Article

BJPS

Brazilian Journal of
Pharmaceutical Sciences
vol. 50, n. 4, oct./dec., 2014
http://dx.doi.org/10.1590/S1984-82502014000400014

Pesticide use and cholinesterase inhibition in small-scale agricultural workers in southern Brazil

Samuel Botião Nerilo[1], Fernanda Andrade Martins[1], Luciana Botião Nerilo[2], Valter Eduardo Cocco Salvadego[1], Renan Yoshio Endo[1], Gustavo Henrique Oliveira Rocha[1], Simone Aparecida Galerani Mossini[1], Vanderly Janeiro[3], Paula Nishiyama[1], Miguel Machinski Junior[1,*]

[1]Department of Basic Health Sciences, State University of Maringa, Maringa, Brazil, [2]Department of Pharmacy, Inga Faculty, Maringa, Brazil, [3]Department of Statistics, State University of Maringa, Maringa, Brazil

A controlled cross-sectional study of family growers of fruit and vegetables was conducted between October 2009 and October 2010 to characterize the use of pesticides, establish the socio-demographic profile, and analyze cholinesterase activity in small-scale agricultural workers in Southern Brazil. Data was collected for 173 workers and 179 controls. A structured questionnaire was applied collecting socio-demographic information and determining knowledge and work practices in relation to pesticide use. The benchmarks for total cholinesterase (ChEs) and butyrylcholinesterase (BuChE) were obtained from the average enzymatic activity of the occupationally unexposed group (control). The mean age of the exposed population was 40.0 ± 11.2 years. The symptoms differed significantly ($p<0.05$) between the exposed and unexposed populations. Forty (23.1%) workers showed symptoms associated with exposure to pesticides. The average value of enzymatic activity in the occupationally unexposed group for ChEs was 6.3 μmol/mL/min among males and 5.6 μmol/mL/min among females while for BuChE was 2.4 μmol/mL/min among males and 2.0 μmol/mL/min among females. Eight (4.6%) workers had high inhibition (>30%) of ChEs activity, whereas no workers showed high inhibition (>50%) of BuChE. Potential factors involved include gender, education, pesticide orientation, exposure, and hygiene measures.

Uniterms: Pesticides/use. Pesticides/occupational exposure. Cholinesterase/evaluation/exposure to pesticides. Agricultural workers/exposure to pesticides. Environmental health. Brazil/southern/environmental health.

Realizou-se um estudo transversal controlado, com hortifruticultores, durante o período de outubro de 2009 a outubro de 2010, a fim de caracterizar o uso de praguicidas, estabelecer o perfil sócio demográfico e analisar a atividade das colinesterases em trabalhadores rurais do sul do Brasil. Os dados foram obtidos de 173 trabalhadores e 179 controles. Um formulário estruturado foi aplicado obtendo informações sócio demográficas, conhecimento e práticas de trabalho relacionadas com o uso de praguicidas. Os valores de referência para colinesterases totais (ChEs) e butirilcolinesterase (BuChE) foram obtidos a partir da média da atividade enzimática do grupo controle. A idade média da população exposta foi de 40,0±11,2 anos. Os sinais/sintomas foram significativamente diferentes ($p<0,05$) entre a população exposta e não exposta. Quarenta trabalhadores (23,1%) apresentaram sinais/sintomas relacionados com a exposição aos praguicidas. O valor médio do grupo controle foi de 6,3 μmol/mL/min para o gênero masculino e 5,6 μmol/mL/min para o gênero feminino na ChEs; 2,4 μmol/mL/min para o gênero masculino e 2,0 μmol/mL/min para o gênero feminino na BuChE. Do total de trabalhadores (n=173), 08 (4,6%) apresentaram inibição elevada (>30%) das atividades das ChEs, e para BuChE nenhum trabalhador apresentou alta inibição (>50%). Dentre os fatores que poderiam estar envolvidos destacam-se o gênero, escolaridade, orientação para trabalhar com praguicidas, exposição e medidas de higiene.

Unitermos: Agrotóxicos/uso. Agrotóxicos/exposição ocupacional. Colinesterase/avaliação/exposição ocupacional a agrotóxicos Hortifruticultores/exposição ocupacional a agrotóxicos. Saúde ocupacional. Brasil/região Sul/saúde ocupacional.

[*]**Correspondence:** Miguel Machinski Junior. Departamento de Ciências Básicas da Saúde, Universidade Estadual de Maringá. Av. Colombo, 5790, 87020-900 - Maringa, Brasil. E-mail: mmjunior@uem.br

INTRODUCTION

In Brazil, pesticides were first used in public health programs in the 1960s to combat vectors. In 1975, the National Development Plan, responsible for Brazil's opening up of trade for pesticides, favored their use in agriculture through rural credit funds upon the inclusion of a set quota for pesticides for each funding application (OPAS, 1996). Thus, by the 1970s Brazil had become the world´s fourth largest consumer of pesticides after the United States (U.S.), Japan and France. Today, Brazil is the world's largest consumer of pesticides. Brazilian rural workers are in contact with large amounts of pesticides (Faria *et al.*, 2005; Faria, Rosa, Facchini, 2009). In developing countries, health problems related to pesticides are common due to low risk awareness among users, misuse of personal protective equipment, lack of care during application, use of highly toxic pesticides and scant mechanisms for health surveillance. This situation is exacerbated by low educational and cultural levels among the majority of rural workers in Brazil (Faria, Rosa, Facchini, 2009; Soares, Porto, 2012).

Accidental or occupational exposure to pesticides can cause acute and chronic poisoning. The acute toxic effects are easily recognized, while the effects of long-term exposure at low doses are hard to diagnose (Chakraborty *et al.*, 2009). These include: temporary and nonspecific symptoms such as eye irritation, dermatitis, asthma, peripheral neuropathy, chronic neurobehavioral motor dysfunction, burning sensation in the face, headache, dizziness, respiratory problems and sialorrhoea, and even chronic diseases such as cancer as well as disorders of the reproductive system (Ohayo-Mitoko *et al.*, 2000; Miranda *et al.*, 2002; Sanborn *et al.*, 2002; Safi, 2002; Salameh *et al.*, 2003; Del Prado-Lu, 2007). Accidental and intentional poisoning have been better studied than occupational exposure, where biological monitoring of workers exposed to pesticides has been widely recommended both to prevent poisoning and for epidemiological studies (Hofmann *et al.*, 2010).

In developing countries, cholinesterase inhibitor pesticides, i.e. organophosphates and carbamates, were found to be primarily responsible for human poisoning caused by pesticides (Chitra *et al.*, 2006; Chakraborty *et al.*, 2009; Mwila *et al.*, 2013). Acute exposure to organophosphates and carbamates has been well documented, because they inhibit neuronal acetylcholinesterase (AChE). The enzyme hydrolyzes the neurotransmitter acetylcholine, and thus plays a critical role in regulating transmission within the central and peripheral nervous system (Costa *et al.*, 2008; Ecobichon, 2001). Enzymatic determinations of cholinesterase (ChEs) present in the blood, of AChE in the erythrocyte membrane of cells and of butyrylcholinesterase (BuChE) in serum are used in the biological monitoring of exposure to cholinesterase inhibitors (EPA, 2000; Wilson, 2001). Nevertheless, results of enzymatic methods have shown broad variability from benchmarks among subjects. It is recommended to determine the level of enzyme activity pre-exposure. However, Brazilian agricultural practice represents a limiting factor, since workers are constantly exposed to pesticides in different crops (Brown, Miller, Keifer, 2006).

In Brazil, the horticulture sector is characterized by being family farming culture. Thus, there is an interest in investigating the socio-demographic working conditions, as well as the health of the agricultural workers exposed to pesticides. The aim of this paper was to analyze the use of pesticides, establish the socio-demographic profile and analyze ChEs activity in workers exposed to cholinesterase inhibitors in the horticulture sector in Brazil, and to establish reference values for ChEs and BuChE activity in the unexposed population.

MATERIAL AND METHODS

A controlled cross-sectional study was conducted from October 2009 to October 2010 in Maringa, Brazil, involving populations occupationally exposed and unexposed to pesticides.

Subjects

Random sampling was used and the exposed group comprised 362 growers of fruit and vegetables residing in Marialva, Maringa and Sarandi (Parana, Brazil). Farmers in three municipalities were invited to attend meetings about the harm posed by pesticides to human health and the environment. Two hundred and seven individuals who showed an interest in the study and were directly involved in planting, harvesting and/or transporting vegetables and fruit were selected. Of those selected, 200 workers attended the collection and underwent clinical and laboratory evaluation by a multidisciplinary team comprising a physician (n=1), pharmacists (n=4) and a nutritionist (n=1). After the rejection of individuals with pathologies or other exclusion factors, this gave a final group of 173 individuals exposed to pesticides. The occupationally unexposed group consisted of 200 blood donors from the Regional Blood Center of Maringa. Of these donors, 179 were included in the analysis after application of the same exclusion criteria used for the population exposed to pesticides.

Ethics

The study was approved by the Committee on Research and Ethics Involving Human Subjects of the State University of Maringa, in accordance with Resolution n°.196/96 of the National Health Counsel of the Ministry of Health, under process 531/2009. All 352 participants signed a detailed consent form. Workers with relevant laboratory findings and possible adverse health effects due to exposure to pesticides were informed about the situation and referred to the Occupational Health Clinic of the Poison Control Center of Maringa.

Interview instrument and data collection

A structured questionnaire was used for all the workers and controls assessed (Nishiyama, 2003). Information on gender, age, education, work practices related to pesticide use, use of personal protective equipment, personal hygiene measures, care for the environment, use of an agronomist's prescription, symptoms and confounding factors (alcoholism, smoking, obesity and others) were collected. Respondents were also asked if they had any previous episodes of poisoning.

Measures

Determinations for ChEs enzyme activity in whole blood and plasma, aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, gamma-glutamyltransferase, creatinine, urea, albumin, total protein and hematocrit were performed in the Laboratory of Toxicology of the State University of Maringa. The determinations of ChEs and BuChE was performed using the method proposed by Ellman *et al.* (1961), incorporating the modifications of Harlin and Ross (1990).

Analytical quality control

Procedures were performed with the adoption of effective internal quality controls (Sera-Pack reference materials, equipment calibration and document control). All samples were processed concomitantly with Precinorm and Precipath controls (Sera-Pack, Tarrytown, NY, USA) and intra and inter-assays were also performed (data not shown).

Establishment of benchmarks

The benchmarks for cholinesterase in whole blood and BuChE were obtained from the enzymatic activity of the enzymes in the control group, stratified by gender. To define high inhibition in the group exposed to pesticides, an inhibition value of 30% was used for ChEs in whole blood (cut-off point) (WHO, 1995) and 50% for BuChE (Brasil, 2005).

Statistical analysis

Statistical analysis was performed using the SAS program (version 9.1.3 Service Pack 3). After stratification of the studied workers into exposed and unexposed groups, Student´s *t*-test was used to compare means. The comparison test for large sample proportions was used to evaluate the signs and symptoms of the occupationally exposed and unexposed groups. Exploratory data analysis using cross-tabulation tables was employed to compare the variables of interest (socio-demographic indicators and use of pesticides) in the variation of cholinesterase activity observed (Casella, Berger, 2002).

RESULTS AND DISCUSSION

Study population characterization

The group exposed to pesticides comprised one hundred and seventy-three individuals while the unexposed group included 179 control subjects. The mean age was 40.0 ± 11.2 years (19-72 years) for the exposed group (Table I).The average length of time working in the field was 23.7 years and personal protective equipment, when used, was deficient. The growers of fruit and vegetables had been exposed to pesticides, on average, for 17.4 years (n=173). Although a sizable number of individuals reported the use of personal protective equipment (PPE), these were not always appropriate or sufficient for protection against the pesticides. Approximately 39.5% of subjects reported the exclusive use of boots, gloves and masks whereas 39.9% reported never having used PPE. The workers also reported that, due to the high temperatures in Brazil, the use of PPE was impractical. Similar results were observed in a study conducted in the Serra Gaucha, Brazil, where the authors showed that 35% of workers did not wear protective gloves, masks or clothing (Faria *et al.*, 2004). The lack of PPE use found in this study reveals the vulnerability of workers in the Brazilian family farming culture (horticulture) to exposure to these chemicals (Chester *et al.*, 1993).

Environmental exposure to cholinesterase-inhibiting pesticides

Seventy-five different commercial products were cited, including several classes of pesticides such as

TABLE I – Socio-demographic characteristics of participants, Southern Brazil, 2009-2010 (*n*=352)

Characteristics	Exposed Group (n=173)		Unexposed Group (n=179)	
	n	%	n	%
Gender				
Male	105	60.7	124	69.3
Female	68	30.3	55	30.7
Age (years)				
18-24	17	9.8	88	49.2
25-34	41	23.7	42	23.5
35-49	80	46.2	39	21.8
≥ 50	35	20.2	10	5.6
Education				
Illiterate	01	0.6	0	0.0
Incomplete Primary School	91	52.6	14	7.8
Complete Primary School	26	15.0	09	5.0
Incomplete High School	03	1.7	13	7.3
Complete High School	45	26.0	63	35.2
University Education	07	4.0	58	32.4
Number of Years Working as Farmer				
1-5	10	5.8		
6-9	09	5.2		
10-15	42	24.3		
≥ 15	112	64.7		
Personal Protective Equipment				
Boots	54	31.2		
Gloves	68	39.3		
Mask	83	48.0		
Protective Overalls	40	23.1		
Visor	16	9.2		
None	69	39.9		
Tasks Performed				
Harvesting	163	94.2		
Diluting Pesticides	103	59.5		
Preparing the Land	86	49.7		
Using Chemicals	84	48.6		
Applying Veterinary Products	38	22.0		
Applying Pesticides	118	68.2		

insecticides, fungicides, herbicides and others. The organophosphates reported were fenthion (3.4%), methamidophos (1.5%), parathion-methyl (1.0%) while carbamates were thiodicarb (0.3%) and carbaryl (0.3%). The occurrence of at least one episode of pesticide poisoning during the lifespan of the farmers was 12.1%, similar to rates reported by other authors. Faria *et al.* (2004) described a prevalence of 12% in rural workers in Serra Gaucha, Brazil. Van der Hoek *et al.* (1998) and London *et al.* (1994) found rates ranging from 7 to 22% in Sri Lanka and 9% for South Africa, respectively.

The growers of fruit and vegetables selected had direct contact with pesticides in 89.0% of cases and 11.0% had indirect contact with these products. Among the workers who had direct contact, 72.3% represented appliers and 54.3% those who prepared the product. After handling pesticides, only 29.5% took a full bath and 49.1% washed their hands and face. The frequency of workers who wore the same clothes the next day was 10.4%. Maintenance of spraying equipment was performed by 72.3% of the workers and 57.8% of the rinsing water for the equipment did not have a specific disposal area, and was often discarded onto the ground. These findings show that Brazilian horticulture workers are unaware of safety practices and do not follow the instructions contained in prescription labels.

The growers of fruit and vegetables reported that 90.2% of farm properties have agronomists in charge of them. By contrast, 43.9% did not follow the instructions contained in the agronomist's prescription and only 50.9% used it to buy pesticides. When purchasing pesticides, agricultural commercial shops were the outlet of choice in 76.9% of cases whereas cooperatives accounted for 8.1%. Although not using the agronomist's prescriptions, 69.9% of the workers reported that agronomists had prescribed pesticides, and 9.3% had purchased them without any professional orientation. With regard to professional instructions for applying pesticides, 53.8% reported receiving advice and 67.6% followed the instructions on the label. Those who did not follow instructions preferred their own methods for preparing and applying the products.

Among workers not using the agronomist's prescription to buy pesticides, 11.5% exhibited changes in ChEs activity, three-fold higher than those who used the prescription. Thus, random use and absence of a technique can lead to high exposure to and poisoning with pesticides and consequent contamination of agricultural produce by chemicals not suitable for the crop (Soares, Porto, 2012). In a study carried out by Castro and Confalonieri (2005), 85.0% of those interviewed stated they did not need a prescription to buy pesticides, even the most toxic types. This kind of conduct reflects the shortcomings in the education of the rural population as well as the lack of supervision by government agencies.

Health effects

Table II shows that the population exposed to pesticides had a high prevalence of the evaluated symptoms. When compared with the unexposed population, it was observed that the symptoms related to respiratory and urinary systems as well as skin and mucous membranes and peripheral nervous system differed significantly ($p<0.05$) for most of the symptoms. These results were similar to the findings of other studies showing that the main symptoms of exposure to pesticides were headache, skin irritation, dizziness, body pain and blurred vision (WHO, 1995; Castro, Confalonieri, 2005; Thundiyil *et al.*, 2008; Faria, Rosa, Facchini, 2009). These complaints can vary widely, depending on an individual's susceptibility. However, the most common diagnosis was acute intoxication when there was immediate post-exposure to pesticides. Nevertheless, chronic poisoning requires greater medical attention for a definitive diagnosis (Thundiyil *et al.*, 2008).

Cholinesterases levels in groups occupationally unexposed and exposed to pesticides

The benchmarks for total ChEs and BuChE were obtained from the average values of the control group (n=179). Among males, values of 6.3 μmol/mL/min (±0.7) for total ChEs and 2.4 μmol/mL/min (±0.4) for BuChE were obtained. Among females, average values of 5.6 μmol/mL/min (±0.7) for total ChEs and 2.0 μmol/mL/min (±0.4) for BuChE were obtained (Table III).

In order to define inhibition of ChEs in males, 30% inhibition for total ChEs and 50% for BuChE were established, representing cut-off points of 4.4 μmol/mL/min for total ChEs and 1.2 μmol/mL/min for BuChE. In females, cut-off values of 3.9 μmol/mL/min for total ChEs and 1.0 μmol/mL/min for BuChE were adopted. Females showed proportionally greater changes in levels of cholinesterase in whole blood compared to males. This difference appeared to be related to the lack of individual protective equipment during times of pesticide spraying, given that those applying the products were generally male whilst exposed females performed other duties within the same environment.

Of the total sample of workers (n=173), 08 (4.6%) had high inhibition (>30%) for cholinesterase activity in whole blood compared to the control group, whereas no workers exhibited high inhibition (>50%) for plasma cholinesterase compared to controls (Table III). According to the exploratory data analysis, related factors were: gender, age, education, agronomist's prescription, exposure and hygiene measures. Among the exposed population, inhibition was detected in 7.3% of women and 2.9% of men. In this study, of the 173 growers of fruit and vegetables selected, 4.6% showed inhibition of cholinesterase in whole blood whereas none showed changes for BuChE. This data differs from other studies conducted in Brazil. Oliveira-Silva *et al.* (2001) evaluated 55 organophosphate and carbamate insecticide applicators and found that 3.6% had inhibited BuChE and 41.8%

TABLE II - Prevalence of symptoms in groups unexposed and exposed to pesticides, Southern Brazil, 2009-2010 (*n*=352)

Symptoms	Exposed	Unexposed	p-value
	%	%	
Peripheral nervous system/general			
Headache	15.0	8.9	0.0779
Dizziness	5.2	0.6	0.0091
Cramps	7.5	2.2	0.0210
General malaise	8.1	0.6	0.0005
Weakness or fatigue	15.6	0.7	0.0000
Loss of appetite	1.7	1.1	0.6298
Blurred vision	10.4	0.6	0.0000
Dry mouth	6.9	1.7	0.0148
Tremulous	5.2	0.8	0.0156
Agitation / irritability	15.6	4.5	0.0004
Torpor	9.3	2.2	0.0044
Insomnia	9.8	3.9	0.0276
Convulsion	0.6	0.0	0.3151
Depression	3.5	0.3	0.0267
Hypothermia	1.7	0.0	0.0810
Drowsiness	8.1	2.2	0.0126
Pale	2.3	0.6	0.1686
Dizziness	5.2	0.0	0.0021
Hypotension	8.7	2.8	0.0172
Gastrointestinal/Digestive System			
Weight Loss	4.1	0.6	0.0291
Colic/Cramps	4.6	1.7	0.1145
Diarrhea	1.7	0.0	0.0810
Nausea	0.6	0.0	0.3151
Vomiting	0.6	0.0	0.3151
Abdominal Pain	6.4	0.6	0.0028
Respiratory System			
Cough	5.2	0.3	0.0042
Nasal discharge/Secretion	8.1	1.0	0.0013
Nasal irritation	12.1	3.9	0.0042
Chest tightness	5.8	0.0	0.0011
Skin and Mucous			
Sweating	11.6	1.7	0.0002
Eye irritation	23.1	3.4	0.0000
Itchy hands and feet	4.6	1.1	0.0492
Irritated skin	11.6	0.6	0.0000
Irritative disease	2.3	0.0	0.0422
Petechiae	5.8	1.1	0.0163

Comparison test for large sample proportions.

TABLE III - Comparison of cholinesterase (ChEs and BuChE) levels in assays of exposed and unexposed populations by gender, Southern Brazil, 2009-2010 (*n*=352)

Gender	Exposed Group				Unexposed Group	
	ChEs		BuChE		ChEs	BuChE
	µmol/mL/min	p-valor	µmol/mL/min	p-valor	µmol/mL/min	
Male	5.86 (0.78) (n=105)	< 0.0001	2.35 (0.45) (n=105)	0.3557	6.30 (0.7) (n=124)	2.40 (0.40) (n=124)
Female	5.18 (0.84) (n=68)	0.0023	2.06 (0.52) (n=68)	0.5979	5.60 (0.7) (n=55)	2.00 (0.40) (n=55)

Results: Mean (standard deviation)

had AChE inhibition. Soares *et al.* (2003) showed that 50% of 1064 rural workers had inhibited enzyme activity for BuChE, however their samples were convenience not random. Moreira *et al.* (2002) found that 11% of a farming community had reduced AChE and 12% reduced BuChE. This disparity might be attributed to the sample selected (random in present study), type of product used on the various crops, methodology applied or the reference values used (Oliveira-Silva *et al.*, 2001; Soares, Almeida, Moro, 2003).

Workers who had incomplete primary education accounted for 75.0% of the alterations while the younger subjects (≤34 years) for 62.5%. Younger professionals (≤34 years) had higher levels of total ChEs inhibition (62.5%) than the more experienced professionals. Low risk awareness in this age group coupled with low educational level were considered variables aggravating the health of these workers (Oliveira-Silva *et al.*, 2001; Faria, Rosa, Facchini, 2009).

Direct contact with the toxicants by spraying was responsible for 100% of the alterations whereas indirectly exposed individuals did not exhibit significant variations in cholinesterase inhibition. Of those involved in spraying who had significant alterations, 66.7% had only washed their hands and face after handling pesticides. Since workers were directly exposed to pesticides, hygiene measures were essential to minimize contact with these toxicants. Washing hands and face was not effective for containing this exposure, and workers who used these procedures were intoxicated more than those who bathed thoroughly after exposure. The data obtained corroborates the findings of Hofmann *et al.* (2010) considering that no association was observed between the practice of washing hands after application of pesticides and BuChE inhibition.

Some workers (4.6%) showed significant inhibition in cholinesterase activity when compared to subjects from the control group. This study also showed that exposure to pesticides allowed the emergence of symptoms more frequently in workers and that handling practices influenced the observed changes. Carelessness in the use of these chemicals appears to have been facilitated by the low educational levels of this population, because the technical terms contained in pesticide labels are difficult for these employees to understand.

Enzymatic concentrations differed significantly (p=0.003) between the group of fruit and vegetable growers and the occupationally unexposed group since the workers showed lower levels of cholinesterase activity in whole blood. The present study demonstrated that this difference was related to gender, age, low education, use of personal protective equipment, non-use of an agronomist's prescription, direct contact with pesticides and poor hygiene after handling these chemicals.

CONCLUSIONS

The results demonstrated that the total ChEs biomarker was appropriate for assessing occupational exposure to pesticides, since it led to the detection of 4.6% of workers with low enzymatic activity. This biomarker detected exposure to pesticides at low doses and for long periods. This study suggests there is a need for future research on reference values and exposure limits for total ChEs in the Brazilian population. Further research is also important to promote the search for new models of agricultural production in an effort to reduce chemical exposure and improve the quality of life of this population.

ACKNOWLEDGEMENTS

The authors thank the Brazilian Coordination for Graduate Studies (Coordenação para Aperfeiçoamento de Pessoal de Nível Superior, CAPES, Brazil) for the graduate scholarship granted to the first author. The project 'Monitoring of Occupational Exposure' of the State University of Maringa (Grant # 7303/2008) provided financial support. We would like to thank all of the workers who participated in this study.

REFERENCES

BRASIL. Ministério do Trabalho e Emprego. 2005. *Normas regulamentadoras de segurança e saúde no trabalho (NRs).* Brasília: Ministério do Trabalho e Emprego. Available at: <http://goo.gl/e1vZO>. Accessed on: 10 Oct. 2011.

BROWN, A.E.; MILLER, M.; KEIFER, M. *Cholinesterase monitoring:* a guide for the health professional. College Park: University of Maryland, 2006. (Pesticide information leaflet, 30).

CASELLA, G., BERGER, R.L. *Statistical inference*. 2ed. Duxbury: Thompson Learning, 2002. 686 p.

CASTRO, J.S.M.; CONFALONIERI, U. Pesticide use in Cachoeiras de Macacu, Rio de Janeiro. *Brazil. Ciênc. Saúde Coletiva.*, v.10, p.473-482, 2002.

CHAKRABORTY, S.; MUKHERJEE, S.; ROYCHOUDHURY, S.; SIDDIQUE, S.; LAHIRI, T.; RAY, M.R. Chronic exposures to cholinesterase-inhibiting pesticides adversely affect respiratory health of agricultural workers in India. *J. Occup. Health*, v.51, p.488-497, 2009.

CHESTER, M.H.; ADAM, A.V.; INKMANN-KOCH, A.; LITCHFIELD, M.H.; SABAPATHY, R.; TUIMAN, C.P. Field evaluation of protective equipment for pesticide appliers in a tropical climate. In: FORGET, G.; GOODMAN, T.; VILLIERS, A. (Eds.). *Impact of pesticide use on health in developing countries*. Ottawa: International Development Research Centre, p.116-123, 1993.

CHITRA, G.A.; MURALEEDHARAN, V.R.; WAMINATHAN, T.; VEERARAGHAVAN, D. Use of Pesticides and Its Impact on Health of Farmers in South India. *Int. J. Occup. Environ. Health,* v.12, p.228-233, 2006.

COSTA, L.G.; COLE, T.B.; JANSEN, K.L.; FURLONG, C.E. Paraoxonase (PON1) and organophosphate toxicity. In: MACKNESS, B. (Ed.). *The paraoxonases:* their role in disease development and xenobiotic metabolism. Dordrecht: Springer, 2008. p.209-220.

DEL PRADO-LU, J.L. Pesticide exposure, risk factors and health problems among cut flower farmers: a cross sectional study. *J. Occup. Med. Toxicol*., v.2, p.9-16, 2007.

ECOBICHON, D.J. Toxic effects of pesticides. In: KLAASSEN, C.D. (Ed.). *Casarett and Doull's toxicology: the basic science of poisons*. New York: McGraw-Hill, 2001. p.763-810.

ELLMAN, G.L.; COURTNEY, K.D.; ANDRES JR., V.; FEATHER-STONE, R.M. A new and rapid colorimetric determination of acetylcholinesterase activity. *Biochem. Pharmacol*., v.7, p.88-95, 1961.

ENVIRONMENTAL PROTECTION AGENCY. EPA. *The use of data on cholinesterase inhibition for risk assessments of organophosphorous and carbamate pesticides*. Washington: U.S. Environmental Protection Agency, 2000. p.7-9.

FARIA, N.M.; FACCHINI, L.A.; FASSA, A.G.; TOMASI, E. Pesticides and respiratory symptoms among farmers. *Rev. Saúde Pública*, v.39, p. 973-981, 2005.

FARIA, N.M.; FACCHINI, L.A.; FASSA, A.G.; TOMASI, E. Rural work and pesticide poisoning. *Cad. Saúde Pública*, v.20, p.1298-1308, 2004.

FARIA, N.M.X.; ROSA, J.A.R.; FACCHINI, L.A. Poisoning by pesticides among family fruit farmers, Bento Gonçalves, Southern Brazil. *Rev. Saúde Pública*, v.43, p.335-344, 2009.

HARLIN, K.S., ROSS, P.F. Enzymatic-spectrophotometric method for determination of cholinesterase activity in whole blood: a collaborative study. *J. Assoc. Off. Anal. Chem*., v.73, p.616-619, 1990.

HOFMANN, J.N.; KEIFER, M.C.; DE ROOS, A.J.; FENSKE, R.A.; FURLONG, C.E.; VAN BELLE, G.; CHECKOWAY, H. Occupational determinants of serum cholinesterase inhibition among organophosphate-exposed agricultural pesticide handlers in Washington State. *Occup. Environ. Med*., v.67, p.375-386, 2010.

LONDON, L.; EHRLICH, R.I.; RAFUDIEN, S.; KRIGE, F.; VURGARELLIS, P. Notification of poisoning in the Western Cape, 1987-1991. *S. Afr. Med. J.*, v.84, p.269-272, 1994.

MIRANDA, J.; MCCONNELL, R.; DELGADO, E.; CUADRA, R.; KEIFER, M.; WESSELING, C.; TORRES, E.; LUNDBERG, I. Tactile vibration thresholds after acute poisonings with organophosphate insecticides. *Int. J. Occup. Environ. Health*, v.8, p.212-219, 2002.

MOREIRA, J.C.; JACOB, S.C.; PERES, F.; LIMA, J.S.; MEYER, A.; OLIVEIRA-SILVA, J.J.; SARCINELLI, P.N.; BATISTA, D.F.; EGLER, M.; FARIA, M.V.C.; ARAÚJO, A.J.; KUBOTA, A.H.; SOARES, M.O.; ALVES, S.R.; MOURA, C.M.; CURI, R. Integrated evaluation of the health impact of pesticide use in a community at Nova Friburgo, RJ. *Ciênc. Saúde Coletiva*, v.7, p.299-311, 2002.

MWILA, K.; BURTON, M.H.; VAN DYK, J.S.; PLETSCHKE, B.I. The effect of mixtures of organophosphate and carbamate pesticides on acetylcholinesterase and application of chemometrics to identify pesticides in mixtures. *Environ. Monit. Assess.,* v.185, p.2315-2327, 2013.

NISHIYAMA, P. *Utilização de agrotóxicos em áreas de Reforma Agrária no Estado do Paraná*. Campinas, 2003. 136 p. [Thesis of PhD degree. Faculty of Medical Sciences, State University of Campinas].

OHAYO-MITOKO, G.J.A.; KROMHOUT, H.; SIMWA, J.M.; BOLEIJ, J.S.M.; HEEDERIK, D. Self-reported symptoms and inhibition of acetylcholinesterase activity among Kenyan agricultural workers. *Occup. Environ. Med*., v.57, p.195-200, 2000.

OLIVEIRA-SILVA, J.J.; ALVES, S.R.; MEYER, A.; PEREZ, F.; SARCINELLI, P.N.; MATTOS, R.C.O.C.; MOREIRA, J.C. Influence of social-economic factors on the pesticide poisoning, Brazil. *Rev. Saúde Pública*, v.35, p.130-135, 2001.

ORGANIZAÇÃO PAN AMERICANA DA SAÚDE. OPAS. *Manual de vigilância da saúde de populações expostas a agrotóxicos*. Brasília: OPAS - Representação do Brasil, 1996. 69 p.

SAFI, J.M. Association between chronic exposure to pesticides and recorded cases of human malignancy in Gaza Governorates (1990-1999). *Sci. Total Environ*., v.284, p.75-84, 2002.

SALAMEH, P.R.; BALDI, I.; BROCHARD, P.; RAHERISON, C.; ABI-SALEH, B.; SALAMON, R. Respiratory symptoms in children and exposure to pesticides. *Eur. Respir. J*., v.22, p.507-512, 2003.

SANBORN, M.D.; COLE, D.; ABELSOHN, A.; WEIR, E. Identifying and managing adverse environmental health effects: 4. Pesticides. *CMAJ*., v.166, p.1431-1436, 2002.

SOARES, W.; ALMEIDA, R.M.V.R.; MORO, S. Rural work and risk factors associated with pesticide use in Minas Gerais, Brazil. *Cad. Saúde Pública*, v.19, p.1117-127, 2003.

SOARES, W.L.; PORTO, M.F.S. Pesticide use and economic impacts on health. *Rev. Saúde Pública,* v.46, p.209-217, 2012.

THUNDIYIL, J.G.; STOBER, J.; BESBELLI, N.; PRONCZUK, J. Acute pesticide poisoning: a proposed classification tool. *Bull. World Health Organ*., v.86, p.205-209, 2008.

VAN DER HOEK, W.; KONRADSEN, F.; ATHUKORALA, K.; WANIGADEWA, T. Pesticide poisoning: a major health problem in Sri Lanka. *Soc. Sci. Med.*, v.46, p.495-504, 1998.

WORLD HEALTH ORGANIZATION. WHO. *International programme on chemical safety:* Basic analytical toxicology. Switzerland: Geneva, 1995. Available at: <http://goo.gl/bwkG8H>. Accessed on: 10 Oct. 2013.

WILSON, B. Cholinesterases. In: KRIEGER, R.I. (Ed.). *Handbook of pesticide toxicology*. San Diego: Academic Press, 2001. p.967-985.

Received for publication on 31st May 2013
Accepted for publication on 16th December 2013