Genetic Analysis of Ecuadorian Population Exposed to Glyphosate

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RESUMEN. Este estudio analiza las consecuencias de las asperciones aéreas con glifosato y surfactantes en la parte norte del Ecuador. Los tests de aberraciones cromosómicas y el ensayo cometa fueron utilizados para el biomonitoreo humano. Un total de 24 individuos expuestos y 21 individuos controles fueron incluidos en el estudio. Los resultados muestran niveles significativamente altos de aberraciones cromosómicas en las muestras de los individuos expuestos (22.42%) comparados con los controles (1.38%). De igual manera, el ensayo cometa mostró un alto grado de daño al ADN en el grupo expuesto (35.5μm) comparado con el grupo control (25.94μm). Estos resultados sugieren que el glifosato, en la formulación utilizada durante las asperciones aéreas, podría tener un efecto genotóxico sobre los individuos expuestos.

PALABRAS CLAVE. Aberraciones cromosómicas, Asperción aérea, Ensayo cometa, Genotoxicidad, Glifosato.

ABSTRACT. This study analyzes the consequences of glyphosate added surfactants for aerial spraying in the northern part of Ecuador. Chromosomal aberrations test and comet assay were used for human biomonitoring. A total of 24 exposed individuals and 21 control individuals were included in this analysis. Results showed significantly high levels of chromosomal aberrations in samples of exposed individuals (22.42%) compared to control individuals (1.38%). Similarly, the comet assay showed a high degree of DNA damage in the exposed group (35.5μm) compared to the control group (25.94 μm). These results suggest that the glyphosate in the formula used during aerial spraying, could have a genotoxic effect over exposed individuals.

KEYWORDS. Aerial spraying, Chromosomal aberrations, Comet assay, Genotoxicity, Glyphosate.

INTRODUCTION

Glyphosate is a non-selective, main chemical component in many systemic herbicides used to control most annual and perennial plants. It controls weeds by inhibiting the synthesis of aromatic amino acids necessary for protein formation, which link primary and secondary metabolism in susceptible plants (1, 2).

According to some reports, glyphosate shows no adverse effects on soil microorganisms, it is relatively non-toxic to fish (2), and it is of relatively low toxicity to birds and mammals, including humans (3, 6, 8). Contrary, a mutagenic effect of pesticides, including glyphosate, has been reported in bovine species, as well as an induction of oxidative stress (7). It is known that genetic damage associated with pesticides, occurs in human populations who are subject to high exposure levels due to intensive use, misuse, or failure of control measures (9).

Since January 2001, the northern area of Ecuador (mainly Sucumbíos) was affected by aerial spraying with Glyphosate + POEA + Cosmoflux, carried out by the Colombian Government (10). The main purpose of using Glyphosate in this formula for the aerial spraying is to eradicate illicit crops on the mentioned area. Several research projects were carried out to investigate the consequences of its use in Ecuador (10, 11).

On the other hand, DNA damage can be evaluated through the study of chromosomal aberrations (12, 13) as well as the use of the comet assay, which provide a useful tool to estimate the genetic risk deriving from exposure to a complex mixture of chemicals (9), and allows detection of direct damage to the DNA. It is also widely applied in investigative studies of genotoxic effects of factors including X-rays and pesticides (9, 11-18).

MATERIALS AND METHODS

Subjects - The exposed group consisted of 24 randomly selected subjects; they lived in the area where glyphosate was applied at the border between Ecuador and Colombia (Table 1).

All of them, sometimes, combined their home activities with cultivating and harvesting. They lived in regions located at 3km or less of the area of aerial fumigations and showed toxic symptoms after several exposures. A clinical history was completed for each one of the exposed individuals who experienced a wide range of reactions (10, 11).

The fumigations were made between February and March (2001), from 7h00 to 12h00 and from 14h00 to 17h00 during 3 days. Other fumigations were made between December (2000) and February (2001), from 8h00 to 16h00; 12.5% of the subjects analyzed were located 200m away from the area of

exposure, 37.5% between 1000m and 3000m away and 50% of them received the spraying directly over their homes. The houses with windows did not have asbestos on the ceilings. The aerial spraying continued for one or two more weeks in the same region (10, 19).

According to the National Narcotic Council for air sprayings on illicit cultures, the mixture was applied in the following amounts: Load of the airplane: 300 – 450 gallons (1137–1705 liters); effective unloading (Roundup Ultra with 43.9% of glyphosate): 23.4 liters/ha (10.3 L/ha of glyphosate) (20).

The control group consisted of 21 unrelated healthy individuals living 80 km away from the application area. Their demographic characteristics and their occupations were similar to those from the exposed individuals (Table 2).

Each subject included in this study, completed a personal and biomedical survey, and gave their consent to be a part of this study. None of them had smoking or drinking habits, nor were exposed to pesticides or drugs.

Alkaline single cell gel electrophoresis-comet assay. - The comet assay was performed under alkaline conditions, according to the methodology described by Singh *et al.* (17) and with modifications implemented in our laboratory (16).

The slides were analyzed at 400x magnification using a Zeiss fluorescence microscope equipped with a calibrated scale in the ocular lens, a 50 W mercury lamp, an excitation filter of 515-560nm, and a barrier filter of 590nm.

Cells were classified by eye into five predefined categories (A-E) according to the amount of DNA in the comet's tail, as previously described by Anderson *et al.* (21). A rank number, ranging from 0 (A) to 400 (E), was assigned to quantify the damage in each cell, in order to calculate a mean on the amount of DNA damage.

In order to measure the comet length, from head to tail, randomly selected cells from the center of the gel were measured using a calibrated scale. According to the protocol published by Singh *et al.*, DNA migration was determined by measuring the nuclear DNA and the migrating DNA (17).

A total of 200 cells per individual were scored and the mean and median comet length from each subject was used for statistical analysis.

Chromosomal analysis.- Peripheral blood was obtained by venipuncture, two weeks after the aerial spraying, and then cultured in RPMI 1640 medium supplemented with 15% fetal calf serum, 10% phytohemagglutinin, L-glutamine and penicillin-streptomycin. Cultures were incubated at 37°C for 48h. Harvesting and staining techniques were performed according to

standard methods implemented in our laboratory (22, 23). The preparations were analyzed using a Zeiss microscope, and 100 metaphases from each subject were scored. The percentage of metaphases per individual was obtained by calculating the percentage of metaphases with structural alterations from the total analyzed. The analysis of structural chromosomal aberrations included breaks, dicentrics, and rings, including or excluding gaps. Chromosomal aberrations and chromosomal variants were classified according to the ISCN (24).

Statistical analysis.- The Mann-Whitney U test was applied to determine the differences between exposed and control groups in the chromosomal aberrations analysis and the comet assay.

The determinant test and the multiple correlation test (SPSS©10.0 for Windows) were used to establish the relationship between DNA damage and chromosomal aberrations.

RESULTS

Alkaline single cell gel electrophoresis-comet assay.- Exposed individuals to glyphosate showed higher DNA migration levels than control individuals ($p<0.001$) as shown by the mean values of total damage, 35.50mm (Table 1) and 25.94mm respectively (Table 2). Comet types D and E were not observed in the control group.

Chromosomal analysis.- As shown in table 3, there is a significant increase in the total percentage of metaphases with breaks and/or gaps, 22.42% in the exposed group compared to the control group (1.38%) ($p<0.001$). No fragments or chromosomal rearrangements were found. The same statistical difference ($p<0.001$) was found when compared the percentage of metaphases, excluding gaps, in exposed and control individuals (15.3% and 1.09% respectively) (Table 4).

DISCUSSION

This report shows the results of the cytogenetic monitoring, DNA damage assessment of individuals exposed to aerial spraying of glyphosate mixture in the Northern part of Ecuador. It has been reported that when people swallow glyphosate, breathe it, bathe in it, drink contaminated water, or get glyphosate on their skin, they show a wide range of acute reactions affecting their eyes, skin, lungs, heart, blood cells and genitals, and causes headaches (25). Data from governmental institutions of Ecuador confirm the existence of these health problems in the spraying zone (10).

Published data show that different pesticides are capable of inducing chromosomal aberrations (9, 26, 27). Chromosomal damage induced by pesticides appears to be transient in acute or discontinuous exposure, but cumula-

tive in continuous exposure to complex agrochemical mixtures (8). Our results also show an increase of chromosomal aberrations in exposed individuals (22.42%) compared to the control group (1.38%) ($p<0.001$), suggesting that substances like glyphosate with their additives are able to increase chromosomal aberrations and DNA damage. The results from comet and chromosomal aberrations show similar percentage of DNA damage. The comet assay is a rapid and sensitive method for the detection of DNA damage induced in vitro (17, 28) or after environmental and occupational exposures (29, 30). We found a higher degree of DNA damage in the exposed group compared to the control group ($p<0.001$). Similar differences were obtained when the mean of the length of DNA migrating was compared between the exposed (35.50 mm) and the control (25.94 mm) individuals ($p<0.001$). The length of the comet is positively correlated with the amount of DNA breakage in the cell (17), which agrees with the results of this work showing an increase of chromosomal aberrations and with the length of the comets.

Factors like age and different endpoints, like chromosomal aberrations and DNA damage, were not found to be related in this work. Regarding to sex influence, we cannot conclude anything because most of the exposed and control groups consists of females. Similar results are reported in other investigations where it is stated that in general terms, sex and age seem to have little, if any, influence on SCE and chromosomal aberrations in control (31, 32) or in pesticide exposed populations. The present findings suggest the existence of genotoxic risk for glyphosate mixture exposure.

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Table 1. DNA damage assessed by the comet assay in individuals exposed to Glyphosate.

Individual no.	Gender	Age (years)	Meana Migration mm	Medianb Migration mm
1	F	53	39.50	32.5
2	F	37	44.05	32.5
3	F	40	56.60	52.5
4	M	27	49.20	37.5
5	F	44	34.59	30.0
6	F	50	30.78	27.5
7	F	38	33.20	30.0
8	F	46	35.18	30.0
9	F	55	32.77	30.0
10	F	50	34.21	30.0
11	F	22	31.99	27.5
12	F	27	33.65	30.0
13	F	28	30.95	30.0
14	F	59	36.36	32.5
15	F	55	32.68	30.0
16	F	17	31.31	37.5
17	F	34	33.40	30.0
18	F	45	33.02	30.0
19	F	28	31.11	27.5
20	F	21	33.22	30.0
21	F	34	31.79	30.0
22	F	23	39.34	37.5
23	F	34	35.45	37.5
24	F	42	27.64	27.5
Mean/Median + SD		38 + 1.22	35.50 + 6.4	30 + 5.4

Abbreviations: F, female; M, male; SD, Standard Deviation
a,b Mean and median value of migrating DNA on 200 cells

Table 2. DNA damage assessed by the comet assay in control individuals.

Individual no.	Gender	Age (years)	Meana Migration mm	Medianb Migration mm
1	F	17	26.29	25
2	F	40	25.40	25
3	F	26	25.70	25
4	M	14	27.30	26.5
5	M	32	25.90	25
6	M	21	25.75	25
7	M	16	25.80	25
8	F	47	25.76	25
9	F	15	25.28	25
10	F	36	25.45	25
11	F	21	26.31	25
12	F	43	26.85	25
13	F	53	26.13	25
14	F	35	27.03	25
15	F	38	26.45	25
16	F	22	25.14	25
17	F	71	25.01	25
18	F	39	25.51	25
19	F	21	25.94	25
20	F	50	25.36	25
21	F	43	26.40	25
Mean/Median + SD		33 + 15	25.94 + 0.6	25 + 0.3

Abbreviations: F, female; M, male; SD, Standard Deviation
a,b Mean and median value of DNA migrating on 200 cells

Table 3. Structural Chromosomal Aberrations in exposed and control individuals

Group	No. Metaphases analyzed	Metaphases with Structural Chromosomal Aberrations	
		Excluding gaps	Including gaps
Exposed	2400	367 (15.3%)	538* (22.42%)
Controls	2100	23 (1.09%)	29* (1.38%)

* Indicates the differences between the exposed and control groups ($p < 0.001$)

Table 4. Percentage of altered metaphases with breaks in exposed and control individuals.

Exposed Individual No.	Metaphases excluding gaps (%)	Control Individual No.	Metaphases excluding gaps (%)
1	18	1	0
2	21	2	0
3	16	3	2
4	24	4	1
5	19	5	1
6	17	6	3
7	18	7	1
8	16	8	0
9	16	9	2
10	7	10	1
11	14	11	1
12	12	12	2
13	9	13	1
14	13	14	1
15	16	15	0
16	14	16	3
17	21	17	1
18	13	18	0
19	12	19	1
20	9	20	2
21	17	21	0
22	15	-	-
23	8	-	-
24	22	-	-
Mean + SD	15.3 + 4.2 *		1.09 + 0.9 *

Abbreviations: SD, standard deviation

* Indicates the differences between the exposed and control groups ($p < 0.001$)

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