% and BChE: 78%	Ellman et al. [20]
Pasiani et al. [35]	54 Horticulturist farmers working	AChE: 1% and BChE: 9%	AChE: 24% and BChE: 3,7%	Ellman et al. [20]
Etges et al. [36]	285 Tobacco farmers working	NI[b]	AChE: 0% and BChE: 25%	NI[b]
Salvi et al. [37]	37 Tobacco farmers working	BChE: 0 %	BChE: 0%	Ellman et al. [20]
Da Silva et al. [38]	27 Tobacco farmers working	BChE: 1%	BChE: 0%	Ellman et al. [20]
Goethel et al. [39]	40 Tobacco farmers working	NI[b]	BChE: 74%	Ellman et al. [20]
Salvador et al. [40]	101 Viticulturists farmers working	BChE: 34%	BChE: 99%	Ellman et al. [20]
Faria et al. [41]	103 Fruitculturists farmers working	NI[b]	BChE: 2,9%	Ni[b]
Benedetti et al. [42]	81 Soybean farmers working	BChE: 0%	BChE: 0%	Ellman et al. [20]

[a]Averange activity among individuals; [b]NI= Not informed.

Table 1: Exposure assesment to a complex mixture of pesticides used in agricultural activities in Brazil.

An important issue to be reflected upon is the methods that may not be in uniform between laboratories (e.g. selected sample and/or type of enzyme evaluated). Some types of exposure, such as absorption through the skin or the respiratory tract, have been reported to be more suitable for an assessment via the AChE rather than the BChE activity, especially in cases of chronic exposure. This is due to the AChE's lower rate of recovery compared to BChE. The effect of AChE inhibition continues, at most, for a few weeks after exposure [43]. However, most of the interviewed farmers in these Brazilian studies were found to be continuously exposed to pesticides, which could prevent a complete recovery of BChE. Furthermore, the separation of erythrocytes from plasma is often difficult to accomplish, and can result in false positives. Levels of erythrocyte AChE do not necessarily reflect the inhibition of AChE in the central nervous tissue accurately, which is in part due to pharmacokinetic factors [4]. Thus, it has been reported that the assessment of serum BChE is more sensitive than that of erythrocyte AChE [44]. Despite the fact that poisoning diagnosis is confirmed by evaluation of AChE and BChE activity levels, these tests are not always readily available in clinical laboratories, or the obtained values cannot be easily related to poisoning, due to difficulties regarding the access of reference values [45]. In general, it is recommended that reference values are defined by evaluations from individuals who are not exposed to OP and/or CBM. As benchmark values, the presented studies use

recommendations from manufacturers. These values are either based on mean population values of unexposed individuals, or on the analysis of exposed individuals outside exposure times. On the other hand, the majority of farmers also live in the work environment, which complicates a determination of exposure time limits. This is important, especially as these workers are exposed continuously to small doses for prolonged periods of time [9]. A more complex challenge is the simultaneous exposure to other groups of chemical compounds, for which the influence on ChE activity levels (since not only OP and CBM are inhibitors of these enzymes) has not yet been determined [46]. Moreover, the variety of pesticides used is different between studies and depend on the type of crop. Some studies consulted show intense exposures to pesticide mixtures, e.g. pyrethroid compounds, atrazine, glyphosate, and paraquat. There, the most toxic health effects for the exposed individuals may not even be registered; as sensitive laboratory tests do not exist in practice (very rarely laboratories have the technological conditions to accomplish this). The reduced activity of AChE and BChE is not compound specific and degrees of poisoning caused by OP or CBM are not yet established, both of which render the access of medical interventions difficult [9]. There is also the possibility of unexplored synergetic effects, arising from the combined exposure to toxic substances [6]. As previously mentioned, farmers are often simultaneously exposed to a variety of different pesticides as well as mixtures of pesticides [26]. Although some of the effects of acute intoxication with these chemical compounds are well known, there are almost no data on the effects of prolonged exposures to low doses. The principal pending questions include the effect of moderate exposure in the absence of overt clinical signs. Several studies have reported significant effects of DNA damage in workers occupationally exposed to pesticides [47] and these effects may well be correlated to the exposure time, due to the cumulative effect that complex mixtures of pesticides may exert over time [47-51]. There is evidence that continuous and frequent exposures to low doses of pesticides are associated with chronic poisoning, which severely compromises the health of exposed individuals over time. The effects may thereby vary, and some are related to e.g. genetic damage and include carcinogenic, neurotoxic processes diseases, reproductive and developmental defects, as well as hormonal, immunological, and neurodegenerative diseases [52,53].

Therefore, our research group addressed studies which evaluate the occupational exposure of individuals exposed to complex mixtures of pesticides. In general, the results show that exposed individuals have higher levels of DNA damage in somatic cells, demonstrating that it is possible to correlate these results with genetic susceptibility [42].

Conclusions

Based on this short literature review, we could observe that laboratory investigations based exclusively on the evaluation of ChE activity generate difficulties for the clinical interpretation, and require the use of all existing resources (laboratory, medical evaluations, information from agricultural workers and technicians in the field), in order to examine poisoning cases. And even then, results may only offer an approximation of reality. Nevertheless, the evaluation of AChE and/or BChE activity levels is reassuring, and serves as a well-defined orientation for the interpretation of results, although it is not always directly related to the risk of exposure to pesticides. Considering the previously described circumstances, the criteria for deleterious effects on the health of occupationally exposed individuals are not restricted to Brazil, but are generally applicable to other developing countries, where the use of complex mixtures of pesticides is increasing and a considerably high percentage of the human work force is involved. This makes biomonitoring studies among exposed individuals an extremely urgent matter. Clinical interpretations still require improvement, and widely available laboratory tests still remain unattainable, especially regarding individual conditions. It is therefore very important to monitor exposures levels, which, albeit considered mild to moderate, may induce significant effects on the human health over time. Subsequently, an evaluation of AChE and/or BChE activity levels in relation to DNA damage could offer a more reliable diagnostic tool. In addition, studies about environmental analysis together with semi-quantitative risk assessment involving crops and farmers in Brazil are limited and need be planned with more attention. Thus, the data that could be generated would generate a greater impact on public health.

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