DOI: 10.1590/1413-81232018246.13142017

Occupational exposure to pesticides and hematological alterations: A survey of farm residents in the South of Brazil

Exposição ocupacional a agrotóxicos e alterações hematológicas: Estudo transversal em moradores rurais do Sul do Brasil

Camila Piccoli (https://orcid.org/0000-0003-0462-557X) [1]
Cleber Cremonese (https://orcid.org/0000-0003-2700-7416) [2]
Rosalina Koifman (https://orcid.org/0000-0003-4391-4542) [1]
Sérgio Koifman (https://orcid.org/0000-0003-1769-3985) [1] (*in memorian*)
Carmen Freire (https://orcid.org/0000-0001-7370-1842) [3]

Abstract *This study sought to investigate the association of exposure to organochlorine (OC) and non-persistent pesticides with hematological parameters in an agricultural population in Southern Brazil. A cross-sectional study was conducted with a random sample of 275 farm workers and their families in Farroupilha-RS. A questionnaire was used to collect information on sociodemographic and lifestyle factors, duration, frequency and type of pesticide used, among others. Blood samples were collected and analyzed for serum concentration of 24 OC pesticides and hematological parameters. Associations were explored through linear regression, controlling for confounders. Lifetime use of chemical classes other than organophosphates and dithiocarbamates were associated with decreased number of lymphocytes, while subjects sampled in the high pesticide use season showed higher number of erythrocytes and hemoglobin level. Detectable serum levels of many OC pesticides were associated with lower counts of white blood cells, particularly eosinophils. Although mostly null associations were observed between pesticide use and hematological parameters, findings may suggest that OC pesticides could lead to hematological alterations among agricultural workers.*

Key words *Agricultural workers, Non-persistent pesticides, Organochlorine pesticides, Hematotoxicity, Hematological alterations*

Resumo *O objetivo deste estudo foi investigar a associação entre a exposição a organoclorados (OC) e agrotóxicos não persistentes e os parâmetros hematológicos em uma população agrícola de Farroupilha-RS. Foi utilizado um questionário para coletar informações sobre fatores sociodemográficos e de estilo de vida, duração, frequência e tipo de pesticidas utilizados, entre outros. Amostras sanguíneas foram coletadas e analisadas quanto a concentração sérica de 24 pesticidas OC e parâmetros hematológicos. As associações foram exploradas através de regressão linear, controlando por confundidores. O uso cumulativo de classes químicas diferentes de organofosforados e ditiocarbamatos associou-se com diminuição do número de linfócitos enquanto indivíduos que tiveram suas coletas sanguíneas realizadas na estação de maior uso de agrotóxicos tinham contagem de eritrócitos e hemoglobina maiores. Níveis séricos de diversos pesticidas organoclorados foram associados com contagens mais baixas de células brancas do sangue, particularmente eosinófilos. Embora as associações com o uso de agrotóxicos tenham sido, em geral, nulas, os resultados podem sugerir que os pesticidas OCs poderiam levar a alterações hematológicas entre os trabalhadores agrícolas.*

Palavras-chave *Trabalhadores agrícolas, Agrotóxicos não persistentes, Pesticidas organoclorados, Hematotoxicidade, Alterações hematológicas*

[1] Escola Nacional de Saúde Pública, Fiocruz. R. Leopoldo Bulhões 1480, Manguinhos. 21041-210 Rio de Janeiro RJ Brasil. cami.piccolli@gmail.com
[2] Centro Universitário da Serra Gaúcha. Caxias do Sul RS Brasil.
[3] Institute of Biomedical Research of Granada. Granada Espanha.

Introduction

Human exposure to pesticides has been linked to several harmful health effects, including endocrine disorders, birth defects, neurological, hepatic, respiratory and immunological effects, and cancer[1,2]. This wide range of adverse outcomes suggests that pesticides exert toxic effects on human health through various mechanisms of action. In this regard, experimental data available indicate that many pesticides may also possess hematotoxic properties, leading to depressed hematopoiesis[3-5].

Animal studies have shown that persistent organochlorine (OC) pesticides can affect the hematopoietic system through oxidative stress and immunological mechanisms inducing apoptosis of mononuclear cells from peripheral blood[6]. According to this hypothesis, findings from some human studies support the existence of a relationship between environmental and occupational exposure to OC pesticides and blood disorders, particularly aplastic anemia[7-14]. On the other hand, data available on effects of non-persistent pesticides on human hematopoiesis are increasing, which include reports of leukopenia, leukocytosis, lymphopenia, lymphocytosis, neutropenia, monocytosis, anemia, and thrombocytopenia associated with occupational exposure to contemporary pesticides[9,11,15-20].

Agricultural populations in developing countries are exposed to increasing amounts of pesticides mixtures, at high concentrations and frequency, including pesticides severely restricted and banned in industrialized countries[21]. We have previously reported associations of cumulative exposure to pesticides, especially herbicides and dithiocarbamate fungicides, with hypothyroidism-like effects and poorer sperm quality in male farm workers in Serra Gaúcha, a family-based agricultural region in the South of Brazil[22,23]. Based on the hypothesis that both persistent and non-persistent pesticides may have the ability to cause hematological disorders in humans, we sought to assess the relationship of agricultural work practices, use of non-persistent pesticides, and serum levels of OC pesticides with hematological parameters in farm residents in this region. It was hypothesized that recent and/or cumulative pesticide exposure of agricultural workers may be related to changes in hematological parameters.

Materials and methods

Study population

This is a survey conducted between 2012 and 2013 in farm workers and their families in Farroupilha, a town with 69.000 inhabitants, localized in Serra Gaúcha, in Rio Grande do Sul state. Agricultural population in this region is involved in activities related to planting, pruning, and harvesting, most commonly in plums, peaches, grapes, and kiwis crops. Assuming a participation rate of around 90% and at least 3 adults per household, 90 residences were randomly selected from the list of rural households of the municipal agriculture office to reach the estimated sample size. The minimum sample size for the study was estimated at 220 individuals. All persons aged 18-69 years living in the selected households were personally invited to participate in the study, representing a total of 301 subjects. Farm owners working in farm work for less than one year and their respective family members were excluded from the study, that is, 5 residents. Among the remaining 296 subjects, 21 (7%) refused to participate in the study, leaving a final sample of 275 adults. The study was approved by the Ethics Committee of the National School of Public Health, Oswaldo Cruz Foundation (ENSP/Fiocruz), in Rio de Janeiro, and written informed consent was obtained from all participants.

Participants underwent a physical examination, provided blood samples, and completed a structured questionnaire on socio-demographics, lifestyle, agricultural work practices, pesticide use, and medical history. Interviews, anthropometric measurements, and blood sampling were conducted during in-home visits to participant.

Questionnaire

Two research staff members administered an extensive structured questionnaire to study participants through face-to-face interviews. Variables gathered through questionnaire and used in the present study were gender, age (continuous and categorized into groups: 18-30, 31-45, 46-60, and > 60 years), years of education (continuous and categorized as ≤ 8, 9-11, and ≥ 12 years), marital status (married; others), household income (categorized as ≤ 10, 11-20, 21-50, and > 50 thousands of Brazilian reais per year), place of birth (Farroupilha; other city), cigarette smoking status (never smoked; ex-smoker; current smoker), number of years of smoking (categorized

as 0, 1-9, and ≥ 10), frequent alcohol use in the past month (no; yes), practiced physical activity regularly in the past 3 months (no; yes), current weight and height, history of hematological disease (no; yes), and history of hematological disease in first-degree relatives (no; yes).

Regarding agricultural work and pesticide use, the following variables were analyzed: currently working in agriculture (no; yes), years working on a farm (categorized as < 1, 1-10, 11-25, 26-50, and > 50), years mixing or applying pesticides (categorized as ≤ 1, 2-10, and >10), days per year mixing or applying pesticides (< 5, 5-39, 40-59, and ≥ 60), season of interview and blood draw (low pesticide use season: from September to March; high pesticide use season: from April to August), use of full personal protective equipment (PPE) (no; yes), current use of pesticides (no; yes), and total number of pesticides currently used (categorized as none, 1, and ≥ 2 products). Information was also gathered on starting and finishing dates of use of specific pesticides from a list including the most commonly-used pesticides in the study area. This list was obtained from the Brazilian Entity for Technical Assistance and Rural Extension (*Empresa de Assistência Técnica e Extensão Rural* - EMATER) and contained 18 commercial products, as previously described[23]. Participants were also asked about the use of pesticides not included in this list. Active ingredients in commercial products were grouped into the following functional and chemical classes: herbicides, insecticides, fungicides, organophosphate (OP) insecticides, dithiocarbamate fungicides, carbamates, and others chemical classes. Number of years of pesticide use was then calculated for overall pesticide use and for each functional and chemical class, regardless of simultaneous use of different pesticides of the same class (for example, if mancozeb and carbendazim were used for 10 years, from 2000 to 2010, we assumed 10 years of fungicide use). Lifetime use of pesticides was categorized as never use, 1-20 years, and > 20 years.

Anthropometric measurements

Standard procedures were followed during anthropometric measurements[24], which included weight (kg), height (cm), and abdominal circumference (cm). Body mass index (BMI) was calculated by dividing weight in kg by height in meters squared and categorized as lower than 25 kg/m^2 (eutrophic) and equal to or greater than 25 kg/m^2 (overweight or obese).

Laboratory analyses

Intravenous blood samples (15 mL) were drawn from participants after a 12-hour overnight fast. Plasma and serum were separated from whole blood by centrifugation, and stored at -20ºC in vacutainer tubes containing EDTA until delivery to the laboratory for toxicological and biochemical analyses.

Hematological parameters were determined by the SYSMEX XS-1000i Automated Hematology Analyzer. The parameters measured included: erythrocyte count (millions/mm^3), hemoglobin (g/dL), hematocrit (%), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), red cell distribution width (RDW), total count of leukocytes, and differential leukocyte count, including neutrophils, eosinophils, lymphocytes, monocytes, and basophils (u/μL). Normal laboratory reference ranges were: erythrocytes,5.3 ± 0.8 million/μL for men and 4.7 ± 0.7 million/μL for women; hemoglobin, 15.3 ± 2.5 g/dL for men and 13.6 ± 2.0 g/dL for women; hematocrit, 46 ± 7% for men and 42 ± 6% for women; MCV, 89 ± 9 fL; MCH, 29 ± 3 pg; MCHC, 33 ± 2 g/dL; leukocyte count, 4,000-11,000 u/μL; neutrophils, 1,500-7,000 u/μL; lymphocytes,1,000-4,500 u/μL; monocytes, 100-1,000 u/μL; eosinophils, 50-500 u/μL; and basophils,0-200 u/μL.

Residues of OC pesticides were measured in blood serum at the Center for Occupational Health, National School of Public Health (ENSP)-Fiocruz, in Rio de Janeiro. Concentration of OC pesticides were determined by gas chromatography with electron-capture detection, following an optimized protocol[25]. Serum samples were analyzed for the following 24 chemicals: α, β, and γ-hexachlorocyclohexane (HCH) isomers, hexachlorobenzene (HCB), α and γ-chlordane, heptachlor epoxide A and B, heptachlor, *trans*-nonachlor, *o,p*'-dichlorodiphenyltrichloroethane (DDT), *p,p*'-DDT, *o,p*'-dichlorodiphenylethane (DDE), *p,p*'-DDE, *o,p*'-dichlorodiphenyldichloroethane (DDD), *p,p*'-DDD, endosulfan I, endosulfan II, aldrin, endrin, dieldrin, methoxychlor, mirex, and pentachloroanisole (an environmental metabolite of pentachlorophenol). Identification of each analyte was based on the mean retention time, established as the mean of retention times in 10 measurements ± three times the standard deviation (SD). According to the IUPAC, limits of detection (LD) were designated as three-fold the SD of the blank, and were the following: 0.05 ng/mL for α-HCH; 0.07

ng/mL for β-HCH, HCB, heptachlor epoxide A, and endosulfan I; 0.04 ng/mL for γ-HCH; 0.13 ng/mL for *o,p*'-DDT; 0.02 ng/mL for *p,p*'-DDT; 0.12 ng/mL for *p,p*'-DDD and *o,p*'-DDD; 0.09 ng/mL for heptachlor epoxide B, *trans*-nonachlor, α-chlordane, γ-chlordane, dieldrin, and *p,p*'-DDE; 0.29 ng/mL for endrin; 0.14 ng/mL for methoxychlor; 0.10 ng/mL for *o,p*'-DDE, aldrin, and mirex; 0.11 ng/mL for endosulfan II; and 0.06 ng/mL for pentachloroanisole. Recovery in the extraction was determined by fortifying 10 aliquots of 4 mL of blank medium to an intermediate point on the calibration curve. Recovery percentage ranged from 80% to 98%. Retention times were confirmed by GC-ECD, using a column HP-1701. For quality control, samples were analyzed in batches of 20 samples, with two replicates in each batch. In addition, to ensure the quality of the method, positive controls and one blank were used. Positive controls were fortified serum samples at 1 ng/mL and 5 ng/mL. The global coefficient of variation between the replicates was 5.6%. No blinded replicates were made. The coefficient of variation of the spiked samples in all batches varied from 7.2% to 9.8%, indicating a good reproducibility of the analytical method for all OC compounds. The Center for Occupational Health (ENSP-Fiocruz), which performs a wide range of toxicological analysis, is accredited by the Joint Commission International (JCI).

Concentrations of total cholesterol and triglycerides (in mg/dL) in serum were determined by colorimetric enzymatic methods. Estimates of total serum lipids were calculated by the formula: Total lipids = 2.27*Total cholesterol + Triglycerides + 0.623[26]. Wet-weight OC pesticide concentrations (ng/mL) were then divided by total lipid serum content (mg/dL) and expressed on a lipid weight basis (ng/g).

Statistical analysis

Characteristics of participants were described by frequency distribution, means, and SD. Given the low percentage of serum samples with quantifiable concentrations for OC pesticides (only 1 pesticide was detected in 50% of samples and 6 were detected in 30-50% of samples), we did not apply any method for dealing with values below the LOD. Quartiles of serum concentrations of OC pesticides were then used for descriptive purposes. None of the study variables had missing values.

Normality of pesticide concentrations above the LOD and hematological parameters was checked by the Kolmogorov-Smirnov test. Pearson and Spearman correlation coefficients were calculated between quantifiable OC pesticide concentrations, and between quantifiable OC concentrations and hematological parameters.

- Multivariate linear regression analysis was performed to assess the association between exposure variables (agricultural work, current and lifetime pesticide use, and OC pesticides) and hematological indices, controlling for potential confounders. Erythrocyte count and hemoglobin were modeled untransformed, while for total leukocytes and neutrophils, lymphocytes, monocytes, and eosinophils we used natural-logarithm transformed variables, which fitted normal distributions. OC pesticides were introduced as dichotomous variables, *i.e.* detected and undetected levels, in regression models. All models were adjusted for sex, age, BMI, smoking habit, alcohol consumption, and categorized *p,p*'-DDE serum levels, regardless of their statistical significance, which are variables identified in the literature as potential confounders. To improve interpretability, regression coefficients (b) and 95% confidence intervals (CI) in white blood cells models were transformed back [exp(b)] on the original scale and expressed as percentage change in dependent variable per one unit change in exposure variable, *i.e.* if exposure variable changes by 1 (unit), dependent variable is expected to change by 100.β percent. Likelihood ratio test was used to test the significance of linear trend in regression models with ordinal exposure variables. Sensitivity analysis was performed by excluding subjects with a history of hematological disorder. Finally, analyzes were also stratified by gender to explore possible interaction with exposures.

Statistical analyses were performed using SPSS version 21.0 (SPSS Inc., Chicago, IL, US) and STATA version 11 was used to estimate linear trends in regression models. A significance level of 0.05 was established.

Results

Fifty-six percent of the study subjects were male. Mean age of participants was 42 years, and 87% of them (92% of men and 81% of women) were directly involved in farming activities. Non-farmer participants were farmers' relatives living on farms (*i.e.* sons, daughters, wives and others not directly involved in field activities). Nearly all participants were white skinned (99.3%). Regarding medical history, only one subject re-

ported a history of hematological disease, while 4 men and 2 women had a family history of hematological disease in first degree (Table 1). For more detailed information on characteristics of study population see Piccoli et al.[23].

Around half of the study population reported more than 25 years of agricultural work, 55% had mixed or applied pesticides for more than 10 years, and37% had mixed/applied pesticides with an average frequency greater than or equal to 60 days per year. Pesticide classes most frequently used by farmers at the time of the interview were herbicides and fungicides, and one third of the respondents were using 2 or more pesticides simultaneously. Regarding specific pesticides, glyphosate and paraquat were the most common herbicides ever used by farmers in the study, while mancozeb (a dithiocarbamate fungicide) and copper sulphate were the most commonly used fungicides (Table 1). Fungicides and dithiocarbamates were the pesticide classes showing the highest number of lifetime exposure years, *i.e.* used for more than 20 years by over 40% of study subjects (Table 2).

Distribution of hematological parameters and OC pesticide serum concentrations are shown in Table 3. Reduced erythrocytes and RDW was seen in 6% and 29% of participants, respectively, whereas MCH and eosinophil count were elevated in 9% of the sample, respectively. All the participants had a basophil count of zero. Half of the study population had detectable γ-HCH in serum, followed by *p,p*'-DDT (42.8%), β-HCH (41.3%), *p,p*'-DDE (39.5%), heptachlor (32.9%), α-HCH (30.9%), and endrin (30.6%). Positive and statistically significant correlations were found between all OC pesticides except for β-HCH, as previously described[23]. Significant negative correlations were observed between **γ**-chlordane and RDW (Spearman coefficient, r: -0.39, p-value: 0.03), **γ**-chlordane and VCM (r: -0.41, p-value: 0.03), *p,p*'-DDT and eosinophils (r: -0.28, p-value: 0.01), *p,p*'-DDD and RDW (r: -0.40, p-value: 0.01), endosulfan I and HCM (r: -0.24, p-value: 0.05), and endosulfan II and lymphocytes (r: -0.25, p-value: 0.05). β-HCH was positively correlated with leukocyte count (r: 0.19, p-value: 0.04) and pentachloroanisole with neutrophils (r: 0.24, p-value: 0.04).

Tables 4 and 5 present results of multivariate analyses. Subjects sampled in the high pesticide use season showed small but significant increases in number of erythrocytes (0.10 m/mm^3, 95%CI: 0.01 to 0.19) and hemoglobin level (0.22 g/dL, 95%CI: 0.00 to 0.45) relative to the low pesticide use season. Duration, frequency, and lifetime years of overall pesticide use did not reveal associations with hematological parameters. Otherwise, long-term use (> 20 years) of pesticides other than OPs and dithiocarbamates was associated with a significant decrease in lymphocytes by 13%, with no evidence of linear trend. No significant associations were found between chronic exposure to non-persistent pesticides and total leucocytes, neutrophils, monocytes, or eosinophils (Table 4). Neither was any significant association of hematological parameters with PPE use or current pesticide use found (data not shown).

Multivariate analysis stratified by gender revealed that long-term use of pesticides other than OPs and dithiocarbamates by men was associated with a significant decrease in total leukocytes by 13%, and frequency of pesticide mixing or applying for 5-39 days/year was associated with decreases in monocytes by 32% also in men (data not shown). Among women, it was observed that those using pesticides for 1-20 years, sampled in the high pesticide use season, and mixing or applying pesticides for 1-10 years had reduced lymphocytes count.

Regarding hematological parameters associated with OC pesticides, eosinophils and monocytes were the parameters most affected by detection of OC pesticide residues in serum (Table 5). Thus, detection of γ-HCH and heptachlor, respectively, was associated with decreases in both monocytes and eosinophils by 13-24%. *Trans*-nonachlor, *o,p*'-DDD, *p,p*'-DDD, endosulfan I, endrin, and methoxychlor were also associated with significantly reduced number of eosinophils by 24-49%, while *p,p*'-DDT, *o,p*'-DDE, and *p,p*'-DDE were related with 9-16% reduction in monocyte count. Additionally, α-HCH showed small but significant association with lower number of total leukocytes, neutrophils, and lymphocytes; aldrin was associated with reduction in lymphocytes by 21%; and γ-chlordane was inversely associated with hemoglobin level, *i.e.* subjects with detectable levels of γ-chlordane had hemoglobin levels averaging 0.40 g/dL lower (95%CI: -0.77 to -0.03) than those with detectable levels. Results of multivariate models did not differ appreciably upon exclusion of the subject with a history of hematological disease (data not shown).

Table 1. Sociodemographic characteristics, lifestyle factors, and hematological disease history of study population.

	N (%)		
	Total	**Men**	**Women**
	N = 275	**N = 155**	**N = 120**
Age (years)			
18-30	77 (28.0)	48 (31.0)	29 (24.2)
31-45	67 (24.0)	33 (21.3)	34 (28.3)
46-60	98 (36.0)	54 (34.8)	44 (36.7)
> 60	33 (12.0)	20 (12.9)	13 (10.8)
Years of education			
< 8	166 (60.0)	85 (54.8)	81 (67.5)
9-11	79 (29.0)	49 (31.6)	30 (25.0)
≥ 12	30 (11.0)	21 (13.5)	9 (7.5)
Marital status			
Married	197 (71.6)	98 (63.2)	99 (82.5)
Single, divorced or widowed	78 (28.4)	57 (36.8)	21 (17.5)
Family income (x1.000 Brazilian reais per year)			
≤ 10	39 (14.2)	18 (11.6)	21 (17.5)
11-20	77 (28.0)	40 (25.8)	37 (30.8)
21-50	96 (34.9)	59 (38.1)	37 (30.8)
> 50	63 (22.9)	38 (24.5)	25 (20.8)
Occupation			
Farmer	239 (86.9)	142 (91.6)	97 (80.8)
Non farmer	36 (13.1)	13 (8.4)	23 (19.2)
Place of birth			
Farroupilha	215 (78.2)	137 (88.4)	78 (65.0)
Other town	60 (21.8)	18 (11.6)	42 (35.0)
Cigarette smoking			
Non-smoker	226 (82.2)	112 (72.3)	114 (95.0)
Ex-smoker	34 (12.4)	31 (20.0)	3 (2.5)
Current smoker	15 (5.5)	12 (7.7)	3 (2.5)
Alcohol intake in the last 30 days			
No	95 (34.5)	36 (23.2)	59 (49.2)
Yes	180 (65.5)	119 (76.8)	61 (50.8)
Regular physical activity in the last 3 months			
No	188 (68.4)	103 (66.5)	85 (70.8)
Yes	87 (31.6)	52 (33.5)	35 (29.2)
Body mass index (BMI)			
Underweight or eutrophic (< 25 kg/m^2)	110 (40.0)	66 (42.6)	44 (36.7)
Overweight or obese (≥ 25 kg/m^2)	165 (60.0)	89 (57.5)	76 (63.3)
History of hematological disorder			
No	274 (99.6)	155 (100)	119 (99.2)
Yes	1 (0.4)	0 (0.0)	1 (0.8)
Family history of hematological disorder in first-degree relatives			
No	269 (97.8)	151 (97.4)	118 (98.3)
Yes	6 (2.2)	4 (2.6)	2 (1.7)

Table 2. Agricultural work-related characteristics and pesticide use.

	N (%)
Years of agricultural work	
< 1	29 (10.5)
1-10	40 (14.5)
11-25	60 (21.8)
26-50	114 (41.5)
> 50	32 (11.6)
Years mixing or applying pesticides	
< 1	62 (22.5)
1-10	62 (22.5)
> 10	151 (54.9)
Days per year mixing or applying pesticides	
< 5	73 (26.5)
5-39	50 (18.1)
40-59	51 (18.5)
≥ 60	101 (36.7)
Sampling season	
Low pesticide use season (April-August)	140 (50.9)
High pesticide use season (September-March)	135 (49.1)
Use full personal protective equipment (PPE)	238 (86.5)
Current use of pesticides	
All pesticides	132 (48.0)
Herbicides	118 (42.9)
Insecticides	40 (14.5)
Fungicides	63 (22.9)
OP insecticides	37 (13.5)
Dithiocarbamates	42 (15.3)
Other chemical classes[a]	127 (46.2)
Total number of pesticides currently used	
None	143 (52.0)
1	39 (14.2)
≥ 2	93 (33.8)
Total lifetime years of use	
All pesticides	
Never	70 (25.5)
1-20	86 (31.3)
> 20	119 (43.3)
Herbicides	
Never	73 (26.5)
1-20	95 (34.5)
> 20	107 (38.9)
Insecticides	
Never	106 (38.5)
1-20	110 (40.0)
> 20	59 (21.5)
Fungicides	
Never	72 (26.2)
1-20	87 (31.6)
> 20	116 (42.2)
OP insecticides	
Never	113 (41.1)
1-20	104 (37.8)
> 20	58 (21.1)
Dithiocarbamates	
Never	71 (25.8)
1-20	92 (33.5)
>20	112 (40.7)
Other chemical classes[a]	
Never	185 (67.3)
1-20	62 (22.5)
> 20	28 (10.2)

[a]Synthetic pyrethroids and carbamates included.

Discussion

In this cross-sectional study of farm workers and their families, mostly null associations were observed between long-term pesticide use and hematological parameters. However, findings may suggest that cumulative exposure to certain classes of pesticides could lead to depleted lymphocyte count. Regarding recent pesticide exposure, residents sampled in the high pesticide use season seem to experience higher levels of hemoglobin and erythrocytes. Results for serum levels of OC pesticides suggest that exposure to certain OC pesticides may lead to lower counts of white blood cells, particularly eosinophils.

Pesticide use and hematological parameters

Results from the current report regarding pesticide use and hematological parameters are consistent with previous studies which failed to reveal a consistent relationship between pesticide exposure and hematological parameters among farmers[21,27,28]. Thus, a Thai study with orchid farmers found no significant differences in hematological parameters between subjects highly exposed to pesticides and non-exposed subjects[21]. An Egyptian study only found increased leukocyte count in farmers compared to non-ex-

Table 3. Hematological parameters and OC pesticide concentrations in serum.

	% > LOD	P25	Median	P75	N (%) < ref[a]	N (%) > ref[b]
Hematological parameters						
Erythrocytes (m/mm^3)	-	4.54	4.91	5.22	16 (5.8)	3 (1.1)
Hemoglobin (g/dL)	-	13.80	14.80	15.70	4 (1.5)	3 (1.1)
Hematocrit (%)	-	41.48	44.30	46.40	7 (2.6)	0 (0.0)
RDW (%)	-	12.20	12.70	13.10	80 (29.1)	3 (1.1)
MCV (fL)	-	86.80	89.95	92.40	1 (0.4)	6 (2.2)
MCH (pg)	-	29.30	30.20	31.20	2 (0.7)	25 (9.1)
MCHC (g/dL)	-	32.98	33.60	34.23	1 (0.4)	21 (7.6)
Leukocytes (u/μL)	-	5,700	6,700	7,900	3 (1.1)	8 (2.9)
Neutrophils (u/μL)	-	2,750	3,418	4,158	0 (0.0)	6 (2.2)
Lymphocytes (u/μL)	-	1,993	2,397	2,842	2 (0.7)	0 (0.0)
Monocytes (u/μL)	-	371	456	560	0 (0.0)	1 (0.04)
Eosinophils (u/μL)	-	118	201	285	0 (0.0)	25 (9.1)
Basophils (u/μL)	-	0	0	0	0 (0.0)	0 (0.0)
OC pesticides (ng/g)						
α–HCH	30.9	<LOD	<LOD	10.10	-	-
β–HCH	41.3	<LOD	<LOD	29.48	-	-
γ–HCH	50.2	<LOD	3.71	12.36	-	-
HCB	28.0	<LOD	<LOD	11.53	-	-
Heptachlor	32.9	<LOD	<LOD	<LOD	-	-
Heptachlor epoxide A	1.8	<LOD	<LOD	21.07	-	-
Heptachlor epoxide B	5.5	<LOD	<LOD	<LOD	-	-
α–chlordane	0.0	-	-	-	-	-
γ–chlordane	10.3	<LOD	<LOD	<LOD	-	-
Trans-nonachlor	1.8	<LOD	<LOD	<LOD	-	-
o,p'-DDT	12.5	<LOD	<LOD	<LOD	-	-
p,p'-DDT	42.8	<LOD	<LOD	<LOD	-	-
o,p'-DDE	10.3	<LOD	<LOD	40.59	-	-
p,p'-DDE	39.5	<LOD	<LOD	<LOD	-	-
o,p'-DDD	3.7	<LOD	<LOD	30.27	-	-
p,p'-DDD	14.4	<LOD	<LOD	<LOD	-	-
Aldrin	2.6	<LOD	<LOD	<LOD	-	-
Endrin	30.6	<LOD	<LOD	<LOD	-	-
Dieldrin	6.6	<LOD	<LOD	<LOD	-	-
Endosulfan I	22.9	<LOD	<LOD	<LOD	-	-
Endosulfan II	2.2	<LOD	<LOD	<LOD	-	-
Methoxychlor	4.4	<LOD	<LOD	<LOD	-	-
Mirex	1.5	<LOD	<LOD	<LOD	-	-
Pentachloroanisole	26.6	<LOD	<LOD	0.71	-	-

LOD: limit of detection; P25, P75: 25th and 75th percentiles. RBC: Red blood cells; RDW: Red cell distribution width; MCV: Mean corpuscular volume; MCH: Mean corpuscular hemoglobin; MCHC: Mean corpuscular hemoglobin concentration. [a]Lower reference limit; [b]Upper reference limit.

posed subjects[27], while an Indian study observed decreased leukocytes count in pesticide-exposed workers vs. unexposed individuals but no significant differences in erythrocytes, hemoglobin, and others hematological parameters[28]. On the other hand, the observed inverse association with lymphocyte counts is in line with an Indian study showing lower counts of lymphocytes in a group of sprayers working in mango plantations relative to unexposed subjects[18]. Nonetheless, they also found altered counts of total leukocytes, monocytes, neutrophils, and erythrocytes, and

Table 4. Adjusted[a] regression coefficients (95% confidence intervals) for change in hematological parameters associated with variables related to the use of pesticides.

Exposure variables	Erythrocytes	Hemoglobin	Leukocytes[b]	Neutrophils[b]	Lymphocytes[b]	Monocytes[b]	Eosinophils[b]
Farmer (ref = non-farmer)	-0.08 (-0.22; 0.07)	-0.17 (-0.54; 0.18)	1.07 (0.96; 1.20)	1.12 (0.99; 1.26)	1.01 (0.91; 1.12)	1.04 (0.91; 1.19)	0.99 (0.76; 1.28)
High pesticide use season (ref = low use season)	0.10 (0.01; 0.19)	0.22 (0.00; 0.45)	0.98 (0.91; 1.05)	1.01 (0.93; 1.08)	1.02 (0.95; 1.08)	0.92 (0.85; 1.01)	0.98 (0.84; 1.16)
Years of agricultural work (ref = < 1)							
1-10	0.09 (-0.12; 0.29)	0.28 (-0.21; 0.77)	0.94 (0.81; 1.09)	0.91 (0.78; 1.08)	0.98 (0.85; 1.13)	0.97 (0.81; 1.15)	0.99 (0.70; 1.43)
11-25	0.07 (-0.11; 0.26)	0.28 (-0.16; 0.72)	0.99 (0.86; 1.12)	1.03 (0.88; 1.19)	0.95 (0.84; 1.08)	0.94 (0.80; 1.10)	0.90 (0.66; 1.25)
26-50	0.14 (-0.07; 0.35)	0.41 (-0.10; 0.92)	0.96 (0.83; 1.12)	0.94 (0.79; 1.12)	1.01 (0.86; 1.16)	1.07 (0.88; 1.27)	0.88 (0.62; 1.30)
> 50	0.15 (-0.46; 0.46)	0.43 (-0.32; 1.18)	0.88 (0.70; 1.10)	0.94 (0.74; 1.21)	0.97 (0.78; 1.20)	1.10 (0.84; 1.46)	0.62 (0.36; 1.06)
p for trend	0.22	0.13	0.55	0.69	0.86	0.59	0.33
Years mixing/applying pesticides (ref = < 1)							
1-10	0.03 (-0.12; 0.19)	0.05 (-0.33; 0.42)	0.94 (0.84; 1.05)	0.97 (0.86; 1.10)	0.92 (0.84; 1.03)	0.98 (0.85; 1.12)	0.82 (0.65; 1.13)
>10	0.10 (-0.05; 0.25)	0.32 (-0.04; 0.68)	1.01 (0.89; 1.12)	1.06 (0.94; 0.19)	0.99 (0.89; 1.09)	1.06 (0.92; 1.20)	0.93 (0.72; 1.20)
p for trend	0.16	0.92	0.85	0.25	0.89	0.36	0.65
Days/year mixing/applying pesticides (ref = < 5)							
5-39	0.15 (-0.04; 0.30)	0.37 (0.02; 0.73)	1.01 (0.90; 1.13)	1.11 (0.99; 1.26)	0.98 (0.89; 1.08)	1.10 (0.96; 1.24)	1.07 (0.80; 1.35)
40-59	0.09 (-0.07; 0.26)	0.21 (-0.19; 0.61)	1.01 (0.90; 1.16)	1.05 (0.91; 1.20)	1.02 (0.90; 1.13)	1.02 (0.88; 1.19)	0.97 (0.74; 1.32)
≥ 60	0.03 (-0.12; 0.18)	0.17 (-0.19; 0.53)	0.99 (0.88; 1.11)	0.99 (0.89; 1.14)	1.01 (0.90; 1.11)	1.02 (0.89; 1.16)	0.89 (0.71; 1.21)
p for trend	0.86	0.67	0.75	0.55	0.78	0.12	0.39
No. of pesticides currently used (ref= none)							
1	-0.08 (-0.22; 0.07)	-0.19 (-0.54; 0.16)	1.00 (0.89; 1.11)	0.94 (0.84; 1.05)	1.06 (0.95; 1.17)	1.05 (0.92; 1.20)	0.98 (0.76; 1.27)
≥ 2	-0.01 (-0.12; 0.11)	-0.11 (-0.39; 0.16)	1.03 (0.95; 1.13)	1.02 (0.92; 1.12)	1.01 (0.93; 1.09)	1.05 (0.94; 1.16)	0.99 (0.83; 1.24)
p for trend	0.89	0.41	0.38	0.71	0.80	0.38	0.90
Lifetime years of pesticide use (ref= none)							
All pesticides							
1-20	-0.10 (-0.23; 0.03)	-0.28 (-0.60; 0.03)	1.01 (0.12; 1.12)	0.95 (0.85; 1.05)	1.06 (0.97; 1.17)	0.92 (0.82; 1.04)	1.01 (0.80; 1.27)
> 20	-0.01 (-0.16; 0.15)	-0.06 (-0.44; 0.33)	1.05 (0.93; 1.18)	1.01 (0.88; 1.16)	1.04 (0.93; 1.16)	0.97 (0.84; 1.11)	0.94 (0.71; 1.24)
p-trend	0.80	0.63	0.45	0.98	0.40	0.59	0.68

it continues

Table 4. Adjusted[a] regression coefficients (95% confidence intervals) for change in hematological parameters associated with variables related to the use of pesticides.

Exposure variables	Erythrocytes	Hemoglobin	Leukocytes[b]	Neutrophils[b]	Lymphocytes[b]	Monocytes[b]	Eosinophils[b]
Fungicides							
1-20	0.11 (-0.03; 0.24)	0.28 (-0.03; 0.60)	0.99 (0.90; 1.09)	1.05 (0.95; 1.17)	0.94 (0.86; 1.03)	1.06 (0.94; 1.18)	1.02 (0.81; 1.28)
> 20	0.03 (-0.13; 0.19)	0.07 (-0.32; 0.45)	0.95 (0.85; 1.08)	1.01 (0.88; 1.14)	0.96 (0.86; 1.07)	1.01 (0.88; 1.16)	1.08 (0.82; 1.43)
p-trend	0.57	0.60	0.49	0.84	0.43	0.82	0.57
Insecticides							
1-20	0.11 (-0.01; 0.22)	0.24 (-0.03; 0.51)	1.04 (0.96; 1.14)	1.07 (0.98; 1.17)	0.96 (0.89; 1.05)	1.07 (0.97; 1.18)	0.97 (0.80; 1.19)
> 20	0.07 (-0.08; 0.22)	0.06 (-0.29; 0.42)	1.01 (0.88; 1.11)	1.01 (0.88; 1.12)	0.91 (0.83; 1.01)	0.99 (0.87; 1.12)	0.99 (0.76; 1.28)
p-trend	0.21	0.49	0.74	0.41	0.08	0.84	0.89
Herbicides							
1-20	0.06 (-0.07; 0.19)	0.17 (-0.15; 0.48)	0.96 (0.87; 1.06)	1.03 (0.93; 1.15)	0.96 (0.88; 1.05)	1.01 (0.90; 1.14)	0.94 (0.75; 1.19)
> 20	0.03 (-0.12; 0.19)	-0.03 (-0.41; 0.35)	0.94 (0.88; 1.07)	0.99 (0.88; 1.13)	0.99 (0.90; 1.10)	1.01 (0.88; 1.16)	0.93 (0.82; 1.42)
p-trend	0.66	0.99	0.48	0.98	0.85	0.85	0.63
OP insecticides							
1-20	0.08 (-0.04; 0.19)	0.19 (-0.08; 0.46)	1.04 (0.95; 1.12)	1.07 (0.98; 1.17)	0.94 (0.89; 1.04)	1.05 (0.95; 1.16)	0.95 (0.78; 1.19)
> 20	0.01 (-0.13; 0.16)	-0.07 (-0.42; 0.28)	0.99 (0.89; 1.10)	1.01 (0.89; 1.13)	0.90 (0.83; 1.01)	0.98 (0.86; 1.11)	0.92 (0.71; 1.16)
p-trend	0.61	0.99	0.90	0.63	0.06	0.99	0.51
Dithiocarbamates							
1-20	0.09 (-0.04; 0.22)	0.28 (-0.03; 0.60)	0.90 (0.89; 1.09)	1.05 (0.94; 1.16)	0.94 (0.86; 1.03)	1.08 (0.94; 1.21)	0.99 (0.79; 1.25)
> 20	0.01 (-0.16; 0.16)	0.14 (-0.25; 0.52)	0.95 (0.84; 1.07)	0.99 (0.88; 1.14)	0.96 (0.85; 1.06)	1.02 (0.88; 1.18)	1.01 (0.75; 1.32)
p-trend	0.85	0.40	0.44	0.95	0.38	0.65	0.99
Other chemical classes							
1-20	0.03 (-0.09; 0.15)	0.25 (-0.03; 0.53)	1.01 (0.93; 1.10)	0.99 (0.89; 1.07)	1.03 (0.95; 1.11)	1.04 (0.93; 1.15)	1.01 (0.82; 1.24)
> 20	-0.11 (-0.27; 0.06)	-0.14 (-0.54; 0.25)	0.88 (0.79; 1.01)	0.88 (0.77; 1.01)	0.87 (0.78; 0.97)	0.90 (0.78; 1.05)	0.99 (0.74; 1.32)
p-trend	0.43	0.81	0.20	0.13	0.12	0.50	0.98

[a]Models adjusted for sex, age, BMI, smoking habit, alcohol consumption and categorized *p,p'*-DDE serum levels. [b]Transformed in natural logarithm: regression coefficients are expressed in percent change (an estimate of 1 equals 100%); ref: Reference category.

Table 5. Adjusted[a] regression coefficients (95% confidence intervals) for change in hematological parameters associated with detectable OC pesticides serum levels.

Levels > LOD (ref = undetected)	Erythrocytes	Hemoglobin	Leukocytes[b]	Neutrophils[b]	Lymphocytes[b]	Monocytes[b]	Eosinophils[b]
α–HCH	0.06 (-0.09; 0.22)	0.03 (-0.07; 0.38)	0.97 (0.94; 0.99)	0.97 (0.94; 0.99)	0.97 (0.94; 0.99)	0.96 (0.93; 1.02)	0.94 (0.88; 1.01)
β–HCH	-0.04 (-0.20; 0.12)	0.11 (-0.13; 0.34)	1.03 (0.96; 1.11)	1.03 (0.96; 1.12)	1.01 (0.95; 1.08)	0.96 (0.87; 1.05)	1.09 (0.93; 1.31)
γ–HCH	0.02 (-0.14; 0.18)	0.04 (-0.19; 0.26)	1.01 (0.94; 1.07)	0.99 (0.92; 1.07)	1.03 (0.96; 1.09)	0.87 (0.81; 0.95)	0.81 (0.69; 0.96)
HCB	0.17 (-0.02; 0.32)	0.28 (-0.03; 0.53)	1.06 (0.98; 1.14)	1.06 (0.98; 1.15)	1.06 (0.98; 1.14)	0.98 (0.88; 1.07)	0.90 (0.75; 1.08)
Heptachlor	-0.04 (-0.19; 0.11)	-0.01 (-0.25; 0.23)	0.96 (0.89; 1.03)	0.96 (0.88; 1.03)	0.83 (0.91; 1.04)	0.83 (0.76; 0.91)	0.76 (0.63; 0.89)
Heptachlor epoxide A	-0.24 (-0.64; 0.16)	-0.43 (-1.26; 0.41)	0.83 (0.64; 1.07)	0.83 (0.64; 1.07)	0.84 (0.66; 1.06)	0.93 (0.68; 1.27)	1.15 (0.63; 2.11)
Heptachlor epoxide B	-0.21 (-0.47; 0.06)	-0.32 (-0.82; 0.18)	0.96 (0.83; 1.13)	0.96 (0.83; 1.13)	1.01 (0.87; 1.16)	0.91 (0.76; 1.09)	0.80 (0.56; 1.16)
γ–chlordane	-0.13 (-0.32; 0.06)	-0.40 (-0.77; -0.03)	0.96 (0.85; 1.07)	0.96 (0.85; 1.07)	0.95 (0.88; 1.09)	0.95 (0.83; 1.08)	0.76 (0.59; 1.01)
Trans-nonachlor	-0.13 (-0.60; 0.34)	-0.75 (-1.59; 0.08)	1.08 (0.84; 1.40)	1.08 (0.84; 1.40)	1.23 (0.94; 1.52)	1.23 (0.91; 1.68)	0.51 (0.28; 0.94)
o,p'-DDT	-0.05 (-0.23; 0.13)	-0.04 (-0.38; 0.30)	0.95 (0.86; 1.06)	0.95 (0.86; 1.06)	0.97 (0.88; 1.07)	0.97 (0.85; 1.09)	0.94 (0.73; 1.21)
p,p'-DDT	0.11 (-0.05; 0.26)	-0.01 (-0.24; 0.23)	0.97 (0.90; 1.05)	0.97 (0.91; 1.05)	0.95 (0.88; 1.01)	0.91 (0.84; 0.99)	0.93 (0.79; 1.10)
o,p'-DDE	-0.19 (-0.38; 0.01)	-0.12 (-0.49; 0.25)	0.93 (0.84; 1.05)	0.93 (0.84; 1.04)	0.94 (0.84; 1.04)	0.84 (0.74; 0.97)	0.77 (0.59; 1.01)
p,p'-DDE	-0.09 (-0.25; 0.07)	-0.09 (-0.33; 0.15)	0.97 (0.89; 1.05)	0.97 (0.90; 1.05)	0.95 (0.88; 1.02)	0.91 (0.84; 0.99)	0.85 (0.72; 1.02)
o,p'-DDD	-0.08 (-0.39; 0.24)	0.07 (-0.53; 0.67)	0.89 (0.75; 1.08)	0.89 (0.75; 1.08)	0.85 (0.72; 1.01)	0.83 (0.66; 1.03)	0.63 (0.41; 0.97)
p,p'-DDD	-0.10 (-0.28; 0.08)	-0.18 (-0.50; 0.15)	0.94 (0.86; 1.05)	0.97 (0.90; 1.05)	1.01 (0.91; 1.09)	0.93 (0.83; 1.05)	0.70 (0.55; 0.88)
Aldrin	0.21 (-0.20; 0.63)	0.22 (-0.49; 0.94)	0.91 (0.73; 1.14)	0.91 (0.73; 1.14)	0.79 (0.65; 0.97)	0.85 (0.66; 1.12)	0.87 (0.52; 1.46)
Endrin	-0.04 (-0.19; 0.11)	-0.06 (-0.30; 0.19)	0.97 (0.91; 1.05)	0.97 (0.89; 1.05)	1.01 (0.93; 1.07)	0.92 (0.84; 1.02)	0.73 (0.61; 0.87)
Dieldrin	0.04 (-0.18; 0.26)	0.07 (-0.38; 0.53)	0.96 (0.84; 1.10)	0.96 (0.84; 1.10)	0.99 (0.87; 1.13)	0.99 (0.84; 1.17)	0.80 (0.58; 1.11)
Endosulfan I	-0.13 (-0.28; 0.02)	-0.11 (-0.38; 0.16)	1.01 (0.93; 1.09)	1.01 (0.92; 1.09)	1.05 (0.97; 1.15)	0.99 (0.89; 1.09)	0.76 (0.63; 0.91)
Endosulfan II	-0.16 (-0.63; 0.31)	-0.39 (-1.15; 0.38)	0.98 (0.77; 1.24)	0.99 (0.77; 1.26)	0.94 (0.76; 1.18)	1.04 (0.79; 1.39)	1.30 (0.75; 2.27)
Methoxychlor	-0.18 (-0.49; 0.14)	0.15 (-0.09; 0.38)	0.91 (0.77; 1.09)	0.91 (0.77; 1.08)	0.91 (0.77; 1.06)	0.97 (0.79; 1.20)	0.66 (0.45; 0.98)
Mirex	-0.25 (-0.83; 0.33)	-0.13 (-1.06; 0.81)	0.99 (0.74; 1.38)	0.99 (0.73; 1.32)	0.91 (0.69; 1.19)	1.05 (0.74; 1.48)	1.07 (0.54; 2.11)
Pentachloroanisole	0.04 (-0.11; 0.19)	0.06 (-0.20; 0.31)	1.03 (0.95; 1.11)	1.03 (0.95; 1.10)	1.01 (0.94; 1.08)	0.95 (0.87; 1.05)	0.87 (0.72; 1.04)

LOD: Limit of detection; ref= Reference category. [a]Transformed in natural logarithm: regression coefficients are expressed in percent change (an estimate of 1 equals 100%). [b]Models adjusted for sex, age, BMI, smoking habit, and alcohol consumption.

reduced hemoglobin, MCV, and MCHC to be associated with pesticide exposure. Additionally, our results regarding current use of pesticides are in partial agreement with a Chinese study showing decreases in monocytes, hemoglobin, and platelets after pesticide exposure, suggesting that pesticides may exert hematotoxic effects due to acute exposures[29]. However, chronic exposure was associated with increased white blood cells count in the Chinese study[29].

By contrast, in a cross-sectional study among cutflowers in Philippines lifetime years of pesticide use and number of hours of pesticide exposure were associated with abnormal MCV and hemoglobin levels[16]. Present data are also inconsistent with an Indian study on OP insecticides sprayers showing lower erythrocyte count, hemoglobin, and hematocrit as compared to unexposed subjects[30]. In addition, decreases in red blood cells indices, but not in leukocytes, were observed among Palestinian farm workers after spraying OP insecticides compared to values before the spraying operations[20], and among pesticide applicators in North America compared to a control group[15].

Overall, human data suggest that both acute and chronic exposure to non-persistent pesticides may induce hematological disorders. Nevertheless, most of the above studies relied on small sample size, used convenience samples, and did not control for confounding. Despite these considerations, it remains possible that equivocal findings across studies, including present data, result from heterogeneity of study designs, variation in exposure doses, pattern of pesticide use, and type of chemicals used by agricultural workers.

Reduced number of total leukocytes and lymphocytes indicates lower ability of the immune system. The observed inverse associations of cumulative exposure to other chemical classes (including carbamates and pyrethroids) with lymphocytes (and total leukocytes in men) could be the result of disruptive action of pesticides in leukopoiesis affecting the viability of the white blood cells. However, the exact mechanisms involved in the hematotoxic action of many modern pesticides remain elusive. Despite this, our results appear to be supported by limited experimental data indicating toxic effects of specific pesticides on bone marrow. For instance, insecticide cypermethrin (synthetic pyrethroid) inhibited erythroid and granulocyte-macrophage progenitors *in vitro*[31], while low doses of the OP mevinphos caused destruction of progenitors in human and rat hematopoietic progenitor cells[32]. There is also experimental evidence that insecticides malathion (OP) and carbaryl (carbamate) may induce anemia, immunosuppression, and altered number of leukocytes and platelets *in vivo*[33,34]. It is noteworthy to mention that previous studies have reported elevated risks for lymphatic and hematopoietic neoplasms, including chronic lymphoid leukemia, Hodgkin and non-Hodgkin lymphoma, and multiple myeloma, among farmers[35,36] and workers occupationally exposed to OP insecticides[37]. This epidemiological association points out that certain pesticides may disrupt normal hematopoiesis and is supported by experimental evidence of genotoxicity on human peripheral blood lymphocytes induced by modern non-persistent pesticides[19,38,39].

There was no evidence for an association between cumulative pesticide exposure and red blood cell indices. Otherwise, a statistically significant positive association of recent pesticide exposure with erythrocytes and hemoglobin was observed. In line with this, a recent study in Spain also showed increased erythrocytes, hemoglobin, leukocytes, platelets, and hematocrit in greenhouse workers in the high (*vs.* low) pesticide exposure season[17]. Conversely, Hassanin et al.[40] found a significant decrease in red blood cells but not hemoglobin levels in Egyptians male farmers compared to a control group. It should be acknowledged that our result could have occurred by chance, reflecting the fact that multiple comparisons were made. This possibility may even be more likely given the lack of significant association with cumulative exposure for red blood cells and recent exposure for white blood cells. In this regard, there is no plausible reason to suggest that there is a causal relationship between recent exposure to pesticides and increase in erythrocytes and hemoglobin.

OC pesticides in serum and hematological parameters

Several case reports and case series have suggested an association of exposure to lindane (γ-HCH), DDT, heptachlor, and chlordane with aplastic anemia and other blood disorders[12-14,37]. Two of the case-control studies mentioned above also reported higher odds of aplastic anemia among subjects occupationally exposed to OC pesticides[10,11]. In addition, exposures to DDT, lindane, chlordane, transnonachlor, and heptachlor, among other OC pesticides, have been associated with increased risks of hematological malignan-

cies, including non-Hodgkin lymphoma and leukemia[41].

In the present study, α-HCH, lindane, and DDT metabolites were associated with lower number of leukocytes. This finding is in line with a previous Brazilian study that found an inverse association between serum levels of *p,p*'-DDE and leukocyte and neutrophil counts among women residing in a rural area heavily polluted with OC pesticides[8]. In addition, our findings are in partial agreement with an Indian study showing higher levels of HCH isomers and *p,p*'-DDE in serum of children with aplastic anemia relative to controls[7].

Stromal fat is an important element in the support of hematopoiesis, and thus bioaccumulation of OC pesticides in adipose tissue of bone marrow may affect lympho-hematopoietic function. In this regard, lindane is the most well-documented hematotoxic OC compound, which has been shown to exert cytotoxic effects in human hematopoietic progenitors[32]. *In vitro* studies have also demonstrated that DDT induces apoptosis in human peripheral blood mononuclear cells (*i.e.* lymphocytes and monocytes) through oxidative stress mechanisms[42]. These data support the inverse association of some OC pesticides (*i.e.* α-HCH, γ-HCH, aldrin, heptachlor, *p,p*'-DDT, *o,p*'-DDE, and *p,p*'-DDE) with lymphocytes and monocytes described here, while associations with reduction in eosinophils, not accompanied by decreased leukocytes, would suggest that OC pesticides may lead to suppression of eosinophils production. As leukopenia, eosinopenia may increase the risk of infections.

Among the OC pesticides analyzed in this study, only chlordane associated with reduced hemoglobin. A possible mechanism to explain this finding could be the impairment of iron utility in erythrocytes induced by certain OC pesticides such as DDT[6], although we cannot rule out the possibility that this result may have occurred by chance, as discussed above. It is also important to note that given that concentrations of OC pesticides were significantly correlated (and thus a regression model including all exposures would present the problem of multicollinearity), potential mechanisms of action explaining the effect of any individual OC pesticide on hematological endpoints are unclear at this time.

Limitations and strong points

This study presents some limitations. Firstly, we cannot exclude the possibility that bias due to misclassification of self-reported use of pesticides have distorted the observed associations. Nonetheless, it's unlikely that participants recall exposures differently depending on their hematological profile, so that exposure misclassification would have resulted in an underestimate of the true associations rather than an overestimate of the effects. Secondly, we cannot disregard the possibility that the observed associations are due to chance, given that multiple comparisons adjustment was not conducted. Third, our analysis was not based on individual pesticides, but instead of this, grouping pesticides according to functional and chemical type allowed us to assess associations with current/recent and cumulative pesticide use and examine exposure-response relationships for pesticides that may have similar modes of action. Fourth, farmers are typically exposed to multiple pesticides during a lifetime, and several pesticides are frequently used at the same time or during the same growing season. For this reason, we cannot rule out the possibility that some of the associations may have resulted from interaction between pesticides or unmeasured confounding by co-exposure to multiple pesticides. In addition, information on history of immunological disorders and infectious or allergic diseases that impact the hematological parameters, as well as data on nutritional status, was not available for the study population.

Despite study limitations, this is the first epidemiological study that has been performed regarding occupational exposure to pesticides and hematological alterations in Brazil, which is the largest consumer of pesticides in the world and where many of the pesticides used have been already banned elsewhere. The study population is representative of the target population, that is, the agricultural population residing in the rural area of Farroupilha. Additionally, a large number of OC pesticides were measured in serum and a comprehensive questionnaire for assessment of recent and past exposure to contemporary-use pesticides was used.

Conclusions

In summary, this study provides little evidence of a relationship between pesticide use and hematological parameters among farm workers and their families. However, findings may suggest that chronic exposure to OC pesticides and certain non-persistent pesticides could lead to changes in the number of lymphocytes, while de-

tectable levels of various OC pesticides in serum were associated with a reduction in the number of different white blood cells. Although cautious interpretation is warranted in light of possible confounding due to unmeasured confounding and multiple comparisons, measures should be taken to minimize occupational exposure to pesticides among small-scale agricultural workers in Brazil.

Collaborations

C Piccoli has contributed to the acquisition, analysis and interpretation of data, and drafting the work. C Freire has contributed to analysis and interpretation of data, and drafting the work. C Cremonese has contributed to the conception of the study, and acquisition and analysis of data. R Koifman and S Koifman have contributed to the conception and design of the study, and interpretation of data. All of the authors revised the work critically and approved the submitted version of the manuscript.

References

1. Androutsopoulos VP, Hernandez AF, Liesivuori J, Tsatsakis AM. A mechanistic overview of health associated effects of low levels of organochlorine and organophosphorous pesticides. *Toxicology* 2013; 307:89-94.
2. World Health Organization (WHO). *Exposure to highly hazardous pesticides: a major public health concern*. Geneva: WHO; 2010.
3. Chatterjee S, Basak P, Chaklader M, Das P, Pereira JA, Chaudhuri S, Law S. Pesticide induced marrow toxicity and effects on marrow cell population and on hematopoietic stroma. *Exp Toxicol Pathol* 2013; 65(3):287-295.
4. Chatterjee S, Basak P, Chaklader M, Das P, Pereira JA, Chaudhuri S, Law S. Pesticide induced alterations in marrow physiology and depletion of stem and stromal progenitor population: an experimental model to study the toxic effects of pesticide. *Environ Toxicol* 2014; 29(1):84-97.
5. Chattopadhyay S, Chatterjee R, Law S. Noncanonical Wnt5a-Ca2+ -NFAT signaling axis in pesticide induced bone marrow aplasia mouse model: A study to explore the novel mechanism of pesticide toxicity. *Environ Toxicol* 2015; 31(10):1163-1175.
6. Tomita M, Yoshida T, Fukumori J, Yamaguchi S, Kojima S, Fukuyama T, Ohnuma-Koyama A, Takahashi N, Takeuchi-Kashimoto Y, Kuwahara M, Nakashima N, Ohtsuka R, Takeda M, Kosaka T, Harada T. p,p'-DDT induces microcytic anemia in rats. *J Toxicol Sci* 2003; 38(5):775-782.
7. Ahamed M, Anand M, Kumar A, Siddiqui MKJ. Childhood aplastic anaemia in Lucknow, India: Incidence, organochlorines in the blood and review of case reports following exposure to pesticides. *Clinic Biochem* 2006; 9(7):762-766.
8. Freire C, Koifman RJ, Koifman S. Hematological and hepatic alterations in Brazilian population heavily exposed toorganochlorine pesticides. *J Toxicol Environ Health* 2015; 78(8):534-548.
9. Kaufman DW, Issaragrisil S, Anderson T, Chansung K, Thamprasit T, Sirijirachai J, Piankijagum A, Porapakkham Y, Vannasaeng S, Leaverton PE, Shapiro S, Young NS. Use of household pesticides and the risk of aplastic anaemia in Thailand. *Int J Epidemiol* 1997; 26(3):643-650.
10. Muir KR, Chilvers CE, Harriss C, Coulson L, Grainge M, Darbyshire P, Geary C, Hows J, Marsh J, Rutherford T, Taylor M, Gordon-Smith EC. The role of occupational and environmental exposures in the aetiology of acquired severe aplastic anaemia: a case control investigation. *Br J Haematol* 2003; 123(5):906-914.
11. Prihartono N, Kriebel D, Woskie S, Thetkhathuek A, Sripaung N, Padungtod C, Kaufman D. Risk of aplastic anemia and pesticide and other chemical exposures. *Asia-Pacific J Pub Health* 2011; 23(3):369-377.
12. Rauch AE, Kowalskey SF, Lesar TS, Sauerbier GA, Burkart PT, Scharfman WB. Lindane (Kwell)-induced aplastic anemia. *Arch Intern Med* 1990; 150(11):2393-2395
13. Rugman FP, Cosstick R. Aplastic anaemia associated with organochlorine pesticide: case reports and review of evidence. *J Clin Pathol* 1990; 43(2):98-101.
14. Srivastava AK, Gupta BN, Bihari V, Mathur N, Pangtey BS, Bharti RS. Chronic effects of hexachlorocyclohexane exposure: clinical, hematological and electrocardiographic studies. *Vet Hum Toxicol* 1995; 37(4):302-305.
15. Casale GP, Scott DM, Anderson JR, Vitzthum EF, Gold RE. A preliminary study of immunologic and hematologic profiles of peripheral blood from Nebraska farmers who apply pesticides to their fields. *J Toxicol Clin Toxicol* 1998; 36(3):183-194.
16. Del Prado-Lu. Pesticide exposure, risk factors and health problems among cutflower farmers: a cross sectional study. *J Occup Med Toxicol* 2007; 2(9):1-8.
17. García-García CR, Parrón T, Requena M, Alarcón R, Tsatsakis AM, Hernández AF. Occupational pesticide exposure and adverse health effects at the clinical, hematological and biochemical level. *Life Sci* 2016; 145:274-283.
18. Fareed M, Pathak MK, Bihari V, Kamal R, Srivastava AK, Kesavachandran CN. Adverse respiratory health and hematological alterations among agricultural workers occupationally exposed to organophosphate pesticides: a cross-sectional study in North India. Plos One. 2013; 8(7):1-10.
19. Jamil K, Shaik AP, Mahboob M, Krishna D. Effect of organophosphorus and organochlorine pesticides (Monocrotophos, Chlorpyriphos, Dimethoate and Endosulfan) on human lymphocyte cultures in vitro. *Drug Chem Toxicol* 2017; 27(2):133-144.
20. Mourad TA. Adverse impact of insecticides on health of palestinian farm workers in the Gaza Strip: a hematologic biomarker study. *Int J Occup Environ Health* 2005; 11(2):144-146.
21. Arronvilairat S, Kespichayawattana W, Sornprachum T, Chaisuriya P, Siwadune T, Ratanabanangkoon K. Effect of Pesticide Exposure on Immunological, Hematologicaland Biochemical Parameters in Thai Orchid Farmers - A Cross-Sectional Study. *Int J Environ Res Public Health* 2015; 12(6):5846-5861.
22. Cremonese C, Piccoli C, Pasqualotto F, Clapauch R, Koifman RJ, Koifman S, Freire C. Occupational exposure to pesticides, reproductive hormone levels and sperm quality in young Brazilian men. *Reprod Toxicol* 2017; 67:174-185.
23. Piccoli C, Cremonese C, Koifman RJ, Koifman S, Freire C. Pesticide exposure and thyroid function in an agricultural population in Brazil. *Environ Res* 2016; 151:389-398.
24. World Health Organization (WHO). Laboratory manual for the examination and processing of human semen. 2010. [cited 2015 Jun 10]. Available at: http://apps.who.int/iris/bitstream/10665/44261/1/9789241547789_eng.pdf?ua=1
25. Sarcinelli PN, Pereira AC, Mesquita SA, Oliveira-Silva JJ, Meyer A, Menezes MA, Alves SR, Mattos RC, Moreira JC, Wolff M. Dietary and reproductive determinants of plasma organochlorine levels in pregnant women in Rio de Janeiro. *Environ Res* 2003; 91(3):143-150.

26. Phillips DL, Pirkle JL, Burse VW, Bernet Jr. JT, Henderson LO, Needham LL. Chlorinated hydrocarbon levels in human serum: effects of fasting and feeding. *Arch Environ Contam Toxicol* 1989; 18(4):495-500.
27. Arafa A, Afify M, Samy N. Evaluation of adverse health effects of pesticides exposure [Biochemical and Hormonal] among Egyptian Farmers. *J Applied Sci Res* 2013; 9(7):4404-4409.
28. Gaikwad AS, Karunamoorthy P, Kondhalkar SJ, Ambikapathy M, Beerappa R. Assessment of hematological, biochemical effectsand genotoxicity among pesticide sprayers in grape garden. *J Occup Med and Tox* 2015; 10(11):1-6.
29. Hu R, Huang Y, Huang J, Li Y, Zhang C, Yin Y, Chen Z, Jin J, Cai J, Cui F. Long- and Short-Term Health Effects of Pesticide Exposure: A Cohort Study from China. *Plos One* 2015; 10(6):e0128766.
30. Rastogi SK, Singh VK, Kesavachandran C, Siddiqui MKJ, Mathur N, Bharti RS. Monitoring of plasma butyrylcholinesterase activity and haematological parameters in pesticide sprayers. *Indian J Occup Environ Med* 2008; 12(1):29-32.
31. Mandarapu R, Prakhya BM. In vitro myelotoxic effects of cypermethrin and mancozeb on human hematopoietic progenitor cells. *J Immunotoxicol* 2015; 12(1):48-55.
32. Parent-Massin D, Thouvenot D. In vitro study of pesticide hematotoxicity in human and rat progenitor. *J Pharmacol Toxicol Met* 1993; 30(4):203-207.
33. Lasram MM, Bini Douib I, Bouzid K, Annabi A, El Elj N, Dhouib H, El Fazaa S, Abdelmoula J, Gharbi N. Effects of N-acetyl-l-cysteine, in vivo, against pathological changes induced by malathion. *Toxicol Mech Methods* 2014; 24(4):294-306.
34. Ramadan G, El-Beih NM, Ahmed RS. Aged garlic extract ameliorates immunotoxicity, hematotoxicity and impaired burn-healing in malathion- and carbaryl-treated male albino rats. *Environ Toxicol* 2016; 32(3):789-798.
35. Blair A, Zahm SH, Pearce NE, Heineman EF, Fraumeni JF. Clues to cancer etiology from studies of farmers. *Scand J Work Environ Health* 1992; 18(4):209-215.
36. Merhi M, Raynal H, Cahuzac E, Vinson F, Cravedi JP, Gamet-Payrastre L. Occupational exposure to pesticides and risk of hematopoietic cancers: meta-analysis of case-control studies. *Cancer Causes Control* 2007; 18(10):1209-1226.
37. Orsi L, Delabre L, Monnereau A, Delval P, Berthou C, Fenaux P, Marit G, Soubeyran P, Huguet F, Milpied N, Leporrier M, Hemom D, Troussard X. Occupational exposure to pesticides and lymphoid neoplasms among men: results of a French case-control study. *Occup Environ Med* 2009; 66(5):291-298.
38. Fleming LE, Timmeny W. Aplastic anemia and pesticides: an etiologic association? *J Occup Med* 1993; 35(11):1106-1116.
39. Undeger U, Basaran N. Effects of pesticides on human peripheral lymphocytes in vitro: introduction of DNA damage. *Arch Toxicol* 2005; 79(3):169-176.
40. Hassanin NM, Awad OM, El-Fiki S, Abou-Shanab RAI, Abou-Shanab ARA, Amer RA. Association between exposure to pesticides and disorder on hematological parameters and kidney function in male agricultural workers. *Environ Sci Pollut Res* 2017; 1:6.
41. Purdue MP, Hoppin JA, Blair A, Dosemeci M, Alavanja MC. Occupational exposure to organochlorine insecticides and cancer incidence in the Agricultural Health Study. *Int J Cancer* 2007; 120(3):642-649.
42. Alegria-Torres JA, Díaz-Barriga F, Gandolfi AJ, Pérez-Maldonado IN. Mechanisms of p,p'-DDE-induced apoptosis in human peripheral blood mononuclear cells. *Toxicol In Vitro* 2009; 23(6):1000-1003.

Artigo apresentado em 20/06/2017
Aprovado em 11/09/2017
Versão final apresentada em 13/09/2017