Rev. Brasil. Genet. 15, 1, 169-182 (1992)
(Brazil. J. Genetics)

ANALYSIS OF SISTER CHROMATID EXCHANGES (SCE) IN HUMAN POPULATION STUDIES

Fernando N. Dulout[1], Jorge S. López Camelo[2] and Huberto N. von Guradze[1]

ABSTRACT

The analysis of sister chromatid exchange frequencies in human population studies is discussed, taking into account: 1) the SCE frequencies in a population of floriculturists exposed to pesticides; 2) the distribution of SCE frequencies; 3) the number of cells scored per individual and 4) the proportion of cells with high SCE frequencies (HFC). SCE data were obtained from a sample of 14 floriculturists with chronic intoxication symptoms and 13 floriculturists without chronic intoxication symptoms. These results were compared with those obtained from a sample of nonexposed donors.

For the analysis of SCE frequencies in symptomatic and asymptomatic floriculturists, the use of parametric and nonparametric statistical tests, as well as the comparison of the distribution curves of SCE frequencies is discussed. As no dramatic increases of SCE frequencies would be expected in population studies, the usefulness of different methods combined with the comparison of distribution curves is demonstrated for the detection of small significant differences.

In the floriculturists and nonexposed donors, the SCE frequencies followed a negative binomial distribution, having a poor fit with the Poisson distribution. This confirms the utility of nonparametric statistical tests for the analysis of the data obtained.

The SCE frequencies of 20 individuals after 20, 25, 30 and 50 cell counts were analysed to determine if the number of cells to be scored per individual depends on the population sample size.

Finally, the detection of cells with high SCE frequencies can be used with large sample size and a great number of cells scored per individual.

1 CIGEBA, Facultad de Ciencias Veterinarias, Universidad Nacional de La Plata, 1900 La Plata, Argentina. Send correspondence to F.N.D.

2 Fundação Oswaldo Cruz, Av. Brasil, 4365, 21040 Rio de Janeiro, RJ, Brasil.

INTRODUCTION

The lymphocyte test is, up to now, the most validated methodology available for monitoring populations exposed to suspected genotoxicants. The analysis of chromosomal aberrations or sister chromatid exchanges (SCE) has been used during the last years to investigate the consequences of exposure to different chemicals.

The characteristics, reliability and limitations of the lymphocyte test have been reviewed by several authors (Natarajan and Obe, 1980; Bloom, 1981; Preston *et al.*, 1981; Evans, 1986) who also gave the appropiate guidelines for chromosomal aberrations and SCE analysis. Moreover, the analysis of SCE frequencies obtained either from *in vivo* or *in vitro* studies has been recently discussed in different papers (Moore and Carrano, 1984; Whorton *et al.*, 1984; Wulf *et al.*, 1984a,b; Anderson *et al.*, 1986; Margolin *et al.*, 1986).

In an attempt to determine the possible mutagenic risk associated with the intensive use of pesticides in Argentina, we carried out a study in a population of 154 floriculturists inhabiting the vicinity of Buenos Aires. Samples of 14 individuals showing chronic intoxication symptoms (fatigue, numbness in higher and lower limbs, leg cramps and abdominal pain) and of 13 individuals without intoxication symptoms were selected for SCE analysis (Dulout *et al.*, 1985; Albiano *et al.*, 1986).

The SCE data obtained from symptomatic and asymptomatic floriculturists had some interesting features. Therefore, we examined the distribution of SCE frequencies in this population and in a group of nenexposed healthy donors, the incidence of the number of cells scored and the influence of cells with high SCE frequencies (HFC) (Moore and Carrano, 1984).

MATERIALS AND METHODS

SCE data were obtained from a population of floriculturists inhabiting the vicinity of Buenos Aires. Cytogenetic studies were performed according to routine protocols for SCE analysis (Dulout *et al.*, 1985).

Statistical analysis of SCE frequencies was made as usual, obtaining the frequency of SCE per cell for each individual and the corresponding variance value. The fitness of SCE frequencies to the Poisson distribution was tested according to Wulf *et al.* (1984a,b), using the expression $(N-1) \cdot s^2 / \overline{x}$ for SCE counts in N cells, with a mean $\overline{x}$, and a variance s^2, which must follow a X^2 distribution. Expected SCE frequencies according to a negative binomial distribution was calculated according to the following formula (Cuadras, 1982):

$$p = \frac{\overline{x}}{s^2} \qquad r = \frac{(\overline{x})^2}{s^2 - \overline{x}} \qquad q = 1 - p \qquad E(X) = \frac{rp}{p}$$

$$\mathrm{var}(X) = \frac{rq}{p^2} \qquad f(0) = p^r \qquad f(k+1) = \frac{(1-p)\,(k+r)}{k+1} f(k) \qquad \text{for } X = 0, N.f(0)$$

To determine the influence of sample size we analysed the SCE frequencies in a group of six healthy donors. Heparinized blood samples were obtained. Cultures were performed by adding one ml of blood to nine ml of Ham F 10 medium supplemented with 15% fetal calf serum, phytohemagglutinin, antibiotics and 10 µg/ml bromodeoxyuridine (BrdU). Culture bottles were incubated at 37°C in the dark during 72 hours. Two hours before fixation the cultures received one µg/ml of colchicine (Merck). Hypotonic treatment and fixation were routinely performed with 0.075 M KCl and methanol-acetic acid 3:1. Slides were stained according to Perry and Wolf (1974) with some minor modifications. The analysis of cells with high SCE frequencies was performed according to Moore and Carrano (1984).

RESULTS AND DISCUSSION

SCE frequencies in floriculturists

Table I shows the results obtained from the SCE analysis of 13 asymptomatic and 14 symptomatic floriculturists. The frequency of SCE in the group of floriculturists without symptoms was 5.47. In the group with chronic intoxication symptoms the frequency of SCE was 6.45. The results were analysed by means of nonparametric tests (Mann-Whitney "U" test and Wilcoxon matched-pairs signed-ranks test) and by means of analysis of variance. A significant difference was found when both groups were compared using the Mann-Whitney "U" test ($p = 0.0401$). Moreover, from the 27 individuals studied it was possible to form 9 pairs matched by sex and age (Table II). Comparison between these two groups by means of the Wilcoxon matched-pairs signed-ranks test showed a significant difference ($p < 0.01$). The analysis of variance also indicated a significant difference between floriculturists with and without chronic intoxication symptoms ($p < 0.05$) (Dulout *et al.*, 1985).

These two comparisons clarly indicated that SCE frequencies in floriculturists with chronic intoxication symptoms are higher than in those floriculturists without symptoms. Moreover, the difference can be visualized in Figure 1, which represents the SCE distribution in both groups. The values of SCE are represented in the abscissa and the number of cells with these SCE values in the ordinate. The areas covered by both curves are different, being higher for floriculturists with symptoms (345.5 against 322.5 in absolute values) ($p < 0.05$ with the X^2 test). Nevertheless, if both curves are divided into two parts, before and after the median value (6 SCE/cell), the area covered before the median is higher for the SCE frequencies of asymptomatic floriculturists (156.5 against 134.6 in absolute values), whereas the area covered after the median value is higher for the SCE frequencies of floriculturists with chronic intoxication symptoms (211.0 against 166.0 in absolute values). This fact indicates that in the group of floriculturists without symptoms, the cells with low SCE values are more frequent and the curve is displaced to the left. In the group of floriculturists with symptoms, the higher values of SCE appear more frequently and consequently the curve is displaced to the right.

Table I - SCE frequencies in floriculturists with and without chronic intoxication symptoms. A total of 25 metaphases per donor were analysed except for probands 2 and 9, where 50 metaphases were scored. Slides were scored "blind" by one observer. Randomly selected slides were checked by two different observers. Differences between observers were not significant (data extracted from Dulout *et al.*, 1985).

Floriculturists without symptoms		Floriculturists with symptoms	
Proband	SCE/cell (s^2)	Proband	SCE/cell (s^2)
20	2.88 (3.09)	11	4.84 (5.76)
8	4.56 (4.24)	37	4.92 (4.08)
6	4.76 (5.10)	34	5.32 (9.79)
25	5.20 (10.17)	24	5.36 (5.81)
33	5.28 (7.95)	15	5.60 (7.56)
1	5.72 (9.79)	2	6.32 (11.48)
5	5.72 (8.04)	4	6.36 (9.74)
32	5.76 (8.76)	12	6.40 (10.24)
19	5.80 (9.36)	14	6.64 (8.82)
9	5.84 (7.67)	7	6.88 (5.43)
35	5.92 (6.70)	13	7.04 (12.32)
10	6.64 (14.28)	30	7.76 (23.71)
16	7.08 (15.13)	3	8.12 (18.86)
		27	8.76 (17.55)
All probands	5.47 (8.48)	All probands	6.45 (10.79)

Table II - Comparison of SCE frequencies in 18 floriculturists paired by sex and age (data from Dulout *et al.*, 1985).

Individuals with symptoms				Individuals without symptoms			
Proband	Sex	Age	SCE/cell	Proband	Sex	Age	SCE/cell
2	F	45	6.32	35	F	48	5.92
4	M	21	6.36	10	M	22	2.57
7	F	53	6.88	25	F	50	5.20
12	M	17	6.41	33	M	11	5.28
13	F	63	7.04	1	F	78	5.72
5	M	65	5.60	4	M	72	5.72
24	M	52	5.36	20	M	55	2.88
27	M	30	8.76	6	M	37	4.76
34	M	29	5.32	16	M	25	7.08

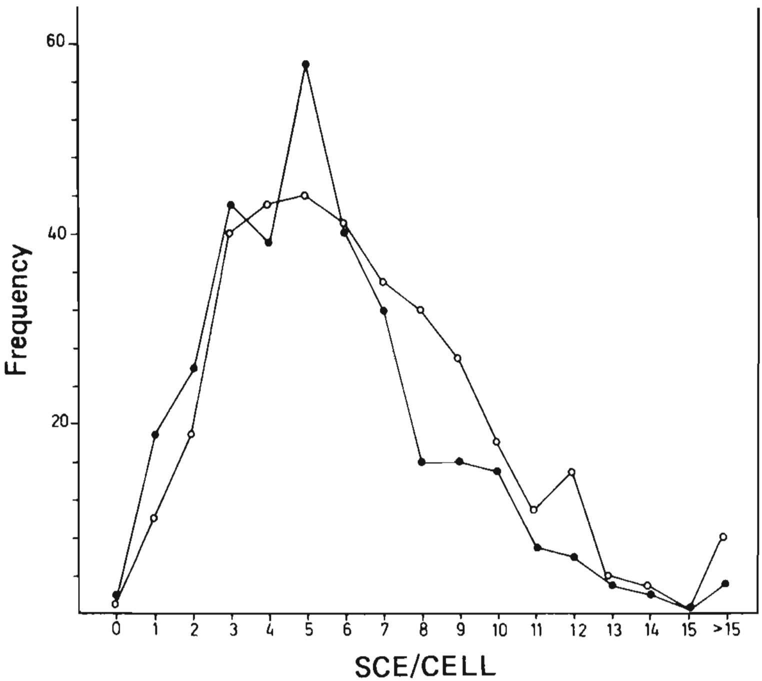

Figure 1 - SCE distribution in the group of floriculturists with chronic intoxication symptoms (___o __o___) and in the group of floriculturists without chronic intoxication symptoms (___● __●___).

Distribution of SCE frequencies

The distribution of SCE was analysed in data obtained from the floriculturist population and from 20 nonexposed healthy donors. The fitness of SCE frequencies to the Poisson distribution was tested according to Wulf *et al.* (1984a,b). Table III summarizes the results of this analysis, and clearly shows that the Poisson distribution gives a poor fit for SCE frequencies. Therefore, we tested the fitness of SCE frequencies to a negative binomial distribution, since in most cases $E(\overline{x}) < E(s^2)$ (only in donors 7, 8, and 37, $E(\overline{x}) > E(s^2)$) (Table II).

Table III - Distribution of SCE frequencies in 14 floriculturists with chronic intoxication symptoms, 13 floriculturists without chronic intoxication symptoms, and 20 nonexposed healthy donors, following their individual X^2 values, which were obtained using the formula $X^2 = (N-1) . s^2/\overline{x}$. Degrees of freedom (DF) for the X^2 intervals depend on the number of cells scored per person. To simplify, only the first 25 cells scored were considered for probands 2 and 9. The number of cells scored in the group of nonexposed healthy donors was 20 per individual.

Group	Chi square interval	Observed number of persons in each X^2 interval	Expected number of persons in each X^2 interval
Without symptoms	< 13.85	0	0.65
(df = 24)	13.85 - 19.94	0	3.25
	19.94 - 23.34	1	2.60
	23.34 - 27.10	2	2.60
	27.10 - 38.42	3	3.25
	> 38.42	7	0.65
	$X^2 = 74.16$	df = 5	$p < 0.001$
With symptoms	< 13.85	0	0.70
	13.85 - 19.94	2	3.50
	19.94 - 23.34	0	2.80
	23.34 - 27.10	1	2.80
	27.10 - 38.42	3	3.50
	> 38.42	8	0.70
	$X^2 = 81.50$	df = 5	$p < 0.001$
Nonexposed probands	> 10.12	1	1.00
	10.12 - 15.35	2	5.00
	15.35 - 18.34	3	4.00
	18.34 - 21.69	4	4.00
	21.69 - 30.14	6	5.00
	> 30.14	4	1.00
	$X^2 = 11.25$	df = 5	$p < 0.05$

Figure 2 represents the comparisons between observed and expected SCE frequencies in floriculturists with and without symptoms, according to a negative binomial distribution. As can be seen, both distributions gave a good fit with no significant differences between observed and expected values.

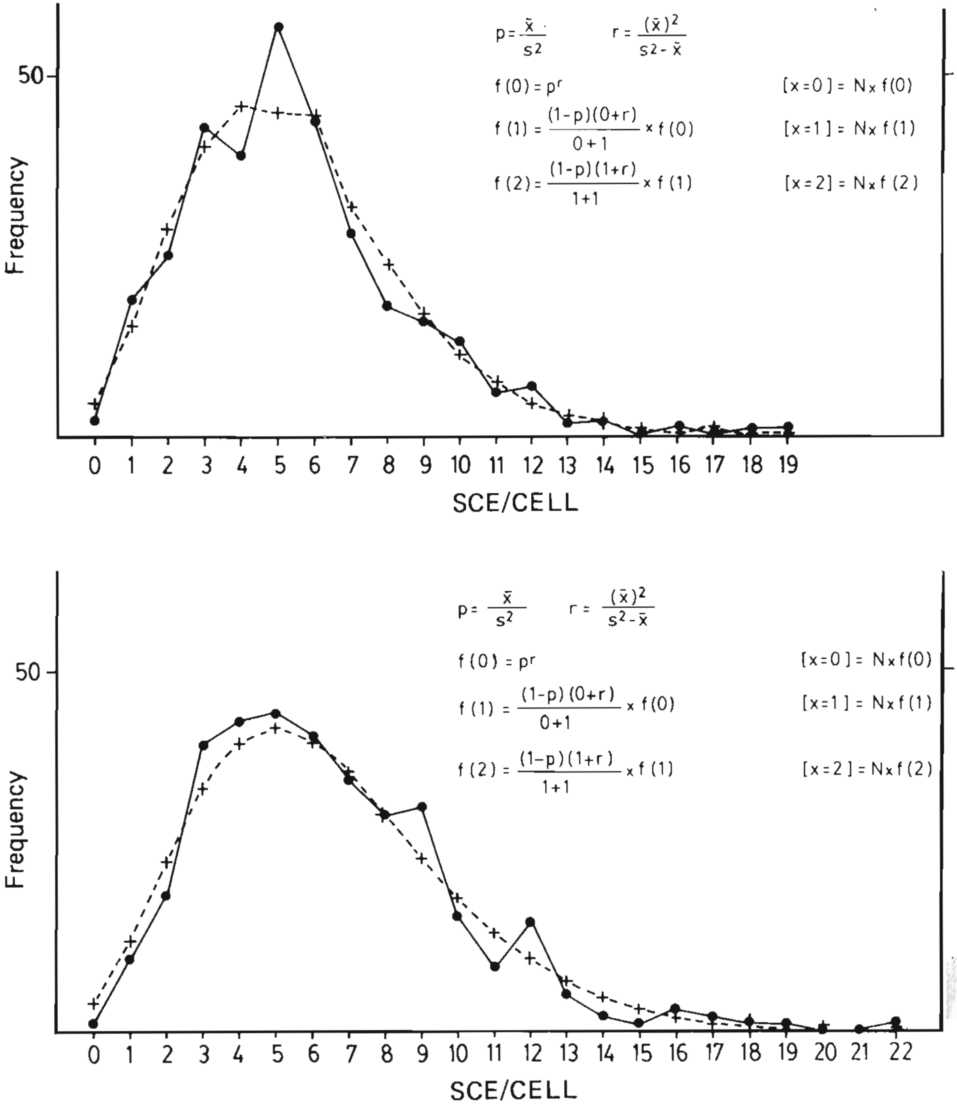

Figure 2 - Comparison of the SCE frequencies scored in floriculturists (___●___●___) with the expected negative binomial distribution (- + - - - + -). Above, floriculturists without chronic intoxication symptoms. Below, floriculturists with chronic intoxication symptoms.

The SCE frequencies of nonexposed donors exhibited a lower discordance with the Poisson distribution than that observed in floriculturists. Nevertheless, its fit to the negative binomial is similar and is also without significant differences between observed and expected values. Figure 3 shows the negative binomial distribution of SCE frequencies of all the floriculturists and of the group of nonexposed donors.

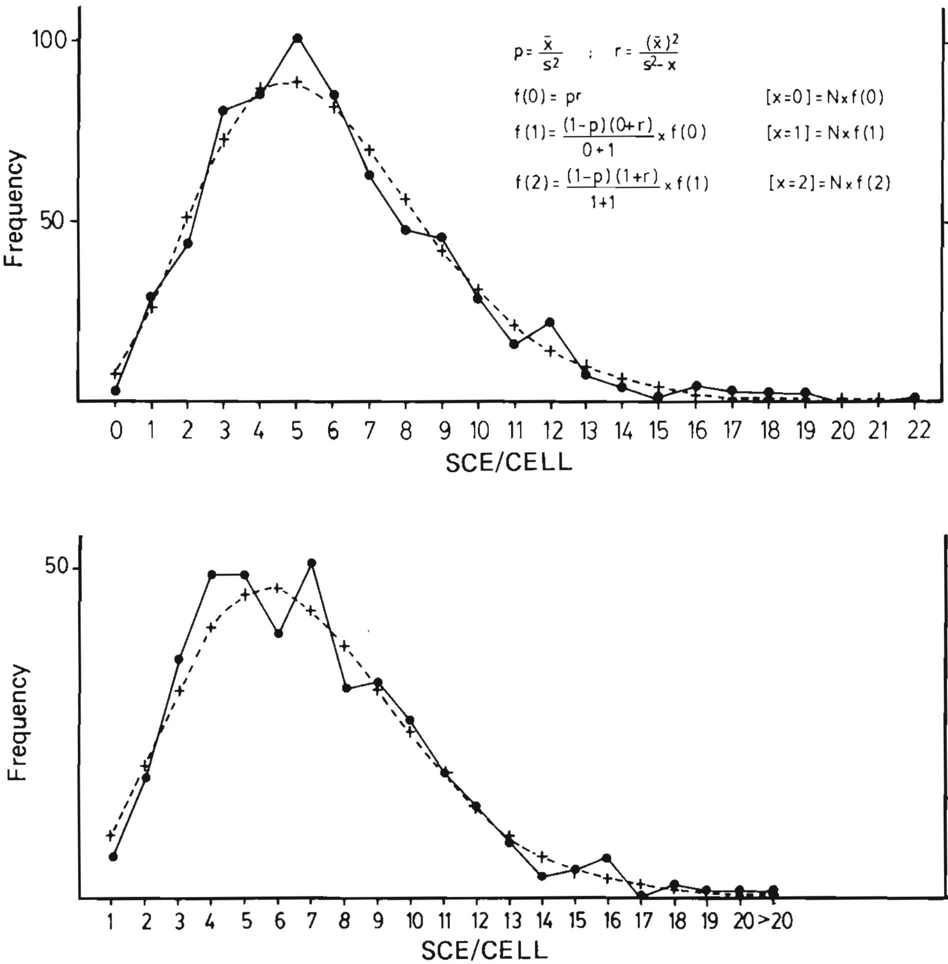

Figure 3 - Fitness of SCE frequencies of all the floriculturists and the group of nonexposed donors (___●___●___) with the negative binomial distribution (- + --- + -). Above, floriculturists. Below, nonexposed donors.

These results are in agreement with those of Wulf *et al.* (1984a,b) in the sense that SCE frequencies in human peripheral blood lymphocytes follow a negative binomial distribution. The situation with cultured cells lines seems to be different since Margolin *et al.* (1986) reported that the Poisson distribution describes well SCE data from CHO cells. The origin of this discrepancy could be originated by the heterogeneity of the lymphocyte system, with cell populations dividing at different rates and a conspicuous intercellular variability in SCE counts. Consequently, the analysis of the statistical distribution of SCE should be important in population studies.

Number of cells scored

There is a general agreement that a sample of 25-30 cells per individual is sufficient for SCE analysis in human populations. Whorton *et al.* (1984) demonstrated that the analysis of 50 cells instead of 25 does not improve the sensitivity of the system. The selection of a correct cell sample size is very important in population studies since duplication of cell sample considerably increases the duration of screening and experimental costs.

We analysed the problem of the number of cells necessary for SCe counts in a group of six healthy donors. A total of 50 cells per individual were scored for SCE counts. SCE frequency, as well as variance, were determined sequentially after the analysis of 20, 25, 30 and 50 metaphases (Table IV). There were no significant differences between SCE frequencies obtained after counting 20, 25, 30, or 50 cells. Consequently, we can conclude that a sample of only 20 cells would be adequate for human population studies. Nevertheless, the example given in Figure 4 could clarify the situation. The histogram represents the SCE frequencies in 50 cells of two floriculturists (numbered 2 and 9 in Table I) and the upper curve the average SCE values after the second count. It is shown that the average values are erratic in the first counts and progressively became stabilized as the number of scored cells increased. But whereas this estabilization is evident after the 25th cell and remains constant after the 30th cell in donor number 2 (upper histogram), it is only reached after the 30th cell in donor number 9, becoming constant after the 38th cell (lower histogram). Therefore, it seems impossible to predict after which cell count the average SCE frequency will become constant in such a way that the average SCE value obtained could be considered as the correct frequency of an analysed individual. Thus, the number of cells to score for SCE frequencies in a population study must be predermined, taking into account population size. The greater the number of individuals, the lower the number of cells to be scored. We believe that with populations exceeding 25 persons, the analysis of 25-30 metaphases per individual would be appropiate even to determine small differences. With small populations the number of cells scored must be increased proportionally.

Table IV - SCE frequencies in six healthy donors after counts of 20, 25, 30, and 50 cells.

Donor	20 cells $\bar{x}$ (s^2)	25 cells $\bar{x}$ (s^2)	30 cells $\bar{x}$ (s^2)	50 cells $\bar{x}$ (s^2)
101	4.70 (4.64)	4.72 (3.79)	4.53 (3.63)	4.48 (3.84)
104	5.25 (9.46)	5.04 (8.54)	5.16 (9.04)	5.02 (6.92)
105	4.50 (7.00)	4.68 (7.31)	4.66 (6.78)	4.72 (5.88)
106	5.10 (7.36)	5.08 (7.33)	4.73 (6.75)	4.53 (5.40)
107	4.70 (4.85)	4.64 (3.99)	4.43 (3.70)	4.78 (4.95)
108	5.50 (6.05)	5.32 (5.22)	5.33 (4.36)	5.60 (5.63)

Cells with high SCE frequency (HFC)

Recently, Moore and Carrano (1984) proposed that the proportion of cells with high SCE frequency could be an important parameter to quantify the exposure to genotoxic agents in human populations. The HFC has been defined as a threshold number of SCE per chromosome that can be determined in terms of a chosen percentile (for instance 95th). Then, increased HFC could be used in detecting low levels of genetic damage. The statistical properties of HFC have been described in a sample of 42 healthy nonsmokers and 29 healthy smokers, analysing 80 cells per individual (Moore and Carrano, 1984).

We analysed the HFC in the population of floriculturists using the formula proposed by Moore and Carrano (1984). Table V shows the results obtained. The threshold of HFC with a percentile of 95 was 13 SCE/cell. In the group of florisculturists with chronic intoxication symptoms, 50% of the individuals had cells with 13 or more SCE/cell. In the group of floriculturists without chronic intoxication symptoms, 5 over 13 individuals had cells with 13 or more SCE/cell. The differences between both groups were not significant ($p < 0.90$). The lack of differences between symptomatic and asymptomatic florisculturists for frequency of HFC could be attributed to two factors: 1) the low number of individuals in both groups and 2) the number of metaphases scored per individual. Moore and Carrano (1984) scored 80 cells per person in a sample of 71 people. This means a triplicate sample in relation to our study in floriculturists. It should be interesting to analyse whether there is a sample threshold or a minimal level of HFC to detect significant differences between exposed and nonexposed populations.

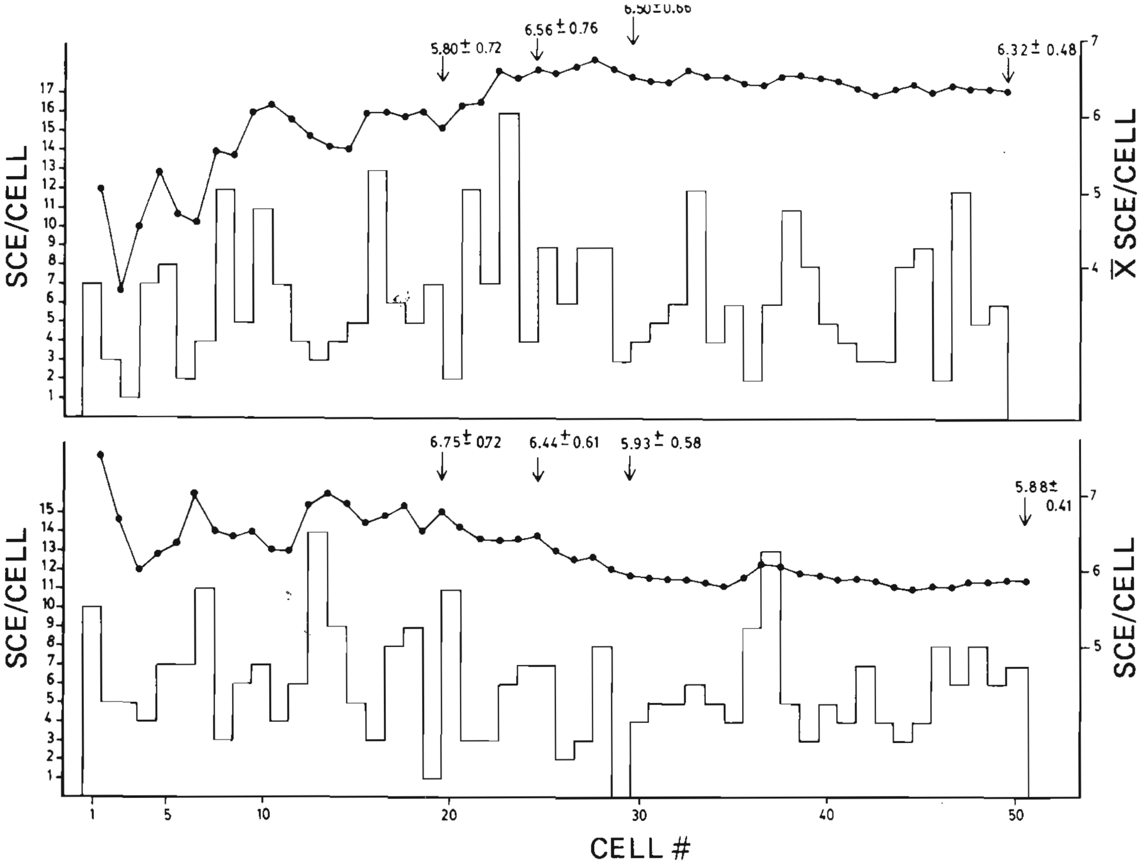

Figure 4 - SCE frequencies in 50 cells of two floriculturists (numbered 2 and 9 in Table I). The histogram represents the SCE counts and the upper curves the average SCE values after the second count. Average SCE values with their corresponding Standard Error of Mean (SEM) are indicated after counting 20, 25, 30, and 50 cells.

Table V - Number of individuals with and without HFCs in the population of floriculturists.

Group	Without HFCs (fewer than 13 SCE)	With HFCs (13 or more SCE)	Total
With symptoms	7	7	14
Without symptoms	8	5	13
Total	15	12	27
	x^2 - 0.364	$p > 0.80$	

CONCLUSIONS

The examples given are a demonstration of the reliability as well as of the limitations of SCE analysis in studies of human populations exposed to chemicals, in the sense that:

1. Small differences in SCE frequencies can be detected using both parametric and nonparametric statistical tests. These differences can be confirmed by comparing the distribution curves of SCE frequencies in exposed and nonexposed populations.

2. SCE frequencies in human blood lymphocytes follow a negative binomial distribution. It would be interesting, however, to check the type of statistical distribution followed by SCE frequencies in every population study in order to confirm this fact. SCE frequencies in human populations are low, even in those exposed to chemicals, where no dramatic increases could be expected. It is not possible to predict that high SCE frequencies, induced, for example, by chemicals under *in vitro* conditions, will also follow a negative binomial distribution.

3. Population sample size as well as the number of cells to be scored per individual must be predetermined. Both parameters are related and, consequently, the number of metaphases analysed per individual must be inversely proportional to sample size.

4. The analysis of HFC would be very useful for detecting small differences. Nevertheless, it seems that a considerable number of individuals as well as a large number of cells per individual are necessary to obtain statistically significant differences.

ACKNOWLEDGMENTS

SCE data from 20 non exposed probands was kindly provided by Dr. N.O. Bianchi, Dr. M.S. Bianchi, and Dr. M.L. Larramendy. The authors also thank Prof. Susana J. Barani for her helpful collaboration in the preparation of the manuscript.

This work was supported by grants from the Consejo Nacional de Investigaciones Científicas y Técnicas (PID 3-050600/88) and the Comisión de Investigaciones Científicas de la Provincia de Buenos Aires.

RESUMO

A análise das frequências da troca de cromátides irmãs (SCE) em estudos de populações humanas é discutida levando em conta: as frequências de SCE em uma população de floricultores expostos à pesticidas; 2) a distribuição das frequências de SCE; 3) o número de células contadas por indivíduo e 4) a proporção com altas frequências de SCE (HFC). Dados sobre SCE foram obtidos a partir de uma amostra de 14 floricultores com sintomas de intoxicação crônica e 13 sem. Esses resultados foram comparados com aqueles obtidos de uma amostra de doadores não expostos.

Para a análise das frequências de SCE em floricultores sintomáticos e não sintomáticos, o uso de testes estatísticos paramétricos e não paramétricos, bem como uma comparação das curvas de distribuição das frequências de SCE são discutidas. Sendo que nenhum aumento da frequência de SCE seria esperado em estudos de populações, a utilidade dos diferentes métodos combinados com a comparação das curvas de distribuição é demonstrada para a detecção de pequenas diferenças significativas.

Nos floricultores e nos doadores não expostos, as frequências de SCE seguiram uma distribuição binomial negativa, tendo pouca correlação com a distribuição de Poisson. Isto confirma a utilidade dos testes estatísticos para a análise dos dados obtidos.

As frequências de SCE de 20 indivíduos após 20, 25, 30 e 50 células foram analisadas para determinar se o número de células a serem contadas por indivíduo dependem do tamanho da amostra da população.

Finalmente a detecção das células com altas frequências de SCE pode ser usada com uma grande amostra e um grande número de células examinadas por indivíduo.

REFERENCES

Albiano, N., Matos, E., Uizich, R., Buján, E.C. de, Loria, D., Sobel, N. and Dulout, F. (1986). Efectos sobre la salud por uso prolongado de plaguicidas. Estudio Clínico Bioquímico. *Acta Bioquímica Clínica Latinoamericana 20*: 65-72.

Anderson, D., Dewdey, R.S., Jenkinson, P.C., Lovell, D.P., Butterworth, K.R. and Conning, D.M. (1986). Sister chromatid exchanges (SCE) analysis in 106 control individuals. In: *Monitoring of Occupational Genotoxicants* (Sorsa, M. and Norppa, H., eds.) Alan R. Liss Inc., New York.

Bloom, A.D. (1981). *Guidelines for studies of human populations exposed to mutagenic and reproductive hazards*. March of Dimes Birth Defects Foundation, New York.

Cuadras, C.M. (1982). *Problemas de probabilidades y estadística*. Editorial Universitaria de Barcelona, Barcelona.

Dulout, F.N., Pastori, M.C., Olivero, O.A., González Cid, M., Loria, D., Matos, E., Sobel, N., Buján, E.C. de, and Albiano, N. (1985). Sister chromatic exchanges and chromosomal aberrations in a population exposed to pesticides. *Mutation Res. 143*: 237-244.

Evans, H.J. (1986). What has been achieved with cytogenetic monitoring? In: *Monitoring of Occupational Genotoxicants* (Sorsa, M. and Norppa, H., eds.) Alan R. Liss Inc., New York.

Margolin, B.H., Resnick, M.A., Rimpo, J.Y., Archer, P., Galloway, S.M., Bloom, A.D. and Zeigler, E. (1986). Statistical analyses for *in vitro* cytogenetic assays using Chinese hamster ovary cells. *Environ. Mutagen. 8*: 183-204.

Moore, D.H. and Carrano, A.V. (1984). Statistical analysis of high SCE frequency cells in human lymphocytes. In: *Sister Chromatid Exchanges* (Tice, R.R. and Hollaender, A., eds.) Plenum Press, New York and London.

Natarajan, A.T. and Obe, G. (1980). Screening of human populations for mutations induced by environmental pollutants: use of human lymphocyte system. *Ecotoxicol. Environ. Safety 4*: 468-481.

Perry, P. and Wolff, S. (1974). New giemsa method for the differential staining of sister chromatids. *Nature 251*: 156-158.

Preston, R.J., Au, W., Bender, M.A., Brewen, J.G., Carrano, A.V., Heddle, J., McFee, A.F., Wolff, S. and Wassom, J.S. (1981). Mammalian *in vivo* and *in vitro* cytogenetic assays. *Mutation Res. 87*: 143-188.

Whorton, E.B., Tice, R.R. and Stetka, D.G. (1984). Statistical design, analysis, and inference issues in studies using sister chromatid exchanges. In: *Sister Chromatid Exchanges* (Tice, R.R. and Hollaender, A., eds.) Plenum Press, New York and London.

Wulf, H.C., Husum, B., Plessner, A.M. and Niebuhr, E. (1984a). Distribution of SCEs in lymphocytes of persons with normal, slightly increased, and heavily increased SCEs. *Mutation Res. 125*: 263-268.

Wulf, H.C., Husum, B., Engberg-Pedersen, H. and Niebuhr, E. (1984b). Guidelines for the statistical evaluation of SCE. In: *Sister Chromatid Exchanges* (Tice, R.R. and Hollaender, A., eds.) Plenum Press, New York and London.

(Received June 4, 1990)