Environmental Science and Pollution Research
https://doi.org/10.1007/s11356-017-0969-z

RESEARCH ARTICLE

Assessment of the health status and risk of genotoxic and cytotoxic damage in Argentinian adolescents living near horticultural crops

Fabián Martínez-Perafán[1] · María Fernanda Santoli[1] · Marcela López-Nigro[1] · Marta Ana Carballo[1]

Received: 3 August 2017 / Accepted: 6 December 2017

Abstract
In some rural areas in Argentina, adolescents may be considered as a group indirectly exposed to agrochemicals because their parents plant small crops near their homes. This could become a health risk to children and adolescents who may be more sensitive to exposure to chemicals than adults. The objective of this study was to evaluate the health status of two different groups of Argentinian adolescents using biochemical parameters, dietary information, and cytogenetic biomarkers of genotoxicity and cytotoxicity. The study groups included 32 adolescents from Montecarlo, who were indirectly exposed to agrochemicals, and 30 unexposed adolescents from Exaltación de la Cruz. The values of total cholesterol, LDL cholesterol, triglycerides, gamma glutamyltransferase, and butyrylcholinesterase (BuChE) were higher ($p < 0.05$) in males from Exaltación de la Cruz compared with those from Montecarlo. The BuChE activity was also higher ($p < 0.05$) in females from this region. Furthermore, the consumption of citrus, vegetable-like fruits, tubers, and red meat was more frequent ($p < 0.05$) in Montecarlo. On the other hand, differences in frequency of biomarkers of genetic damage in lymphocytes were not found ($p > 0.05$). However, the cytome assay in buccal cells showed that karyorrhectic and pyknotic cells were more frequent ($p < 0.05$) in the Montecarlo group; whereas, the frequencies of cells with nuclear buds, condensed chromatin and karyolysis were higher ($p < 0.05$) in the Exaltación de la Cruz group. Despite the differences between the parameters and biomarkers evaluated, the adolescents of Montecarlo did not present health impairment probably due to the type and level of exposure to agrochemicals.

Keywords Biomonitoring · Adolescents · Environmental exposure · Agrochemicals · Blood biochemical parameters · Cytome assay

Introduction

When an individual is exposed to a xenobiotic, it can enter the body through different routes: inhalation, ingestion, or dermal absorption. The agent is metabolized to be subsequently excreted; however, many metabolites can interact with the organism, eventually damaging important biomolecules which preserve the homeostasis and therefore the health of an individual (World Health Organization 2015). These damages can be assessed by biomonitoring, which is the evaluation of the exposure to an environmental chemical agent, and its effects on health through the measurement of different biological markers.

In recent years, the environmental/occupational exposure to agrochemicals has become a health problem. This set of chemical compounds is widely used in the agricultural industry for the control of pests and weeds that affect crops. In South America, glyphosate-based broad spectrum herbicides are used largely (López et al. 2012). Many studies in vitro, in vivo, and ex vivo show the genotoxic effects of glyphosate and its metabolites in different experimental models (Alvarez-Moya et al. 2014; Mensah et al. 2015) as well as its presence in several pesticide mixtures. However, the use of these pesticides remains common in many countries, mainly due to the absence of clear regulations and lack of awareness about the possible toxic effects of agrochemicals on human health.

Most biomonitoring studies in populations exposed to agrochemicals analyze the effects of these compounds on adult health (Bolognesi 2003; Eastmond and Balakrishnan 2010),

Responsible editor: Philippe Garrigues

✉ Marta Ana Carballo
macarballo11@gmail.com

[1] Facultad de Farmacia y Bioquímica, INFIBIOC—Departamento de Bioquímica Clínica, CIGETOX (Citogenética Humana y Genética Toxicológica), Universidad de Buenos Aires, Junín 956, C1113AAD Buenos Aires, Argentina

Published online: 13 December 2017

assuming that farmers and individuals who handle the pesticides belong to this age group. However, children and adolescents may also be direct and indirectly exposed to agrochemicals. In Argentina, small farmers usually grow grounds around their homes, where women, children, and adolescents are exposed to chemicals because of the proximity of their application. They are also exposed when pesticide applicators take work clothes and chemical containers home without any safety considerations for cleaning and storage. Accordingly, children and adolescents may also be considered a vulnerable population susceptible to this type of exposure.

It should be noted that environmental factors can affect the health of children very differently from adult's health. Considering the World Health Organization data (2011), 24% of the overall morbidity rate is attributed to the environment, while this percentage increases to 33% if only the child population is considered. Furthermore, the World Health Organization (2006a) recommends studies in children's populations at different development stages. This is important especially in developing countries, in addition to the evaluation of aggregate and cumulative exposure to diverse pesticides using different biomarkers.

The exposure to environmental genotoxins can induce genomic damage. The cytokinesis-block micronucleus cytome (CBMNcyt) assay is a method widely used to measure this damage in different cell types. This technique evaluates three biomarkers of genotoxicity in lymphocytes: micronuclei (MNi), nucleoplasmic bridges (NPBs), and nuclear buds (NBUDs), which reflect clastogenic and/or aneugenic damage to chromosomes. MNi mainly originates from chromosome fragments or whole chromosomes that lag behind at anaphase during nuclear division. NPBs are caused by (a) dicentric chromosomes that result from telomere end-fusions and/or DNA misrepair or (b) impairment of chromatid separation due to defects in cohesins and/or separases at anaphase/telophase, while NBUDs are caused by elimination of amplified DNA and/or DNA repair complexes (Fenech et al. 2016; Thomas and Fenech 2011a). On the other hand, the buccal cell micronucleus cytome (BMNCyt) assay is a minimally invasive method for evaluating genetic damage, cytokinetic defects, cell death, and the regenerative potential of buccal mucosal tissue. The DNA damage is assessed by MN and NBUD biomarkers, while cytokinesis failure is estimated by the frequency of binucleated (BN) cells. Finally, condensed chromatin (CC), karyorrhectic (KHC), pyknotic (PYK), and karyolitic (KYL) cells are considered cell death parameters (Fenech et al. 2016; Thomas and Fenech 2011b).

Taking into account the above-described life conditions and following the World Health Organization recommendations, the aim of this work was to evaluate the health status of two different groups of Argentinean adolescents: 32 adolescents from Montecarlo (Misiones Province), who lived in close proximity to horticultural crops and therefore could be indirectly exposed to agrochemicals, and 30 adolescents from Exaltación de la Cruz (Buenos Aires Province), who were not exposed in any way to pesticides, using biochemical parameters, dietary information, and cytogenetic biomarkers of genotoxicity and cytotoxicity.

Materials and methods

Study population

We carried out a cross-sectional descriptive study involving school-age adolescents of both sexes: 32 from Montecarlo, Misiones province and 30 from Exaltación de la Cruz, Buenos Aires province. Both groups lived in rural areas. It should be noted that in Misiones province, the problem of exposure to xenobiotics from agro-industrial activity could be considered as a health risk factor. Adolescents with any infection, chronic disease, or familiar cancer history, as well as those with known exposure to antibiotics and/or other administered xenobiotics, or exposed to ionizing or non-ionizing radiation for diagnostic or therapeutic purposes, were excluded from the study.

Anamnesis and food consumption frequency questionnaire

Parents of adolescents signed the informed consent for their participation in this study, which followed the guidelines established in the Declaration of Helsinki (World Medical Association 2013). Besides, adolescents and their parents responded to an anamnesis and a food frequency questionnaire (FFQ). The anamnesis included socio-demographic data: age, sex, region and schooling, type and location of their house, source of water consumption, and current or chronic disease, among others. The FFQ was designed for this study taking into account the format of the questionnaires reported in nutrition studies and the type of feeding that we presumed had the adolescents in each region. We made a list of the main foods that are part of the Argentine diet and a table of frequency of consumption per week of each one of them. Afterwards, foods were then classified into groups according to their nutritional characteristics (macronutrient content); the average frequency of consumption per week of each food was multiplied by the variety/number of foods consumed in each group to obtain a frequency-variety index, which allowed knowing if the adolescents had a balanced or biased diet towards few foods.

Samples testing

Peripheral blood samples (12 mL) were taken from each adolescent by venipuncture using three vacutainer tubes: one

serum separator tube (3 mL) for biochemical parameters, one EDTA tube (3 mL) for blood count, and one heparin tube (6 mL) for the cytome assay in peripheral blood lymphocytes (PBLs). Samples of buccal epithelium were obtained by scraping both cheeks with different cytological brushes; later, they were placed into centrifuge tubes with Saccomannos' fixative (Biopur, Rosario, Argentina). All samples were transported to the laboratory within 24 h and stored at 4 °C until their processing.

Hematological and serum biochemical parameters

The Wiener lab. Counter 19 was used to count the plasma blood cells. The Cobas®6000 analyzer (Roche) was used to measure glucose, urea, creatinine, total cholesterol, HDL cholesterol, LDL cholesterol, triglycerides, total proteins, albumin, alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALKP), gamma glutamyltransferase (GGT), butyrylcholinesterase (BuChE), phosphate, magnesium, and calcium in serum.

Cytome assay in PBLs

The lymphocyte cytokinesis-block micronucleus cytome (L-CBMN) assay was carried out according to the protocol described by Fenech (2007). The lymphocytes were isolated from the collected blood samples by standard method on a ficoll-paque density gradient (GE Healthcare Bio-Sciences, Uppsala, Sweden). Later, they were cultured in a multi-well plate for 72 h at 37 °C, 5% CO_2. After 44 h of culture, cytochalasin B (4.5 μg/mL; Sigma-Aldrich, Steinheim, Germany) was added to each well to block cytokinesis (Fenech and Morley 1985). At 72 h of incubation, the lymphocytes were harvested and fixed with methanol (Merck, Darmstadt, Germany) on a slide. Finally, the cells were stained with a 10% Giemsa solution and 2000 binucleated cells were scored per subject (1000 cells for each of two duplicate wells) using a transmitted light microscopy (Olympus CX31). The frequency of micronuclei (MNi), nuclear buds (NBUDs), and nucleoplasmic bridges (NPBs) was reported in 1000 BN cells.

Cytome assay in buccal cells

The buccal cell micronucleus cytome assay (BMNCyt) was done following the protocol by Thomas et al. (2009) with minor modifications. The buccal mucosa samples were centrifuged for 10 min at 1200 rpm, the supernatant was aspired, and the cells resuspended in buffer (0.1 M EDTA, Sigma-Aldrich; 0.01 M Tris–HCl, Sigma-Aldrich and 0.02 M NaCl, Merck). After three washes with this buffer, the cells were transferred onto slides and stained with propidium iodide (30 μg/mL; Sigma-Aldrich, Steinheim, Germany). Finally, 2000 cells were scored per subject (1000 cells for each of two duplicate slides) at × 400 magnification with a fluorescence microscope (Olympus BX-40F4). The frequency of micronuclei (MNi), nuclear buds (NBUDs), binucleated (BN), condensed chromatin (CC), karyorrhexis (KHC), pyknosis (PYK), and karyolysis (KYL) was reported in 1000 cells.

Statistical analysis

The data were analyzed using the statistical and graphical functions of SPSS version 11.5 software package (Chicago, IL). Kolmogorov–Smirnov and Levenes' tests were used to evaluate the normality and variance homogeneity of data, respectively. Chi-square and Student's t tests were used to compare the demographic characteristics of the study groups. Non-parametric Mann-Whitney U-test was used to examine the differences between the dietary profiles and the frequency of cytogenetic biomarkers of both adolescent groups. On the other hand, the values of hematological and serum biochemical parameters were analyzed using the Student's t tests. Statistical significance was set at $p < 0.05$ with power at ≥ 0.8.

Results and discussion

Demographic characteristics

This study was carried out in two groups of adolescents from two different regions: Montecarlo (Misiones Province) and Exaltación de la Cruz (Buenos Aires Province). Although both lived in rural areas, only in Montecarlo was observed a close proximity (< 100 m) between agricultural crops and farmers' homes. According to anamnesis data, at Montecarlo's crops, agrochemicals used included glyphosate, chlorpyrifos, 2,4-dichlorophenoxyacetic acid (2,4-D), and paraquat individually or in mixtures. This information is consistent with a previous report about use of pesticides in Misiones Province (Gonzáles 2007). On the other hand, the International Agency for Research of Cancer classified the glyphosate as probably carcinogenic to human (2017) and the 2,4-D as possibly carcinogenic to humans (2016); therefore, the direct and/or indirect exposure to these compounds could suggest a health risk to vulnerable populations such as adolescents.

The main demographic characteristics of the adolescent groups are shown in Table 1. The average age of adolescents in Montecarlo (12.03 years) was significantly higher ($p = 0.001$) than the group from Exaltación de la Cruz (11.23 years). The anthropometric measures did not exhibit significant difference between both groups for height and weight. It was also noted that in Exaltación de la Cruz, we found the same percentage of females and males (50%) while in Montecarlo, 59.4% were females and 40.6% males.

Table 1 Demographic characteristics of the study groups

Characteristics	Montecarlo ($n = 32$)		Exaltación de la Cruz ($n = 30$)		p value[b]
Age (years, mean ± SE)	12.03 ± 0.16		11.23 ± 0.18		0.001*
Weight (kg, mean ± SE)	45.31 ± 1.90		49.07 ± 3.14		0.302
Height (m, mean ± SE)	1.53 ± 0.02		1.50 ± 0.02		0.323
Gender (%)					
Female	19 (59.4)		15 (50.0)		0.610
Male	13 (40.6)		15 (50.0)		
BMI per sex (kg/m^2, mean ± SE)	♀ 19.42 ± 0.66	♂ 18.83 ± 0.89	♀ 22.03 ± 1.79	♂ 20.65 ± 1.03	♀ 0.189 ♂ 0.200
Exposure to second hand smoke (%)[a]					
Passive smokers at home	14 (48.3)		20 (66.7)		0.192
Unexposed adolescents	15 (51.7)		10 (33.3)		

BMI body mass index

*Significant difference, $p = 0.001$ (Student's t test)

[a] Some data was not provided in the questionnaire

[b] Significance between groups

According to the mean values of the body mass index (BMI) and the criteria of the World Health Organization (2007), adolescents from Exaltación de la Cruz of both sexes were overweight, while the participants from Montecarlo were in a normal range weight for their age. Despite the above, there was no significant difference between populations for sex distribution and BMI values. The possible role of cigarette smoke was also evaluated, and we observed that 66.7% of Montecarlo adolescents were exposed as passive smokers, whereas in Exaltación de la Cruz, the proportion was lower 48.3%. However, there was no significant difference between the groups for this variable.

Different factors (anatomical, physiological, and/or behavioral) can influence children and adolescents' exposure to environmental pollutants such as agrochemicals. Their developmental stage is an important variable that could generate variations related to their susceptibility to xenobiotics. According to the classification of stages in human development proposed by the World Health Organization (2006a), participants in the present study were in the transition from late childhood (5–12 years old) to adolescence (12–18 years old), the Montecarlo group having a significantly higher age mean than Exaltación de la Cruz group. Despite the subtle age differences between both groups, every individual had undergone the same physiological changes and it can be considered that both were comparable since the participants are beginning their adolescence and at the same time are going through puberty, considering that the effects of chemical exposure during puberty are largely unknown (World Health Organization 2006a).

Biochemical parameters

Regarding the evaluation of different biochemical parameters in both groups, it was observed that the mean values of all parameters in both groups corresponded to normal values for healthy adolescents (Reference ranges of the Hospital de Clínicas José de San Martín, Universidad de Buenos Aires). Table 2 shows the values that were found when we stratified both groups according to sex. We observed that there was no significant difference ($p > 0.05$) between sexes for the Exaltación de la Cruz group, except for the activity of the alkaline phosphatase enzyme (ALKP), which was higher in males. In literature, this dimorphic pattern has been described as characteristically of the onset of puberty, being possibly related to hormonal changes at this stage of life (Adeli et al. 2015; Turan et al. 2011). In the Montecarlo group, significant higher values of hemoglobin concentration and ALKP enzyme activity in blood were observed in males, while the triglyceride concentration was significantly higher in females of this group. These differences may be related to the menarche in adolescent females which is associated with decreased red blood cell count and hemoglobin (Rushton et al. 2001). In contrast, triglycerides were higher in women of this region, which may be associated with the sexual maturity, knowing that puberty usually starts earlier in girls than in boys (Bertrais et al. 2000).

On the other hand, when comparing the biochemical parameter values of males for both groups, we observed higher concentration of hemoglobin in the Montecarlo group, whereas the concentrations of total and LDL cholesterol, triglycerides, and the activity of gamma glutamyltransferase (GGT) and butyrylcholinesterase (BuChE) enzymes were higher in the Exaltación de la Cruz group. These slight differences between analyte concentrations can be attributed to factors such as nutrition and physical activity of each individual (Huang et al. 2011; Zachrisson 2000). Despite the GGT activity

Table 2 Blood biochemical parameters of adolescents from both regions

Parameter	Montecarlo		*p* value[a]	Exaltación de la Cruz		*p* value[b]	Reference ranges	
	Females ($n = 19$)	Males ($n = 13$)		Females ($n = 15$)	Males ($n = 15$)		Females (11–14 years)	Males (11–14 years)
Hematocrit (%)	39.71 ± 0.55	40.27 ± 0.86	0.566	40.86 ± 0.65	39.62 ± 0.68	0.202	33–47	
Hemoglobin (g/dL)	13.25 ± 0.18	13.91 ± 0.19**	0.024*	13.46 ± 0.23	13.30 ± 0.19	0.588	12–15	
White blood cells count (10^9/L)	6.66 ± 0.38	6.44 ± 0.43	0.701	7.15 ± 0.41	6.16 ± 0.37	0.088	4–11	
Glucose (mg/dL)	79.42 ± 1.66	84.46 ± 2.14	0.069	84.71 ± 2.49	86.20 ± 1.41	0.602	60–100	
Urea (mg/dL)	21.47 ± 1.39	22.38 ± 1.70	0.681	21.00 ± 1.25	21.73 ± 1.41	0.702	10–40	
Creatinine (mg/dL)	0.48 ± 0.01	0.52 ± 0.02	0.154	0.49 ± 0.02	0.53 ± 0.02	0.183	≤ 1	
Total cholesterol (mg/dL)	143.05 ± 6.60	128.46 ± 8.46**	0.179	146.86 ± 3.83	152.40 ± 5.41	0.416	Desirable < 170 Borderline high 170–199 High > 200	
HDL cholesterol (mg/dL)	47.89 ± 3.92	45.92 ± 2.45	0.705	42.71 ± 1.64	41.33 ± 2.71	0.672	Desirable > 45 Borderline low 40–45 Low < 40	
LDL cholesterol (mg/dL)	84.68 ± 6.94	75.23 ± 7.86**	0.380	89.93 ± 3.14	95.73 ± 4.90	0.335	Desirable < 110 Borderline high: 110–129 High > 130	
Castelli Index (Total cholesterol/HDL)	3.20 ± 0.20	2.87 ± 0.21**	0.273	3.50 ± 0.14	3.93 ± 0.31	0.225	< 4.5	< 5.0
Triglycerides (mg/dL)	78.74 ± 6.29	60.23 ± 4.80**	0.039*	77.86 ± 6.72	86.93 ± 11.67	0.426	Desirable < 90 Borderline high 90–129 High > 130	
Total proteins (g/dL)	7.41 ± 0.08	7.43 ± 0.09	0.876	7.34 ± 0.22	7.29 ± 0.07	0.819	6.2–8.1	
Albumin (g/dL)	4.74 ± 0.06	4.73 ± 0.05	0.937	4.69 ± 0.06	4.75 ± 0.05	0.475	3.6–5.2	
ALT (UI/L)	13.16 ± 0.92	19.85 ± 3.71	0.103	13.07 ± 1.01	13.20 ± 2.32	0.961	≤ 31	
AST (UI/L)	20.32 ± 0.85	25.23 ± 2.76	0.058	20.36 ± 1.73	23.07 ± 1.06	0.195	≤ 31	
ALKP (UI/L)	212.16 ± 18.48	325.77 ± 34.41	0.004*	243.57 ± 13.95	316.47 ± 27.13	0.026*	≤ 329	
GGT (UI/L)	10.32 ± 0.44	10.92 ± 0.58**	0.404	12.07 ± 1.22	14.53 ± 1.48	0.212	5–36	
BuChE (UI/L)	8455.63 ± 254.77**	8511.69 ± 353.59**	0.896	9379.93 ± 321.92	10,292.60 ± 411.03	0.095	4260–11,250	
Phosphorus (mg/dL)	4.53 ± 0.12	4.52 ± 0.19	0.959	4.66 ± 0.13	4.83 ± 0.14	0.398	2.8–5.3	2.9–5.7
Magnesium (mg/dL)	2.02 ± 0.02	2.07 ± 0.03	0.215	1.94 ± 0.05	1.94 ± 0.09	0.950	1.6–2.6	
Calcium (mg/dL)	9.73 ± 0.08	9.62 ± 0.07	0.309	9.66 ± 0.12	9.38 ± 0.16	0.173	8.4–10.8	

Values are expressed as mean ± SE

*Significant difference between both sexes, $p < 0.05$ (Student's *t* test)

**significant difference in comparison to the group of same sex from Exaltación de la Cruz, $p < 0.05$ (Student's *t* test)

[a] Significance between females and males from Montecarlo

[b] Significance between females and males from Exaltación de la Cruz

difference, it did not represent a possible liver damage since it may be related to its enzymatic dynamic throughout the development of each individual (Adeli et al. 2015; Heiduk et al. 2009).

Concerning females, only a significantly lower activity of the butyrylcholinesterase enzyme was observed in the Montecarlo group. The inhibition of acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE) enzymes is a widely used biomarker in the monitoring of organophosphorus (OP) and carbamates pesticide exposure (Jintana et al. 2009). Our results showed that BuChE activity was significantly lower in adolescents from Montecarlo, who lived near horticultural and fruit crops. However, these values were still within the reference range for healthy adolescents. These results suggest chronic low exposure to agrochemicals, which increase the risk of diseases later in life (Valcke et al. 2017).

Dietary information

Table 3 shows the FV (frequency-variety) index of some groups of foods related with adolescent's dietary habits. We observed that citrus fruit (orange, grapefruit, lemon), vegetable (bell pepper, zucchinis, aubergine, tomato), tuber (potato, cassava), bulb, root, and red meat consumption was significantly higher ($p < 0.05$) in the Montecarlo group.

Macro- and micronutrients are necessary for optimal body function. Citrus fruits are mainly a source of vitamin C, fiber, folate, and potassium, while fruit-like vegetables, tubers, bulbs, and roots provide fiber, carbohydrates, and a variety of vitamins and minerals. Red meats also contribute with protein, fat, and minerals (US Departament of Agriculture 2017). Macronutrients (carbohydrates, proteins and fats) are vital in adolescence to supply the energy demand used in gain of

Table 3 Dietary information of adolescents from both regions

FV-Index for some food groups	Montecarlo ($n = 32$)	Exaltación de la Cruz ($n = 30$)	p value[a]
Citrus	5.80 ± 0.92	1.97 ± 0.35	0.001**
Tropical fruits	4.07 ± 0.67	2.52 ± 0.45	0.198
Vegetables-like fruits	5.53 ± 0.87	4.52 ± 1.36	0.041*
Green leafy vegetables	1.98 ± 0.23	3.05 ± 0.56	0.441
Tubers, bulbs and roots	12.46 ± 1.29	7.77 ± 1.15	0.009*
Grains and legumes	2.22 ± 0.35	3.22 ± 0.70	0.706
Dairy products and eggs	11.73 ± 1.26	14.38 ± 1.05	0.101
Red meat	3.50 ± 0.39	1.81 ± 0.28	0.004*
White meat	3.44 ± 0.39	3.07 ± 0.39	0.525
Lunch meat	2.09 ± 0.42	2.55 ± 0.65	0.807
Flour and cereals	20.14 ± 1.79	16.99 ± 1.28	0.282
Oil and fat	4.08 ± 0.64	4.90 ± 0.64	0.256
Candy	4.09 ± 0.48	4.86 ± 0.40	0.230

Values are expressed as mean ± SE

FV-Index average frequency of consumption per week multiplied by the variety of foods consumed in each food group

***Significant difference, $p < 0.05$ (Mann-Whitney U test); significant difference, $p = 0.001$ (Mann-Whitney U test)

[a] Significance between groups

weight and height during this stage of life (World Health Organization 2006b). In relation to micronutrients (vitamins and minerals), these function as cofactors for different metabolic enzymes and are necessary for DNA synthesis and oxygen transport; therefore, their consumption is essential (Miller and Rayalam 2017).

Cytogenetic biomarkers

Cytome assay in PBLs

Considering genotoxicity biomarkers, Table 4 shows the parameters of the cytome assay in peripheral blood lymphocytes (PBLs) for adolescents from Exaltación de la Cruz and Montecarlo. According to these results, there were no significant differences ($p > 0.05$) between the frequencies of micronuclei, nuclear buds, and nucleoplasmic bridges in both groups.

One of the widely used genotoxicity biomarkers is the micronucleus test (MN) in two cell types, peripheral blood lymphocytes (PBLs) and exfoliated oral cells through the cytome assay evaluating the frequency of different nuclear abnormalities (Bolognesi et al. 2013; Fenech et al. 2003). Furthermore, increased MNi formation in PBLs is associated with early events in carcinogenesis (Bonassi et al. 2011). However, we did not find significant differences between the frequencies of MNi, nuclear buds (NBUDs), and nucleoplasmic bridges (NPBs) in PBLs (Fig. 1) between the studied groups, which could be related with a low level of exposure of the adolescents living near horticultural crops. In addition, MN, NBUD, and NPB frequencies observed in both groups were within the reference range proposed by Fenech (2007) for untreated human lymphocytes and the baseline frequency of MNi in children suggested by Neri et al. (2005).

Cytome assay in buccal cells

Table 5 shows biomarker frequencies of genotoxicity and cytotoxicity by cytome assay in exfoliated buccal cells (BMNCyts) for adolescents of both locations. We observed

Table 4 Frequency of genotoxicity biomarkers by cytome assay in PBL of adolescents from both regions

Biomarker/1000 cells	Montecarlo ($n = 32$)	Exaltación de la Cruz ($n = 30$)	p value[a]
MNi	1.58 ± 0.23	1.36 ± 0.15	0.632
NBUDs	0.11 ± 0.05	0.14 ± 0.04	0.333
NPBs	0.29 ± 0.08	0.18 ± 0.05	0.414

Values are expressed as mean ± SE

MNi micronuclei, *NBUDs* nuclear buds, *NPBs* nucleoplasmic bridges

[a] Significance between groups (Mann-Whitney U test)

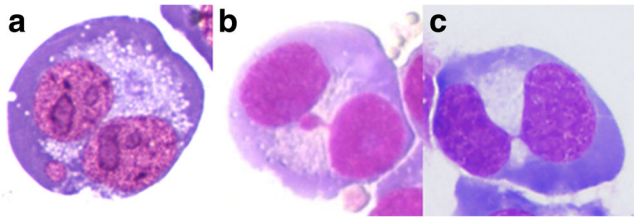

Fig. 1 Cells observed by cytome assay in peripheral blood lymphocytes (PBLs). **a** Cell with micronucleus. **b** Cell with nuclear bud. **c** Cell with nucleoplasmic bridge

that micronucleated (MN) and binucleated cell (BN) frequencies were not significantly different between both groups, whereas the number of cells with nuclear buds (NBUDs) was significantly higher in the Exaltación de la Cruz adolescents. MN and NBUD frequencies are considered as biomarkers of genotoxic damage, whereas BN cells indicate cytokinesis failure (Thomas et al. 2009). In this study, both tissues (blood and mucosa) exhibited the same behavior in relation to MN frequencies; in addition, we did not find a significant difference between both groups for this parameter.

BMNCyt is also used to analyze cytotoxicity biomarkers (Fig. 2) such as the frequency of cells with condensed chromatin (CC), karyorrhesis (KHC), karyolysis (KYL), and pyknosis (PYK) (Thomas et al. 2009). A higher number of CC and KYL cells were observed in Exaltación de la Cruz's volunteers, while KHC and PYK cell frequencies were higher in Montecarlo's. These biomarkers would indicate cell death processes, where CC represents an early phase; KHC would be a late stage and KYL an even later point of apoptosis. On the other hand, PYK cells could be associated with a single cell death process; however, the biological significance of these biomarkers is still unknown (Thomas et al. 2009). Considering our results, the Exaltación de la Cruz group exhibited a larger number of cells in early and late stages of the cell death process, while the Montecarlo group presented the intermediate phase of cell death with more frequency. This pattern could be related to the cellular desquamation, the rate of renewal of the buccal epithelium, and the possible increase of antioxidant levels in adolescents from Montecarlo due to their diet (more frequent and varied consumption of fruits and vegetables). However, the association of each marker with these variables needs further studies.

Despite the fact that we observed significant differences in both groups for cytome assay values, all these biomarker frequencies were within the reference ranges in buccal cells reported by Thomas et al. (2009) for healthy young people. Therefore, the adolescents included in this study residing near horticultural crops did not present any genotoxic or cytotoxic damage evidenced by the biomarkers mentioned above. However, other reports show increased damage using BMNCyt in children and adolescents living in rural areas near fumigated crops that are presumably exposed to high levels of pesticides (Castañeda-Yslas et al. 2016; Gómez-Arroyo et al. 2013). These differences could be attributed to variations in exposure since in our study, the agrochemicals were applied using a backpack sprayer, while in the other studies, the children and adolescents were exposed to aerial fumigation. Exposure levels and absorption differences could be an explanation for our results evidenced with BMNCyt biomarkers.

In the last years, the use of the MN test in the monitoring of children and adolescents has increased; therefore, the influence of variables such as lifestyle, diet, age, and sex should be taken into account in the interpretation of results (Holland et al. 2011). We did not find influence of the body mass index (BMI), sex, or exposure to second-hand smoke on genotoxicity and cytotoxicity biomarkers assessed by the cytome test in PBLs and buccal cells ($p > 0.05$). On the other hand, the Spearman's correlation analysis showed an association between age and some of the biomarkers of the cytome assay in both PBLs and oral cells (data not shown); however, these results are not conclusive considering that the age range of participants was very small.

Table 5 Frequency of genotoxicity and cytotoxicity biomarkers by cytome assay in exfoliated buccal cells (BCMNCyts) of adolescents from both regions

Biomarker/1000 cells	Montecarlo ($n = 32$)	Exaltación de la Cruz ($n = 30$)	p value[a]
MNi	3.59 ± 0.53	5.50 ± 1.25	0.932
NBUDs	0.06 ± 0.04	0.18 ± 0.06	0.048*
BN	0.63 ± 0.18	0.53 ± 0.14	0.987
CC	9.53 ± 1.42	33.40 ± 3.82	< 0.001**
KHC	27.63 ± 4.21	12.93 ± 1.78	0.003*
PYK	1.53 ± 0.36	0.53 ± 0.18	0.008*
KYL	3.38 ± 0.41	10.83 ± 2.75	0.007*

Values are expressed as mean ± SE

MNi micronuclei, *NBUDs* nuclear buds, *BN* binucleated cells, *CC* condensed chromatin cells, *KHC* karyorrhectic cells, *PYK* pyknotic cells, *KYL* karyolytic cells

***Significant difference, $p < 0.05$ (Mann-Whitney U test); significant difference, $p < 0.001$ (Mann-Whitney U test)

[a] Significance between groups

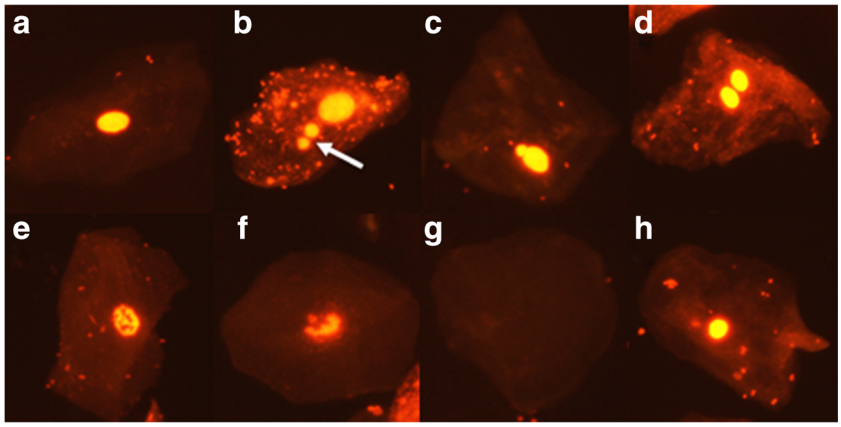

Fig. 2 Cells observed by cytome assay in exfoliated buccal cells stained with propidium iodide. **a** Normal differentiated cell. **b** Cell with two micronuclei (arrow). **c** Cell with nuclear bud. **d** Binucleated cell. **e** Condensed chromatin cell. **f** Karyorrhetic cell. **g** Karyolitic cell. **h** Pyknotic cell

Nutrition plays an important role in the maintenance of genomic stability. The consumption of a certain food group in a greater or lesser proportion influences the cellular concentration of micronutrients that are necessary for the processes of DNA synthesis and repair (Fenech and Bonassi 2011). Regarding diet, we observed that the frequency of consumption of some food groups was different between both groups of adolescents. Some authors report that fruit and vegetable consumption provide protection against various chronic impairment health outcomes including cardiovascular diseases, type-II diabetes, and various types of cancer (Valcke et al. 2017). Therefore, there may be a possible association between the consumption of different food groups and the cytogenetic biomarkers provided by the cytome test in PBLs and buccal mucosa cells; however, more extensive studies are needed to elucidate this correlation.

Conclusion

This study provides information on the health status of two groups of adolescents in rural areas, one of them indirectly exposed to pesticides used in vegetable crops. Both groups showed differences between biochemical parameters as well as food consumption and cytogenetic biomarker frequencies; however, none of these variables indicated any kind of current health impairment. However, the adolescents from the location where pesticides were used showed lower activity of butyrylcholinesterase, which suggest a chronic exposure that could have clinical consequences later in life. Biomarkers of genetic damage associated with increased risk for cancer were negative in both groups. Diet seems to play an important role in carcinogenic risk prevention. According to the above, if adolescents continue to be exposed to agrochemicals, the chronic exposure could lead to disease development in the future; thus, further studies about this subject should be carried out. Besides, bad habits such as using pesticides near the home, mixing clothes used in agrochemical application with other clothes, storing chemicals inside the home, or allowing children/adolescents to handle these compounds should be avoided.

Acknowledgements We thank PhD. Aldo Squassi and BDent. Carlos Capuano of the Cátedra de Odontología Preventiva y Comunitaria, Facultad de Odontología, Universidad de Buenos Aires, for providing the samples for this study. We also acknowledge the collaboration of phD. Beatriz Perazzi in the biochemical determinations and the valuable help of Biochemist Victoria Schiariti Lampropulos in the methodological adjustments and English editions. Finally, we are grateful to all the volunteers who participated in this study.

Funding information This work was supported by the Universidad de Buenos Aires [grant number UBACYT 20920160100154BA, 2014–2017].

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

References

Adeli K, Higgins V, Nieuwesteeg M, Raizman JE, Chen Y, Wong SL, Blais D (2015) Biochemical marker reference values across pediatric, adult, and geriatric ages: establishment of robust pediatric and adult reference intervals on the basis of the Canadian health measures survey. Clin Chem 61(8):1049–1062. https://doi.org/10.1373/clinchem.2015.240515

Alvarez-Moya C, Silva MR, Ramírez CV, Gallardo DG, Sánchez RL, Aguirre AC, Velasco AF (2014) Comparison of the in vivo and in vitro genotoxicity of glyphosate isopropylamine salt in three different organisms. Genet Mol Biol 37(1):105–110. https://doi.org/10.1590/s1415-47572014000100016

Bertrais S, Balkau B, Charles MA, Vol S, Calvet C, Tichet J, Eschwege E (2000) Puberty-associated differences in total cholesterol and triglyceride levels according to sex in French children aged 10–13 years. Ann Epidemiol 10(5):316–323. https://doi.org/10.1016/s1047-2797(00)00056-9

Bolognesi C (2003) Genotoxicity of pesticides: a review of human biomonitoring studies. Mutat Res Rev Mutat Res 543(3):251–272. https://doi.org/10.1016/S1383-5742(03)00015-2

Bolognesi C, Knasmueller S, Nersesyan A, Thomas P, Fenech M (2013) The HUMNxl scoring criteria for different cell types and nuclear anomalies in the buccal micronucleus cytome assay—an update and expanded photo gallery. Mutat Res Rev Mutat Res 753(2): 100–113. https://doi.org/10.1016/j.mrrev.2013.07.002

Bonassi S, El-Zein R, Bolognesi C, Fenech M (2011) Micronuclei frequency in peripheral blood lymphocytes and cancer risk: evidence from human studies. Mutagenesis 26(1):93–100. https://doi.org/10.1093/mutage/geq075

Castañeda-Yslas IJ, Arellano-García ME, García-Zarate MA, Ruíz-Ruíz B, Zavala-Cerna MG, Torres-Bugarín O (2016) Biomonitoring with micronuclei test in buccal cells of female farmers and children exposed to pesticides of Maneadero agricultural valley, Baja California, Mexico. J Toxicol 2016:1–8. https://doi.org/10.1155/2016/7934257

Eastmond DA, Balakrishnan S (2010) Chapter 11—genotoxicity of pesticides. In: Krieger R (ed) Hayes' handbook of pesticide toxicology. Academic Press, New York, pp 357–380. https://doi.org/10.1016/B978-0-12-374367-1.00011-2

Fenech M, Morley AA (1985) Measurement of micronuclei in lymphocytes. Mutat Res 147(1-2):29–36. https://doi.org/10.1016/0165-1161(85)90015-9

Fenech M, Chang WP, Kirsch-Volders M, Holland N, Bonassi S, Zeiger E (2003) HUMN project: detailed description of the scoring criteria for the cytokinesis-block micronucleus assay using isolated human lymphocyte cultures. Mutat Res Genet Toxicol Environ Mutagen 534(1-2):65–75. https://doi.org/10.1016/S1383-5718(02)00249-8

Fenech M (2007) Cytokinesis-block micronucleus cytome assay. Nat Protoc 2(5):1084–1104. https://doi.org/10.1038/nprot.2007.77

Fenech M, Bonassi S (2011) The effect of age, gender, diet and lifestyle on DNA damage measured using micronucleus frequency in human peripheral blood lymphocytes. Mutagenesis 26(1):43–49. https://doi.org/10.1093/mutage/geq050

Fenech M, Knasmueller S, Bolognesi C, Bonassi S, Holland N, Migliore L, Palitti F, Natarajan AT, Kirsch-Volders M (2016) Molecular mechanisms by which in vivo exposure to exogenous chemical genotoxic agents can lead to micronucleus formation in lymphocytes in vivo and ex vivo in humans. Mutat Res Rev Mutat Res 770(Pt A):12–25. https://doi.org/10.1016/j.mrrev.2016.04.008

Gómez-Arroyo S, Martínez-Valenzuela C, Calvo-González S, Villalobos-Pietrini R, Waliszewski SM, Calderón-Segura ME, Martínez-Arroyo A, Félix-Gastélum R, Lagarda-Escarrega A (2013) Assessing the genotoxic risk for Mexican children who are in residential proximity to agricultural areas with intense aerial pesticide applications. Rev Int Contam Ambie 29:217–225

Gonzáles CO (2007) La problemática de los agroquímicos y sus envases, su incidencia en la salud de los trabajadores, la población expuesta y el ambiente: región mesopotámica, provincia de Misiones, Ministerio de Salud, Secretaría de Ambiente y Desarrollo Sustentable. OPS, AAMMA, Buenos Aires, pp 137–180

Heiduk M, Päge I, Kliem C, Abicht K, Klein G (2009) Pediatric reference intervals determined in ambulatory and hospitalized children and juveniles. Clin Chim Acta 406(1-2):156–161. https://doi.org/10.1016/j.cca.2009.06.021

Holland N, Fucic A, Merlo DF, Sram R, Kirsch-Volders M (2011) Micronuclei in neonates and children: effects of environmental, genetic, demographic and disease variables. Mutagenesis 26(1):51–56. https://doi.org/10.1093/mutage/geq064

Huang J, Frohlich J, Ignaszewski AP (2011) The impact of dietary changes and dietary supplements on lipid profile. Can J Cardiol 27(4): 488–505. https://doi.org/10.1016/j.cjca.2010.12.077

IARC (2016) 2,4-dichlorophenoxyacetic acid (2,4-D) and some organochlorine insecticides, IARC monographs on the evaluation of carcinogenic risk to humans. Int Agency Res Cancer, Lyon, France 113: 1–126

IARC (2017) Some organophosphate insecticides and herbicides, IARC monographs on the evaluation of carcinogenic risks to humans. Int Agency Res Cancer, Lyon, France 112:321–412

Jintana S, Sming K, Krongtong Y, Thanyachai S (2009) Cholinesterase activity, pesticide exposure and health impact in a population exposed to organophosphates. Int Arch Occup Environ Health 82(7): 833–842. https://doi.org/10.1007/s00420-009-0422-9

López SL, Aiassa D, Benítez-Leite S, Lajmanovich R, Mañas F, Poletta G, Sánchez N, Simoniello MF, Carrasco AE (2012) Chapter two—pesticides used in south American GMO-based agriculture: a review of their effects on humans and animal models. In: James CF (ed) Advances in molecular toxicology. Elsevier, pp 41–75

Mensah PK, Palmer CG, Odume ON (2015) Chapter 4—ecotoxicology of glyphosate and glyphosate-based herbicides—toxicity to wildlife and humans. In: Larramendy M (ed) Toxicity and hazard of agrochemicals. InTech, pp 93–112. https://doi.org/10.5772/60767

Miller CN, Rayalam S (2017) The role of micronutrients in the response to ambient air pollutants: potential mechanisms and suggestions for research design. J Toxicol Environ Health B Crit Rev 20(1):38–53. https://doi.org/10.1080/10937404.2016.1261746

Neri M, Ceppi M, Knudsen LE, Merlo DF, Barale R, Puntoni R, Bonassi S (2005) Baseline micronuclei frequency in children: estimates from meta- and pooled analyses. Environ Health Perspect 113(9):1226–1229. https://doi.org/10.1289/ehp.7806

Rushton DH, Dover R, Sainsbury AW, Norris MJ, Gilkes JJH, Ramsay ID (2001) Why should women have lower reference limits for haemoglobin and ferritin concentrations than men? BMJ 322(7298):1355–1357. https://doi.org/10.1136/bmj.322.7298.1355

Thomas P, Holland N, Bolognesi C, Kirsch-Volders M, Bonassi S, Zeiger E, Knasmueller S, Fenech M (2009) Buccal micronucleus cytome assay. Nat Protoc 4(6):825–837. https://doi.org/10.1038/nprot.2009.53

Thomas P, Fenech M (2011a) Cytokinesis-block micronucleus cytome assay in lymphocytes. In: Didenko VV (ed) DNA damage detection in situ, ex vivo, and in vivo: methods and protocols. Humana Press, Totowa, NJ, pp 217–234. https://doi.org/10.1007/978-1-60327-409-8_16

Thomas P, Fenech M (2011b) Buccal micronucleus cytome assay. In: Didenko VV (ed) DNA damage detection in situ, ex vivo, and in vivo: methods and protocols. Humana Press, Totowa, NJ, pp 235–248. https://doi.org/10.1007/978-1-60327-409-8_17

Turan S, Topcu B, Gökçe I, Güran T, Atay Z, Omar A, Akçay T, Bereket A (2011) Serum alkaline phosphatase levels in healthy children and evaluation of alkaline phosphatase z-scores in different types of rickets. J Clin Res Pediatr Endocrinol 3(1):7–11. https://doi.org/10.4274/jcrpe.v3i1.02

US Departament of Agriculture (2017) Macronutrients. US Departament of Agriculture. Available at: https://www.nal.usda.gov/fnic/macronutrients. Accessed May-23-2017

Valcke M, Bourgault M-H, Rochette L, Normandin L, Samuel O, Belleville D, Blanchet C, Phaneuf D (2017) Human health risk assessment on the consumption of fruits and vegetables containing residual pesticides: a cancer and non-cancer risk/benefit perspective. Environ Int 108:63–74. https://doi.org/10.1016/j.envint.2017.07.023

World Health Organization (2006a) Principles for evaluating health risks in children associated with exposure to chemicals. World Health Organization, Geneve, Switzerland, pp 1–329

World Health Organization (2006b) Adolescent nutrition: a review of the situation in selected south-east Asian countries. WHO Regional Office for South-East Asia, New Delhi, pp 1–84

World Health Organization (2007) Growth reference data for 5–19 years. World Health Organization. Available at: http://www.who.int/growthref/en/. Accessed May-23-2017

World Health Organization (2011) Summary of principles for evaluating health risks in children associated with exposure to chemicals. 978 92 4 150117 0, World Health Organization, Geneva, Switzerland, pp. 1–50

World Health Organization (2015) Human biomonitoring: facts and figures. World Health Organization, WHO Regional Office for Europe, Copenhagen, pp 1–88

World Medical Association (2013) World Medical Association Declaration of Helsinki: ethical principles for medical research involving human subjects. JAMA 310(20):2191–2194. https://doi.org/10.1001/jama.2013.281053

Zachrisson I (2000) Thesis: diabetes and puberty. Umeå University, Stockholm, Sweden, Studies on hormonal factors of importance to the blood glucose control, pp 7–13