Cytogenetic Monitoring of Farmers Exposed to Pesticides in Colombia

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We have monitored 30 pesticide-exposed workers and 30 matched controls for expression of chromosome aberrations (CA) and sister chromatid exchanges (SCE) in their lymphocytes. Peripheral blood cultures were set up within 3 hr after the collection of samples, and four cultures were set up from each donor. For CA analysis, 100 complete metaphase cells from each donor were evaluated. For the SCE assay, 50 complete metaphase cells from each donor were analyzed. The CA and SCE data were analyzed for differences between the two groups using the χ² and the Student’s t-test, respectively. From the CA analysis it was obvious that the overwhelming majority of aberrations were chromatid breaks and isochromatid breaks; therefore, only these data are presented and used for statistical analysis. Isochromatid breaks were counted as two breaks each and chromatid breaks as one in calculating the total chromatid break frequencies. Statistical evaluation of the data indicates that there is no significant difference (p>0.05; χ² test) between the exposed and the nonexposed groups based on chromatid breaks per 100 cells (1.2 ± 0.3 and 1.5 ± 0.2, respectively) and total chromatid breaks per 100 cells (1.7 ± 0.3 and 2.1 ± 0.2, respectively). No significant difference between the two groups (p>0.05, Student’s t-test) was observed with SCE frequencies (5.0 ± 1.1 and 4.8 ± 0.9, respectively). Linear regression analysis indicates that the data were not influenced by age, cigarette smoking, or alcohol consumption. It is assuring that the exposure conditions among these Indian farmers have not caused detectable increases of chromosome damage using standard assays; this suggests the lack of serious long-term health problems. However, periodic monitoring of such exposed populations should be conducted using the same or other more sensitive assays. In addition, other populations with exposure to different types of pesticides in Colombia should also be investigated. — Environ Health Perspect 104(Suppl 3):535–538 (1996)

Key words: pesticide exposure, chromosome aberrations, sister chromatid exchanges, Colombian farmers

Introduction

Pesticides are some of the most widely used chemicals throughout the world. Due to inadequate management of their use in the past, substantial exposure of humans and extensive contamination of natural resources (e.g., rivers) have occurred. Toxicity to individuals who have been exposed to excessive amounts of pesticides have been documented. Many pesticides are suspected to have mutagenic, carcinogenic, and teratogenic activities as reported by the International Agency for Research on Cancer (J) and Kourakis et al. (2). Negative findings have also been reported. These negative effects may be due to reduced exposure or to exposure to less toxic pesticides. On the other hand, contaminants in pesticides may be contributors to the observed genotoxicity (3). Because a population in one country may be exposed to different types and quantities of pesticides from similarly employed populations in a different country, the potential health risk from exposure to pesticides from each country should be systematically investigated.

In Colombia the use of pesticides/fungicides is prevalent, widespread exposure is expected, and toxic effects from accidental exposure have been documented. It has been reported by Vargas and Vallejo (4) that organochlorine insecticide residues in milk of humans and cows in certain areas of Colombia exceed regulatory limits. Increased risk of hemangioma is detected among children born to a population exposed occupationally to pesticides (5). Therefore, long-term health problems from environmental exposure to pesticides/fungicides may exist in Colombia. This problem should be identified from systematic scientific studies, and exposed workers are an appropriate group to conduct the study. Workers in Colombia who are exposed to hazardous pesticides under current conditions should be monitored using relevant biomarkers to provide early warning signals for potential health effects.

In our study we have targeted a population residing in Paletara and Coocuco, which are located in the central-eastern region of the State of Cauca in Colombia; they are ethnic native Indians and are predominantly farmers. Over 10 years ago, their farming practice was changed to the cultivation of a single crop, the potato, and to the extensive use of pesticides/fungicides as listed in Table 1. The list contains organophosphates and carbamates. Many of them have been shown to be genotoxic and potentially carcinogenic (6).

The farm workers in this area usually do not wear gloves or have protective covering when they apply the pesticides; therefore, they are definitely exposed. Furthermore, due to the mild weather year round in the region, these farmers work on the field throughout the year; thus, the population has rather uniform exposure conditions. Such conditions should facilitate studies in establishing a cause–effect relationship; therefore these workers were chosen as a model population for our investigation.
Table 1. Pesticides and fungicides frequently used by the studied population.

| Trade name | Chemical group |
|------------|----------------|
| Furadon    | Carbamate      |
| Curacon    | Organophosphate|
| Tamaron    | Organophosphate|
| Monitor    | Organophosphate|
| Curater GR3| Parathion      |
| Parathion  | Parathion      |
| Garrafos   | Organophosphate|
| Fungicides |                |
| Ridoimil   | Dithiocarbonate|
| Dithane    | Dithiocarbonate|
| Duter 20%  | Dithiocarbonate|
| Manzate    | Dithiocarbonate|
| Patafol    | Dithiocarbonate|
| Curzate    | Dithiocarbonate|
| Oxiclonuro | Carbamate      |

We have monitored 30 pesticide-exposed workers and 30 matched controls for expression of chromosome aberrations (CA) and sister chromatid exchanges (SCE). Our data indicate that there are no exposure-related cytogenetic effects.

Materials and Methods

Selection of Participants

Several organizational meetings were held with community leaders and members of the City Council of Cauca to present the rationale and the logistics of the study. With their approval, announcements were made to recruit volunteers to participate in the study. After obtaining informed consent from the volunteers, our trained interviewers collected their personal information according to the procedures of Au et al. (7). Demographic information regarding age, gender, marital status, residential history, occupational history, lifestyle activities, health information, etc., were collected and used to qualify individuals for the study. Criteria for acceptance of exposed individuals were exposure to pesticides as a full-time farmer for over 5 years, no current or previous exposure to radiotherapy or to cytotoxic chemotherapeutic drugs, and no recent illness. Criteria for acceptance of nonexposed individuals were the same as for exposed individuals except that they were not exposed to pesticides. Consumption of cigarettes and alcohol was prevalent so they could not be used as criteria for exclusion of participants; however, their consumption was light and similarly reported by both the exposed and nonexposed groups. Cigarette smokers claimed to consume about 1 to 2 cigarettes per day. Drinkers claimed to drink approximately twice per month.

Accepted individuals from the exposed and the nonexposed groups were matched for age (+5 years), gender, and smoking and drinking habits. Matched pairs were interviewed again to verify the collected information. Those who had no current illness were asked to donate blood samples for laboratory analysis.

Blood Sample Collection

Four matched pairs of individuals were asked to come to a local clinic at the same time for donation of blood samples. Each individual donated 10 ml of blood, which was collected into heparinized syringes from a vein in the arm. The samples were packed in boxes, maintained at room temperature, and transported to the laboratory by car immediately after the collection.

Blood Culture and Cytogenetic Assays

Upon arrival in the laboratory, blood cultures (four from each donor) were set up immediately using conditions similar to those reported by Au et al. (7). The cultures were set up within 3 hr after the collection of samples. For setting up blood cultures, 0.5 ml whole blood was added to 9.5 ml RPMI 1640 medium (Gibco, Buffalo, NY) supplemented with 10% heat-inactivated fetal bovine serum (Gibco), 1% L-Glutamine (stock concentration 29.2 mg/ml; Gibco), 1% streptomycin–penicillin antibiotics (Gibco), and 0.25 ml reagent grade phytohemagglutinin (stock concentration 9 mg/ml; Burroughs Wellcome, Research Triangle Park, NC). All cell culture medium and reagents were from the same lot from the suppliers. The cultures were maintained with tightened caps and incubated at 37°C. At 6 hr after initiation of cultures, bromodeoxyuridine was added to each culture to reach a final concentration of 10 μM. Cultures were then returned to the incubator and maintained until harvest time.

At 50 hr and 70 hr after initiation of blood cultures, respectively, colcemid (0.1 μl per culture from stock concentration of 0.1 μg/ml) was added to two of the cultures. Two hours later, cultures were harvested for CA and SCE assays. Cells were centrifuged to remove culture medium, swollen with 0.075 M KCl for 15 min at 37°C, and treated twice with Carnoy’s fixative (3:1 ratio of methanol:acetic acid). Cytological preparations were made by dropping 2 to 3 drops of concentrated cell suspension onto slides wet with ice cold water. These preparations were then dried on a hot plate and stored at room temperature. Later these slides were stained using the fluorescence-plus-Giemsa technique.

Stained slides were coded by an assistant who was not involved in microscopic analysis of the slides. The coded slides were then evaluated for frequencies of CA and SCE by two assistants. For CA analysis, 100 complete metaphase cells from each donor showing the first metaphase staining pattern were evaluated under high power (1000× magnification). For the SCE assay, 50 complete metaphase cells from each donor, showing differential staining patterns, were selected and evaluated, and the frequency of SCE per cell was recorded. Each assistant analyzed the same number of cells from each donor. All types of chromosome aberrations were recorded. The data were recorded on standard score sheets and later entered into computer files.

Statistical Analysis

The CA and SCE data were analyzed for differences between the two groups using the χ2 and the Student’s t-test, respectively. The relationship between the two cytogenetic effects and lifestyle factors were evaluated using regression analysis.

Results

A total of 179 volunteers were interviewed. The collected information was used for selection of exposed and nonexposed individuals for our study based on our predetermined criteria. The qualified individuals from one group were matched with those from the other group based on described criteria. As a result, 30 pairs of participants were selected. The demographics of the two populations are summarized in Table 2. As shown in the table, the ages of the exposed group range from 17 to 52 years with a mean of 33.6 ± 10.2 years, and the ages of
the nonexposed group range from 18 to 53 years with a mean of 32.2 ± 10.6 years. The gender ratio is 26 to 4 for male to female for both groups. The mean occupational exposure time to pesticides/fungicides for the exposed group is 16.5 ± 8.8 years. The nonexposed group did not have previous occupational exposure to pesticides. There were 10 cigarette smokers and 23 alcohol drinkers in each of the two groups.

From the CA analysis, it was obvious that the overwhelming majority of aberrations were chromatid breaks and isochromatid breaks; therefore, only these data are presented and used for statistical analysis. Isochromatid breaks were counted as two breaks each and chromatid breaks counted as one in calculating the total chromatid break frequencies. A summary of the CA and SCE data for each subject is presented in Tables 3 and 4 for the exposed and nonexposed groups, respectively. Statistical evaluation of the data indicates that there is no significant difference (p > 0.05, χ² test) between the exposed and nonexposed groups based on chromatid breaks per 100 cells (1.2 ± 0.3 and 1.5 ± 0.2, respectively), and total chromatid breaks per 100 cells (1.7 ± 0.3 and 2.1 ± 0.2, respectively). No significant difference between exposed and nonexposed groups (p > 0.05, Student’s t-test) was observed with SCE frequencies per cell (5.0 ± 1.1 and 4.8 ± 0.9, respectively). Linear regression analysis indicates that data were not influenced by age, cigarette smoking, or alcohol consumption.

### Discussion

We have conducted a population monitoring study using an adequate number of individuals. The participants from the exposed and nonexposed populations were carefully selected and well matched. The results from this study indicate that there is no exposure-related induction of chromosome damage as indicated by our CA and SCE assays. The lack of influence from cigarette smoking is consistent with the observation of Kourakis et al. (2). Although many previous studies demonstrated cytogenetic effects from exposure to pesticides, our negative finding suggests that, perhaps, our population is exposed to lower concentrations of pesticides or that the current pesticides are less mutagenic than before. However, these suggestions need to be confirmed by environmental monitoring of work conditions and by assays to investigate the genotoxic activities of our pesticides. It is also possible that the diet of these workers, which is made up of large

### Table 3. Chromosome aberrations and sister chromatid exchanges in the exposed population.

| #  | Age (years) | Sex | Chromatid breaks/100 cells | Isochromatid breaks/100 cells | Total chromatid breaks/100 cells | Mean SCE/cell |
|----|-------------|-----|---------------------------|-------------------------------|---------------------------------|--------------|
| 1  | 28          | M   | 1                         | 1                             | 3                               | 4.26         |
| 2  | 44          | M   | 0                         | 0                             | 0                               | 3.3          |
| 3  | 52          | F   | 1                         | 0                             | 1                               | 5.6          |
| 4  | 37          | F   | 1                         | 0                             | 1                               | 5.98         |
| 5  | 22          | M   | 2                         | 0                             | 2                               | 3.96         |
| 6  | 47          | M   | 0                         | 0                             | 0                               | 4.7          |
| 7  | 23          | M   | 0                         | 1                             | 2                               | 4.66         |
| 8  | 33          | M   | 1                         | 0                             | 1                               | 4.08         |
| 9  | 25          | F   | 1                         | 0                             | 1                               | 5.56         |
| 10 | 34          | F   | 4                         | 0                             | 4                               | 7.1          |
| 11 | 43          | M   | 1                         | 1                             | 3                               | 5.6          |
| 12 | 30          | M   | 1                         | 0                             | 1                               | 4.86         |
| 13 | 50          | M   | 4                         | 0                             | 4                               | 4.4          |
| 14 | 29          | M   | 1                         | 0                             | 1                               | 6.46         |
| 15 | 30          | M   | 6                         | 0                             | 6                               | 4.58         |
| 16 | 25          | M   | 2                         | 1                             | 4                               | 5.14         |
| 17 | 48          | M   | 1                         | 0                             | 1                               | 6.34         |
| 18 | 50          | M   | 1                         | 0                             | 1                               | 6.52         |
| 19 | 33          | M   | 0                         | 0                             | 0                               | 5.22         |
| 20 | 36          | M   | 2                         | 0                             | 2                               | 4.16         |
| 21 | 26          | M   | 0                         | 0                             | 0                               | 2.88         |
| 22 | 13          | M   | 1                         | 0                             | 1                               | 4.05         |
| 23 | 30          | M   | 1                         | 0                             | 1                               | 4.18         |
| 24 | 17          | M   | 1                         | 0                             | 1                               | 6.16         |
| 25 | 20          | M   | 0                         | 0                             | 0                               | 4.2          |
| 26 | 32          | M   | 0                         | 0                             | 0                               | 2.72         |
| 27 | 18          | M   | 2                         | 0                             | 2                               | 5.88         |
| 28 | 44          | M   | 2                         | 0                             | 2                               | 5.64         |
| 29 | 40          | M   | 0                         | 1                             | 2                               | 6.1          |
| 30 | 24          | M   | 0                         | 1                             | 1                               | 4.24         |

Mean: 1.233, SD: 1.382, SEM: 0.252

### Table 4. Chromosome aberrations and sister chromatid exchanges in nonexposed populations.

| #  | Age (years) | Sex | Chromatid breaks/100 cells | Isochromatid breaks/100 cells | Total chromatid breaks/100 cells | Mean SCE/cell |
|----|-------------|-----|---------------------------|-------------------------------|---------------------------------|--------------|
| 1  | 23          | M   | 3                         | 0                             | 3                               | 5.12         |
| 2  | 43          | M   | 1                         | 2                             | 5                               | 4.12         |
| 3  | 53          | F   | 1                         | 0                             | 1                               | 4.5          |
| 4  | 40          | F   | 2                         | 0                             | 2                               | 5.54         |
| 5  | 26          | M   | 3                         | 0                             | 3                               | 3.82         |
| 6  | 53          | M   | 0                         | 1                             | 1                               | 4.8          |
| 7  | 7           | M   | 2                         | 0                             | 2                               | 3.78         |
| 8  | 34          | M   | 0                         | 0                             | 0                               | 5.42         |
| 9  | 26          | M   | 1                         | 0                             | 1                               | 4.78         |
| 10 | 28          | F   | 1                         | 0                             | 1                               | 5.76         |
| 11 | 40          | M   | 1                         | 0                             | 1                               | 6.08         |
| 12 | 28          | M   | 4                         | 0                             | 4                               | 3.48         |
| 13 | 47          | M   | 2                         | 0                             | 2                               | 4.96         |
| 14 | 25          | M   | 3                         | 1                             | 5                               | 5.08         |
| 15 | 32          | M   | 1                         | 1                             | 3                               | 4.36         |
| 16 | 24          | M   | 2                         | 0                             | 2                               | 4.8          |
| 17 | 52          | M   | 1                         | 0                             | 1                               | 4.12         |
| 18 | 45          | M   | 1                         | 0                             | 1                               | 6.34         |
| 19 | 29          | M   | 2                         | 0                             | 2                               | 6.16         |
| 20 | 35          | M   | 0                         | 0                             | 0                               | 4.4          |
| 21 | 21          | M   | 3                         | 0                             | 3                               | 4.04         |
| 22 | 37          | M   | 1                         | 0                             | 1                               | 5.44         |
| 23 | 24          | M   | 1                         | 0                             | 1                               | 3.36         |
| 24 | 18          | M   | 2                         | 0                             | 2                               | 5.18         |
| 25 | 20          | M   | 0                         | 1                             | 2                               | 5.56         |
| 26 | 26          | M   | 2                         | 1                             | 3                               | 4.66         |
| 27 | 22          | M   | 1                         | 1                             | 3                               | 3.76         |
| 28 | 40          | M   | 1                         | 0                             | 1                               | 6.38         |
| 29 | 34          | M   | 1                         | 1                             | 3                               | 5.1          |
| 30 | 20          | M   | 2                         | 0                             | 2                               | 3.72         |

Mean: 1.5, SD: 1.009, SEM: 0.184

Abbreviations: M, male; F, female.
amounts of natural food and vegetables, may reduce the clastogenic effects of toxicants. There is evidence that certain vegetables contain anticlastogenic agents such as galangin (8). It is also possible that the standard assays are not sensitive enough for detecting small differences between the exposed and nonexposed groups. In this case, new techniques such as the fluorescence in situ hybridization (FISH) assay (9) and a challenge assay (10,11) should be used.

In summary, it is assuring that the exposure conditions among the Indian farmers have not caused a detectable increase of chromosome damage using standard assays; this suggests the lack of serious long-term health problems. However, periodic monitoring of such exposed populations should be conducted using the same or other more sensitive assays. In addition, other populations with exposure to different types of pesticides in Colombia should also be investigated.

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