Cytogenetic Monitoring in a Population Occupationally Exposed to Pesticides in Ecuador

César Paz-y-Miño,[1,2] Gabriela Bustamante,[1] María Eugenia Sánchez,[1,2] and Paola E. Leone[1,2]

[1]Laboratorio de Genética Molecular y Citogenética Humana, Departamento de Ciencias Biológicas, and [2]Unidad de Genética, Facultad de Medicina, Pontificia Universidad Católica del Ecuador, Quito, Ecuador

We analyzed the incidence of structural and numerical chromosomal aberrations (CAs) in workers of a plantation of flowers located in Quito, Ecuador, in South America. This study included 41 individuals occupationally exposed to 27 pesticides, some of which are restricted in many countries and are classified as extremely toxic by the World Health Organization; among these are aldicarb and fenamiphos. The same number of individuals of the same age, sex, and geographic area were selected as controls. Workers exposed to these pesticides showed an increased frequency of CA compared with control group (20.59% vs. 2.73%; $p < 0.001$). We conclude that screening for CA is an adequate biomarker for evaluating and detecting genotoxicity resulting from exposure to pesticides. Levels of erythrocyte acetylcholinesterase were also determined as a complementary metabolic study. Levels below the optimal (> 28 U/mL blood) were found in 88% of exposed individuals; this clearly shows the effect of organophosphate pesticides. When comparing the levels of acetylcholinesterase and structural CA frequencies, there was a negative linear correlation ($r = 0.416$; $p < 0.01$). We conclude that by using both analyses it may be possible to estimate damage produced by exposure to organophosphate pesticides. *Key words:* **chromosomal aberrations, erythrocyte acetylcholinesterase, mutagenic and carcinogenic risk, pesticide exposure.** *Environ Health Perspect* **110:1077–1080 (2002). [Online 12 September 2002]**
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The World Health Organization (WHO) has classified pesticides according to their potential health risks (WHO 2001). Despite all the known risks of some of these pesticides, many of those catalogued as extremely toxic are still being used in Ecuador—for example, aldicarb and fenamiphos.

Evidence of the carcinogenic effects of certain pesticides in animals and an increase in the risk of developing malignancies in occupationally exposed populations have made necessary studies in exposed workers (Lucas et al. 2001; Mills and Zahm 2001). Some demographic characteristics may also be useful in categorizing pesticide exposure (Echols et al. 2001), in the same manner as studies such as a detailed questionnaire survey concerning the lifetime use of pesticides by workers (Engel et al. 2001).

Methods used to evaluate exposed individuals include chromosomal aberration (CA) analysis, which is considered a reliable test because of the association between the frequency of CAs and the risk of developing cancer (Bonassi et al. 2000; Lando et al. 1998).

This type of analysis has been used previously to evaluate populations that are occupationally exposed to pesticides. Cytogenetic damage related to pesticide exposure has been reported in various populations. Some investigators have reported significant differences in the percentage of CAs in exposed individuals (range, 2.66–10.30%) compared with control (range, 0.53–5.52%) (Balaji and Sasikala 1993; Brega et al. 1998; De Ferrari et al. 1991; Joksic et al. 1997; Kourakis et al. 1992; Rupa and Reddi 1991; Yoder et al. 1973); others have not (de Cassia Stocco et al. 1982; Hoyos et al. 1996; Steenland et al. 1986).

Erythrocyte acetylcholinesterase level is used as a marker to evaluate the exposure to organophosphates (Brega et al. 1998; Gomes et al. 1998; Lakew and Mekonnen 1998; Tinoco-Ojanguren and Halperin 1998). The inactivation of acetylcholinesterase causes overstimulation of the nervous system, which produces such symptoms as headache, dizziness, nausea, stomachache, and weakness (Ballantyne et al. 1994; Dulout et al. 1985). Exposure to some organophosphate pesticides may additionally cause alteration in noncholinergic neurochemical processes (Pope 1999) and have been associated with risk of developing non-Hodgkin lymphomas (Sierra-Torres et al. 1998). Evaluation of acetylcholinesterase levels complements cytogenetic analysis, which is the traditional monitoring method to establish a relation between genetic damage and the critical events leading to carcinogenesis (Miller and Shah 1983). This study evaluates the cytogenetic damage of farmers according to the place of work and the relationship with levels of acetylcholinesterase.

Materials and Methods

This study was carried out in a group of 41 workers exposed to pesticides (group Gp), and the same number of nonexposed individuals who served as the control group (group Gc). The Gp group included in this study comprised 28 men and 13 women, 29.54 years old on average (range, 17–52 years; SD ± 9.55). They were exposed to 27 kinds of pesticides, which are detailed in Table 1 with their corresponding WHO classification by hazard. Duration of pesticide exposure ranged from 6 to 66 months. Data, including age, sex, work area, and duration of exposure, are shown in Table 2.

Individuals who presented symptoms of toxicity, such as fatigue, weakness, cramps, abdominal pain, dizziness, and headaches, constituted 24.3% of group Gp. Interestingly, four women exposed to pesticides had incurred miscarriages.

The control group was made up of nonexposed individuals, living in the same area and with no history of occupational exposure to pesticides. They were of the same age and sex and had similar socioeconomic conditions as the exposed group. The average age of the group was 30.4 years (SD ± 8.89; data not shown). No individual of this group had had contact with known genotoxic agents.

All study participants completed a questionnaire in which personal data, working activity, type and duration of contact with pesticides, viral infections, smoking and alcohol habits, and recent exposure to X-rays, chemicals (other than pesticides), drugs, or medicines were recorded.

Cytogenetic analysis. Peripheral blood was obtained and cultured in RPMI-1640 medium supplemented with fetal calf serum, phytohemagglutinin, penicillin–streptomycin, L-glutamine, and HEPES buffer in standard concentrations. The cultures were maintained for 48 hr at 37°C. Harvesting and staining were performed according to standard techniques implemented in our laboratory (Paz-y-Miño et al. 1995, 2000). One hundred metaphases per individual were scored. The percentage of CAs was obtained by calculating the percentage of metaphases from the total analyzed, per individual, that showed structural and numerical alterations.

Address correspondence to C. Paz-y-Miño, Laboratorio de Genética Molecular y Citogenética Humana, Departamento de Ciencias Biológicas, Facultad de Ciencias Exactas y Naturales, Pontificia Universidad Católica del Ecuador, Avda. 12 de Octubre y Patria, Quito, Ecuador, P.O. Box 17-01-2184. Telephone: (593-2) 565627 ext. 1193. Fax: (593-2) 509680. E-mail: cpazymino@puceuio.puce.edu.ec

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The analysis of structural CA included breaks, dicentrics, and rings. In relation to numerical CAs, only metaphases with a loss or gain of one or two chromosomes and endoreduplications were considered. CA and cytogenetic variants were classified according to the ISCN (Mitelman 1995). Statistical comparative analysis between the two groups was performed using the chi-square test ($p < 0.001$).

Erythrocyte acetylcholinesterase evaluation. The Test-Mate OP kit (EQM Research Inc., Cincinnati, OH, USA) cholinesterase assay is based on the original assay of Ellman (Ellman et al. 1961). Thiocholine ester is used as substrate, which reacts with cholinesterase, producing thiocholine. Thiocholine then reacts with DTNB [Ellman's reagent, 5,5′-dithiobis(2-nitrobenzoic acid)] to produce a yellow color that is measured at 470 nm by the Test-Mate OP photometric analyzer (Ellman et al. 1961). The Test-Mate OP kit determines biologic exposure by measuring inactivation of blood cholinesterase (U/mL blood); the optimal level of erythrocyte acetylcholinesterase established for our group of study is > 28 U/mL blood.

The Test-Mate blood analyzer (TA-20; EQM Research Inc.) is a computer-controlled light-emitting diode source calorimeter. The Test-Mate contains an electronic clock and thermometer, which are used by its computer to perform a kinetic temperature-corrected Ellman-type cholinesterase assay. Cholinesterase activities were automatically corrected to 25°C using the internal thermometer of the Test-mate analyzer.

After all variables were adjusted to the conditions of our study, the people to be tested were taken into the laboratory of the plantation, where the blood samples were taken and processed immediately with medical supervision. Cholinesterase activity was determined specifically for erythrocytes.

The test was performed using blood from the fingertip; buffer was added to the blood, and then the sample was inserted in the chamber, where the 30-sec hemoglobin analysis began. Four drops of water were added to a reagent well (erythrocyte acetylcholinesterase reagent; 96 assays/plate). At this point, the cholinesterase analysis of the blood sample began.

The Pearson correlation analysis was applied to determinate the relationship between levels of acetylcholinesterase and the percentage of structural CAs, using the MSTAT-C program (Michigan State University, East Lansing, MI, USA) ($p < 0.05$).

Table 1. List of pesticides used in the flower plantation.

Active ingredient	Trade name	WHO classification[a]
Acephate	Orthene	III
Aldicarb	Temik	Ia
Benomyl	Benlate	U
Captan	Captan	U
Carbendazim	Bavistin	U
Carbendazim	Derosal	U
Carbofuran	Furadan	Ib
Carboxin + captan	Vitavax	U
Cartap	Padan	II
Chlorothalonil	Daconil	U
Copper hydroxide	Kocide	III
Cypermethrin	Cipermetrina	II
Cyromazine	Trigard	U
Deltamethrin	Decis	II
Endosulfan	Thiodan	II
Fenamiphos	Nemacur	Ib
Fosetyl	Aliette	U
Iprodione	Rovral	U
Metalaxyl	Ridomil	III
Methyl bromide	—	F
Oxyfluorfen	Goal	U
Prochloraz	Sportac	III
Profenofos	Curacron	II
Profenofos + cypermethrin	Fenom	II
Propineb	Antracol	U
Thyocyclam	Evisect	II
Vinclozolin	Ronilan	U

[a]Classification according to WHO (2001): Ia, extremely hazardous; Ib, highly hazardous; II, moderately hazardous; III, slightly hazardous; U, unlikely to present acute hazard in normal use; F, gaseous fumigant not classified by the WHO.

Results

Cytogenetic analysis. Table 2 shows the percentage of structural CA per individual (18.29%). The mean values of percentage of structural and numerical CAs per group analyzed in this study are shown in Table 3, which compares the Gp (exposed) and Gc (control) groups. Individuals in the exposed group presented a frequency of single chromatid-type

Table 2. Characteristics of the population exposed to pesticides, percentage of structural chromosomal aberrations, and erythrocyte acetylcholinesterase levels.

Individual no.	Sex	Age (years)	Work area	Time working in plantation (months)	Structural CA (%)	Erythrocyte acetylcholinesterase levels
1	M	20	Fumigation	6	43	19.00
2	M	52	Fumigation	40	34	19.20
3	M	22	Fumigation	48	30	20.00
4	M	51	Field supervisor	60	34	20.00
5	M	25	Fumigation	60	21	20.20
6	F	22	Harvesting	48	15	21.10
7	F	24	Supervisor	12	31	21.10
8	M	22	Storage manager	12	28	22.00
9	M	31	Fumigation/field[a]	24	22	22.10
10	M	27	Fumigation supervisor	12	6	22.30
11	M	21	Field	24	11	23.00
12	F	38	Field	24	15	23.21
13	M	22	Office	11	7	23.30
14	M	22	Watering	66	12	23.30
15	F	21	Field	60	14	23.64
16	F	25	Field	17	12	23.65
17	M	43	Field supervisor	60	20	23.70
18	F	23	Harvesting	60	12	23.87
19	M	26	Chemical storage room	50	24	24.00
20	M	28	Field	60	16	24.20
21	F	34	Field	36	18	24.20
22	M	17	Refrigerated room	24	14	24.53
23	M	25	Field	60	20	24.53
24	M	49	Field	36	14	24.80
25	M	28	Field	60	18	24.80
26	M	50	Cleaning	27	24	25.10
27	F	30	Quality control	60	12	25.30
28	F	30	Field	24	17	25.30
29	M	33	Supervisor	7	11	25.50
30	F	19	Field	48	17	26.10
31	M	40	Field	48	16	26.51
32	M	31	Production manager	24	12	26.84
33	M	40	Mixing chemicals	24	13	27.00
34	M	29	Field	60	18	27.00
35	M	34	Fumigation	9	15	27.50
36	M	39	Supervisor	65	24	28.00
37	F	23	Office	54	8	28.43
38	F	23	Supervisor	60	18	28.90
39	M	33	Supervisor	19	9	29.00
40	F	20	Harvesting	60	14	30.02
41	M	24	Fumigation storage	60	31	32.00
Mean ± SD		29.54 ± 9.55		39.49 ± 20.25	18.29 ± 8.23	24.49 ± 2.99
Pearson's r (structural CA and acetylcholinesterase)					–0.416 ($p < 0.01$)	

Abbreviations: F, female; M, male.
[a]Individuals who have worked in both fumigation and field activities.

alterations seven times higher than those involving both chromatids.

We did not find any correlation between structural CA and duration of employment. We did, however, find a correlation between structural CA and place of work. The frequency of CA was higher in individuals who worked in the chemical storage room, were in charge of mixing pesticides, or performed fumigation (Table 2).

The frequency of numerical CAs was 30 times higher in the exposed group (2.29%) than in the control group (0.07%; $p < 0.001$; Table 3).

The overall percentage of CA frequency was 20.59% in the exposed group and 2.73% in the control group (Table 3).

Levels of erythrocyte acetylcholinesterase. Five of the exposed individuals (12.2%) presented optimal levels of erythrocyte acetylcholinesterase (> 28). Five presented levels that indicated overexposure to organophosphate pesticides (< 21), and levels of the remaining 31 individuals ranged from 21 to 28 (Table 2).

When comparing levels of acetylcholinesterase with the percentage of structural CAs, we found that individuals with greater cytogenetic damage showed low levels of acetylcholinesterase ($r = 0.416$; $p < 0.01$; Table 2).

Discussion

The percentage of CAs was 20.59%, which exceeds the percentages reported by other researchers. Carbonell et al. (1995) found CAs between 3.7% and 6.93% in a group of workers highly exposed to pesticides during part of the year (spring–summer). Because of the favorable weather conditions in Ecuador, farming activities take place year-round, so workers are exposed all the time, rather than seasonally. This phenomenon could explain the higher level of CAs compared with results reported by other investigators such as Joksic et al. (1977). On the other hand, Joksic et al. (1997) reported 0.13% CAs in the exposed group. However, they took only dicentric and ring chromosomes into account, which were not findings reported in our study. Another study (Kourakis et al. 1992) reported 2.66% CAs in the exposed group and also found higher frequencies in those who work exclusively in green houses compared with those who work in the open air. Similar studies show 6% and 11% CAs in exposed individuals (De Ferrari et al. 1991; Rupa and Reddi 1991).

The increase in CAs in the exposed group analyzed in our study may stem from the fact that some of the highly toxic pesticides used in Ecuador are restricted in other countries; levels of exposure may also be higher in our country, and protection measures are not enforced.

The results we obtained from this study clearly demonstrate the harmful effects that pesticides have on chromosome structure in exposed individuals. Because we found a higher proportion of chromatid-type aberrations than chromosomal ones, we were also able to confirm that pesticides as well as other genotoxic chemical agents are S-phase dependent.

Numeric alterations reflect genomic instability and are 33 times more frequent in the exposed group than in the control group. Hypodiploidies and hyperdiploidies may involve oncogenes and tumor suppressor genes, which control cell cycles and differentiation processes, in turn may cause an unbalance at the cellular level with serious biologic consequences. Pesticides may be able to induce a new S phase in the cell cycle and bypass mitosis, explaining the finding of endoreduplications only in the exposed group.

Our findings show that the control group presented 2.73% CAs. This number is high compared with those findings in other studies (Kourakis et al. 1992). A possible explanation for this could be that the control group consisted of people living in regions close to the plantation we studied; therefore, risk of contamination by pesticides cannot be discarded.

The cytogenetic findings in the exposed group lead us to consider workers exposed to pesticides as a population with potential carcinogenic risk. This affirmation is based on the association between the frequency of CAs and the risk of developing cancer (Bonassi et al. 2000; Lando et al. 1998).

Results of epidemiologic studies indicated that exposure to pesticides was associated with increased risk from cancer (Blair and White 1981; Blair et al. 1983; Brown et al. 1990; Burmeister 1981; Council on Scientific Affairs 1988; Lucas et al. 2001; Mills and Zahm 2001). No congenital malformations were reported in the offspring of workers occupationally exposed to pesticides. Results of epidemiologic studies did not provide sufficient evidence to associate exposure to pesticides in mothers with congenital defects in children (García et al. 1998).

The four women who suffered miscarriages represent 30.77% of the women population in the study, which is considered to be higher than normal ranges of spontaneous miscarriages occurring during the first trimester of pregnancy (Simpson and Golbus 1992). The number of individuals analyzed is small; this observation is worth further investigation with larger number of subjects.

In the study, we found a connection between CAs and location of work performed. Individuals who worked in the chemical storage room or were in charge of preparing and mixing pesticides for fumigation presented a higher frequency of CA compared with those who worked in administrative functions. This difference may be due to direct exposure to pesticides.

Levels of acetylcholinesterase were below the optimal in 88% of the individuals in the exposed group, which reveals the detrimental effects of exposure to organophosphate pesticides. This exposure may have caused the overstimulation of the nervous system, resulting in symptoms found in 24.3% of the exposed individuals. Studies in chemical plant workers producing dust pesticides and performing ancillary jobs under conditions of lower pesticide exposure have showed changes in some components of humoral and cellular immunity, resulting in chronic bronchitis in some of the cases (Klucinski et al. 2001).

When relating levels of erythrocyte acetylcholinesterase and the percentage of structural chromosome alterations, there is a highly significant negative correlation ($r = 0.416$; $p < 0.01$), indicating that individuals with low erythrocyte acetylcholinesterase levels also show an increase in chromosome aberrations.

From the results of our investigation, we conclude that CA analysis provides a useful biomarker for occupational exposure to pesticide as indicated by the increased incidence of CA in the exposed group compared with the controls.

Table 3. Structural and numerical CAs in the Gp and Gc groups.

Group	No. metaphases analyzed	Altered metaphases (%)								
		Structural CA			Numerical CA					
		Chromosome type	Chromatid type	Total	End	Hyper	Hypo	Total	TOTAL CAs	*p* Gp/Gc
Gp	4,100	88 (2.15)	662 (16.15)	750 (18.29)	44 (1.07)	43 (1.05)	7 (0.17)	94 (2.29)	844 (20.59)	$p < 0.001$*
Gc	4,100	41 (1)	68 (1.66)	109 (2.66)	0 (0)	2 (0.05)	1 (0.02)	3 (0.07)	112 (2.73)	

Abbreviations: End, endoreduplications; Hyper, hyperdiploids; Hypo, hypodiploids.

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