EXHIBIT 111

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Baseline determination in social, health, and genetic areas in communities affected by glyphosate aerial spraying on the northeastern Ecuadorian border

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Abstract

The northeastern Ecuadorian border has undergone aerial spraying with an herbicide mix that contains surfactants and adjuvants, executed by the Colombian Government. The purpose of this study was to diagnose social, health, and genetic aspects of the people affected by glyphosate. For this objective to be achieved, 144 people were interviewed, and 521 medical diagnoses and 182 peripheral blood samples were obtained. Genotyping of GSTP1 Ile105Val, GPX-1 Pro198Leu, and XRCC1 Arg399Gln polymorphisms were analyzed, using PCR-RFLP technique. The assessment of chromosomal aberrations was performed, obtaining 182 karyotypes. Malnutrition in children was 3%. Of the total population, 7.7% had children with malformations, and the percentage of abortions was 12.7%. Concerning genotyping, individuals with GSTP1 Val/Val obtained an odds ratio of 4.88 ($p<0.001$), and Ile/Val individuals, together with Val/Val individuals, had an odds ratio of 2.6 ($p<0.05$). In addition, GPX-1 Leu/Leu individuals presented an odds ratio (OR) of 8.5 ($p<0.05$). Regarding karyotyping, the 182 individuals had normal karyotypes. In conclusion, the study population did not present significant chromosomal and DNA alterations. The most important social impact was fear. We recommend future prospective studies to assess the communities.

Keywords: Arg399Gln; GPX-1; GSTP1; Ile105Val; Pro198Leu; XRCC1.

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Introduction

Glyphosate (N-phosphonometyl glycine) is a nonselective, broad spectrum, postmergence organophosphorus herbicide effective in controlling annual, biennial, and perennial herb species, pastures, and broadleaf weeds (1). Glyphosate is one of the world's most widely used herbicides with 20,000 tons year used in Europe and 51,000 tons year in the USA (2, 3). The glyphosate activity is primarily due to the inhibition of 5-enolpyruvylshikimate-3-phosphate synthase, resulting in a retardation of the shikimate pathway that is involved in the synthesis of aromatic amino acids in plants and microorganisms (4, 5). The herbicide is commonly formulated with surfactants that decrease the surface tension of the solution and increase penetration into the tissues (6). Roundup® (Monsanto, St. Louis, MO, USA) is an aqueous solution of the isopropylamine salt of glyphosate with a polyethoxylated tallowamine surfactant (POEA) and the adjuvant Cosmoflux 411F (Monsanto, St. Louis, MO, USA) (7, 8).

Several research studies worldwide demonstrated that the use of glyphosate formulations develops high and low levels of toxicity in different organisms. Glyphosate can interfere with certain enzymatic functions in animals, but the symptoms of poisoning depend on the dose and exposure time. In humans, Roundup® is toxic in placental and embryonic cells and sexual steroid biosynthesis (9). This pesticide mixed with adjuvants was cytotoxic through alteration of succinate dehydrogenase and was toxic to human peripheral blood mononuclear cells (10). The results of four case-control studies suggested an association between glyphosate and the risk of non-Hodgkin's lymphoma (11–14). In amphibians, *Rana pipiens* Schreber tadpoles showed decreased snout-vent length at metamorphosis and increased time for metamorphosis to occur, tail damage, and gonadal abnormalities. Pesticide toxicity is often proposed as a contributing factor to the worldwide decline of amphibian populations (15, 16). In sea urchin eggs development, glyphosate prevents the hatching enzyme transcription synergistically and activates the DNA damage checkpoint CDK1/cycline B of the first cell cycle of development for commitment to cell death by apoptosis (9, 17, 18). In rabbits, glyphosate treatment resulted in a decline in body weight, sperm concentration, and semen osmolality (19). In isolated rat liver mitochondria, Roundup® depresses the mitochondrial complexes II, III and is able to induce a dose-dependent formation of DNA adducts in the kidney and the liver (20).

Among the research studies showing a low toxicity of glyphosate, an outstanding study conducted by Bolognesi

et al. (8) executed a cytogenetic analysis of agricultural workers from five Colombian regions; a study conducted by Sanin et al. (21) proved a non-association between glyphosate and the prolongation of pregnancy in women; and another study proved the genotoxicity of glyphosate at a low-risk level in the environment, compared with the harmful products used during cocaine production in Colombia (22).

During the period 2000–2007, the Ecuadorian northern border suffered from repeated aerial spraying with an herbicide mix composed of high doses of glyphosate, the surfactant polyethoxylated tallowamine (POEA), and the adjuvant Cosmoflux 411F. After analyses were conducted in 2004 and 2006, in which an increase in DNA damage and genetic risk was detected, biomonitoring established a baseline for social, health, genetic, and environmental areas in the Ecuadorian communities bordering Colombia, to determine what occurred at the biological level once aerial spraying with a broad spectrum herbicide was suspended two years after the last aerial spraying with a herbicide mix with glyphosate.

Experimental

Area of study

This research was carried out in the province of Sucumbios located in the Ecuadorian Amazon basin bordering Colombia. Baseline determination in social, health, and genetic areas was performed in the following communities: Chone-2, Yanamarum, Playera Oriental, Fuerzas Unidas, Puerto Escondido, Corazon Orense, Santa Marianita, San Francisco, and Las Salinas 5 de Agosto in the province of Sucumbíos (Figure 1).

Biological samples and field data collection

Subjects (n=144) were interviewed, and 521 medical diagnoses of men (47.8%) and women (52.2%) were obtained. The origin of the population from the study area corresponds to 53.4% of those born in the Amazonian region, 46.6% come from other Ecuadorian regions, and 16.1% are Colombian immigrants; the presence of immigrants from said country has increased over the last 10 years, when aerial spraying of illegal crops in Colombia started. Psychological assessment in children from different schools belonging to the study communities consisted of

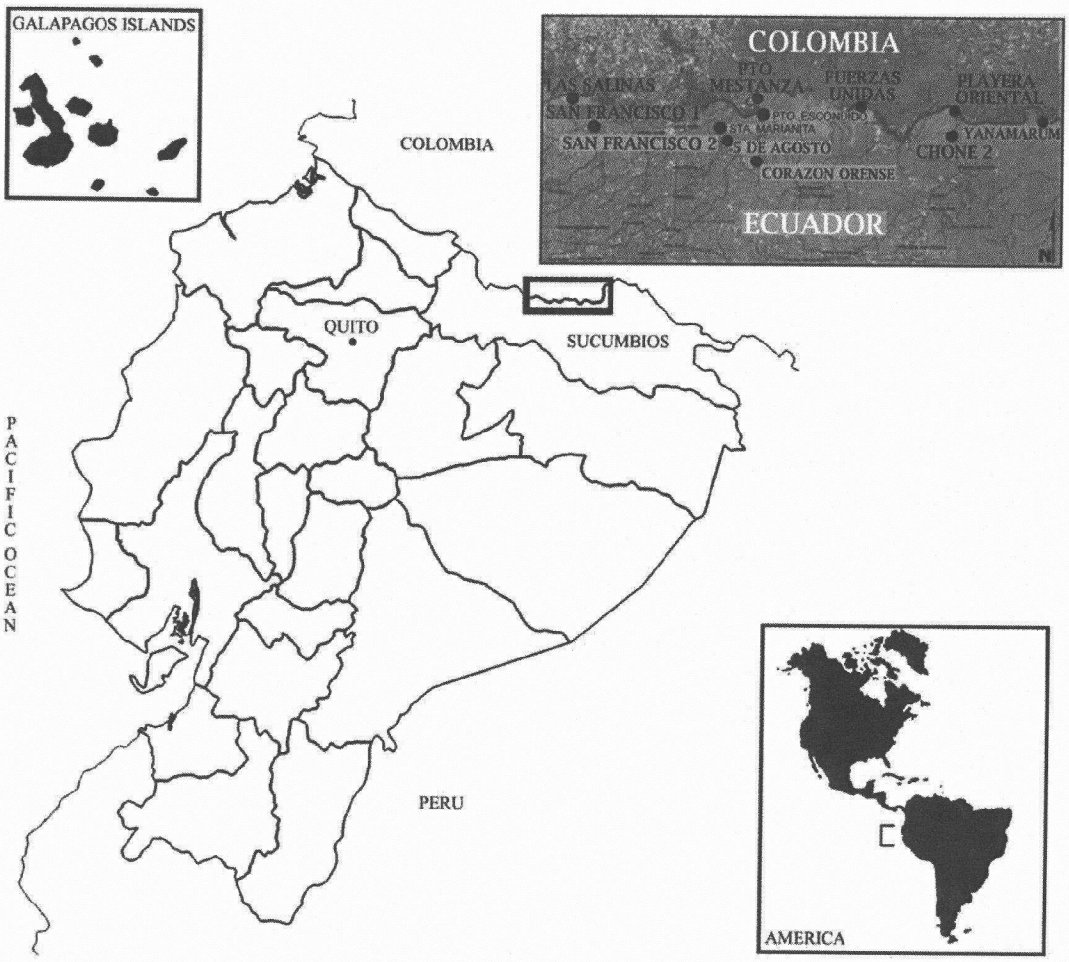

Figure 1 Studied communities in Ecuador.

the analysis of drawings made by the children, for which the following formal features were considered – transparency, contrast, proportionality, symmetry, support base, concealment, confusion, motion, rigidity, lines, presentation, chromatic expression, outline, and texture.

For the analysis of chromosomal aberrations and the study of GSTP1 (glutathione S-transferase pi 1), GPX-1 (glutathione peroxidase 1), and XRCC1 (X-ray repair cross-complementing group 1) genes, 92 peripheral blood samples in vacutainer tubes with heparin and EDTA were obtained from individuals exposed to the aerial spraying of an herbicide mix with glyphosate. The genetic study also required the analysis of 90 DNA samples from healthy individuals who belonged to several provinces of the country who did not have a background of smoking or exposure to genotoxic substances, such as hydrocarbons, X-rays, or pesticides. Each one of these study individuals signed their corresponding informed consent.

Genotyping

DNA from individuals exposed to an herbicide mix with glyphosate and that of healthy individuals, stored in the nucleic acid data bank of the Biomedical Research Institute at the Universidad de las Américas, was extracted from peripheral blood samples using PureLink™ Genomic DNA Kit (Invitrogen). The mean concentration of the DNA samples was 100 ng mL^{-1} measured in a Qubit® Fluorometer (Invitrogen). Because the affected communities had a background involving spraying with an herbicide mix with glyphosate, we proceeded to study single nucleotide polymorphisms (SNPs) in the GSTP1 (Ile105Val), GPX-1 (Pro198Leu), and XRCC1 (Arg399Gln) genes. Genotyping was performed through the polymerase chain reaction–restriction fragment length polymorphism technique (PCR-RFLP). For GSTP1, GPX-1, and XRCC1 genes amplification, a PCR final volume of 50 µL was prepared, containing 4 µL of DNA template, 34 µL H_2O Milli-Q, 0.4 µM of forward and reverse primers, 1.5 mM $MgCl_2$, 5 µL 10× buffer (200 mM Tris-HCl pH 8.4, 500 mM KCl), 0.2 µM each deoxynucleotide triphosphate (dNTPs), and 2.5 U Taq DNA polymerase (Invitrogen). For the 177 bp fragment amplification and the analysis of the Ile105Val polymorphism found in chromosome 11, codon 105, exon 5, we used the following primers: FW 5′-ACCCCAGGGCTCTATGGGAA-3′ and RV 5′-TGAGGGCACAAGAAGCCCCT-3′. Once the PCR reaction was obtained, the samples were placed in the MultiGene Thermal Cycler TC9600-G for amplification (Labnet, Edison, NJ, USA). The initial denaturation lasted 5 min at 95°C, followed by 35 cycles of 45 s at 94°C, 30 s at 62°C, 30 s at 72°C, and 1 min at 72°C. Digestion of the amplified fragment was performed during 2 h at 37°C with 5 U of the Alw26I (Promega, Madison, WI, USA) restriction enzyme. Electrophoresis analysis revealed homozygous individuals (Ile/Ile), (Val/Val) or heterozygous (Ile/Val) (23). For a 191 bp fragment amplification and the analysis of the Pro198Leu polymorphism found in chromosome 3, we used FW 5′-AAGGTGTTCCTCCCTCGTAGGT-3′ and RV 5′-CTACGCAGGTACAGCCGCCGCT-3′ primers (24, 25). In the thermal cycler, the initial denaturation step lasted 10 min at 95°C, then 35 cycles of 30 s at 56°C, 30 s at 56°C, 45 s at 72°C and 3 min at 72°C were needed. Digestion of PCR product was carried out during 2 h at 37°C with the ApaI (Promega) restriction enzyme. The PCR-RFLP test revealed homozygous individuals (Pro/Pro), (Leu/Leu) or heterozygous (Pro/Leu) (25, 26), whereas for a 242 bp fragment amplification and the analysis of the Arg399Gln polymorphism found in chromosome 19, codon 399, exon 10, the following primers FW 5′-CCCCAAGTACAGCCAGGTC-3′ and RV 5′-TGCCCCGCTCCTCTCAGTAG-3′ were used (27). The initial denaturation step lasted 5 min at 95°C, then 35 cycles of 45 s at 94°C, 1 min at 59°C, 30 s at 72°C and 3 min at 72°C. Digestion of amplicon was performed during 2 h at 37°C with the MspI (Promega) restriction enzyme. The analysis revealed homozygote individuals (Arg/Arg), (Gln/Gln), or heterozygote individuals (Arg/Gln) (27).

Karyotyping

For the cytogenetic analysis, we used techniques that we modified in our laboratory from previously standardized protocols (28, 29). The 92 individuals belonging to the 10 communities in the study area in Ecuador's northern border area were karyotyped to assess the existence of chromosomal alterations, according to the 'An International System for Human Cytogenetic Nomenclature' (30). Peripheral blood (5 mL) in vacutainer tubes with heparin was extracted, the samples were cultured at 37°C using RPMI 1640 medium (Gibco Laboratories, Grand Island, NY, USA), complemented with 10% phytohemagglutinin, 15% fetal bovine serum, 0.5 mL L-glutamine, 1.5 mL penicillin-streptomycin, and 1.5 mL HEPES buffer for the stimulation of cell division. After 48 h, 200 µL of colcemid was placed in the culture medium to collect metaphase cells. For harvesting the cells: we used hypotonic solutions (KCl) to increase cell volume, which spreads apart the chromosomes, and methanol-acetic acid to fix them for study. The fixed cells were dropped onto slides, stained with Giemsa 8% diluted with buffer solution (KH_2PO_4 0.025 M, pH 6.8) and ready to be observed with an Olympus BX51 microscope at 100×. The CytoVision® System (Applied Imaging, Santa Clara, CA, USA) allowed us to order the chromosomes in homologous pairs to obtain the karyotyping of the individuals.

Statistical analysis

The allelic and genotypic frequencies of each single nucleotide polymorphism were calculated from the information provided by the genotypes, and the Hardy-Weinberg equilibrium was determined by using software available on the Internet (http://www.genes.org.uk/software/hardy-weinberg.shtml). All the information obtained from the individuals studied was compiled in a database, and the statistical analysis was carried out using PASW Statistical 17 for Windows (SPSS, Chicago, IL, USA). The allelic and genotypic frequencies of the GSTP1, GPX-1, and XRCC1 genes were calculated. The chi-square (χ^2) analysis was performed to determine significant differences between the presence of Ile105Val, Pro198Leu, and Arg399Gln polymorphisms and the studied population. The relative risk of dysfunction in the DNA detoxification or repair process, in the presence of the polymorphisms in individuals exposed and non-exposed to the aerial spraying with glyphosate, was determined using the odds ratio test (OR). The data were analyzed using a 2×2 contingency table.

Results

Social and health analysis

A descriptive study was conducted to determine the population baseline in the social and health areas. The health and housing general conditions in the communities studied here are not very appropriate for the environment found in the Amazon Basin. Houses are built with zinc roofs, 73.9% of the houses are barely open to the air, and 43.3% have no awning that protects them from vectors. The population consumes water that comes mainly from such natural sources as rivers, marshes, or springs (38.8%), whereas 25.2% of water comes

from rain, 21.58% from open wells, and drinking water consumption represents only 14.42%. Of the families, 38.4% have facilities for the elimination of feces, whereas 61.4% eliminate feces in the open land.

As for the global nutritional status (weight-for-age), 2 years after the last aerial spraying with pesticide (2007), we observed that the global malnutrition status of children aged between 6 and 17 years old decreased from 10.3% to 3%, and the risk of slight malnutrition diminished from 36.3% to 23.2%. The group of children under 6 years old had the largest percentage of malnutrition (14.9% in boys and 13.6% in girls); the percentage of malnutrition decreased markedly in the group of children between 6 and 11 years old (2% boys and 1.8% girls); whereas the percentage of malnutrition in the group between 12 and 17 years old increased to 3.8% in boys and 6.7% in girls. Concerning chronic malnutrition (height-for-age), we found numbers very similar to those obtained in young people between the ages of 6 and 17 years old in 2006, when the percentage of chronic malnutrition decreased from 29% to 28%. The most malnourished group is the one between the ages of 12 and 17 years old (41%), in comparison with the group of children under 6 years old (30%), and the group of children between 6 and 11 years old (22%). Regarding acute malnutrition (weight-for-height), a slight change is seen now if we compare the data obtained in 2006, in which the percentage of acute malnutrition decreased from 1.87% to 1% and the risk went down from 7.17% to 5.8%. The body mass index (BMI) in adults demonstrates that, after 2 years without aerial spraying, no malnutrition occurred in adults over 18 years old, but rather a surge in the tendency to obesity in women (29.7%) and in men (7.8%). As for family health, we observed that during the aerial spraying the percentage of abortions rose from 8.4% to 12.7%, whereas in the same period the percentage of child mortality decreased from 12% to 9.1%. The main causes of child mortality were diseases (40%), unknown reasons (17%), labor (13%), violence (9%), malaria (6%), aerial spraying with glyphosate (5%), cancer (4%), traffic accidents and congenital malformations (2%), and finally, pesticides and snakebites (1%).

Concerning the health conditions caused by aerial spraying with glyphosate, we found that in 84.7% of families, an individual fell ill during the spraying, and the symptoms were respiratory, digestive, and ophthalmological problems, cephalea, and skin conditions, whereas a little after the spraying, the latter became the most important problem. Psychological tests determined that 84.86% of the population had psychological manifestations, with fear being the most frequent reaction (51.3%). After the spraying, fear diminished and concern about the future of the crops rose (18.6%), as well as depression (16.7%).

Genotyping

Table 1 shows the Hardy-Weinberg equilibrium and the genotypic and allelic frequency of the studied polymorphisms. Table 2 shows the statistical analysis through χ^2 and OR tests. The study population was found in Hardy-Weinberg equilibrium. Regarding the GSTP1 Ile105Val polymorphism, we observed that the frequency of the Val allele was higher in exposed individuals (0.48) than in control individuals (0.28) (Table 1). The presence of the Val/Val variant was associated with a 4.88-fold risk of acquiring detoxification problems (OR=4.88, 95% CI, 2.0–11.8, $p<0.001$), whereas the combination of the Ile/Val and Val/Val alleles was associated with a 2.6-fold risk of presenting a GSTP1 gene dysfunction (OR=2.6, 95% CI, 1.4–4.8, $p<0.05$) (Table 2). As for the GPX-1 Pro198Leu polymorphism, we observed that the Leu allele had a higher frequency in exposed individuals (0.41), unlike control individuals (0.32) (Table 1). The presence of the Leu/Leu variant was associated with an 8.5-fold risk of having problems in the function of the GPX-1 gene (OR=8.5, 95% CI, 1.8–39.9, $p<0.05$) (Table 2). Concerning the XRCC1 Arg399Gln polymorphism, we observed that the frequency of the Gln allele was higher in control individuals (0.98), unlike the population exposed to glyphosate (0.54) (Table 1). None of the variables of the Arg399Gln polymorphism presented a significant OR (Table 2).

Chromosomal analysis

After analyzing the metaphases and karyotyping the 92 individuals who belonged to the different communities of the province of Sucumbios located in Ecuador's northeastern border, we observed that all the analyzed women obtained a normal karyotype (46, XX). We also observed that 33% of the 92 individuals with normal karyotype had a low percentage of chromosomal fragility (<5%), whereas 67% of the individuals did not present this feature. All the studied population came within the normal parameters considered for studies of chromosomal fragility (30) (Table 3).

Discussion

During the years 2000–2007, the communities located in the Ecuadorian northern area bordering Colombia suffered from involuntary exposure to aerial spraying with a broad spectrum herbicide mix containing high doses of glyphosate (main herbicide), as well as surfactants and adjuvants to strengthen its power. The aerial spraying with this herbicide mix is part of a program provided by the Colombian National Police (DIRAN-CNP) to eliminate cocaine production (*Erythroxyum coca*) in Colombia. Involuntary exposure to this herbicide mix with high doses of glyphosate has triggered a political, social, and economic conflict between the two countries. Therefore, the Instituto de Investigaciones Biomédicas at the Universidad de las Américas has conducted a descriptive study to determine the baseline on the aerial spraying system and its impact on the social, health, genetic, and environmental areas in the communities located along Ecuador's northeastern border, affected by the aerial spraying with an herbicide mix containing high doses of glyphosate.

The communities studied here do not have health and housing general conditions appropriate for the environment found in the Amazon basin because many lack ventilation systems, as well as protection systems against vectors. The

Table 1 Genotype distribution and allelic frequency of GSTP1 Ile105Val, GPX-1 Pro198Leu and XRCC1 Arg399Gln polymorphism.

Genes	Genotype	Genotypic frequency			Allelic frequency			HWE (χ^2)
		Case	Control	All	Case	Control	All	
GSTP1 Ile105Val	Ile/Ile	0.32	0.54	0.43	0.52	0.72	0.62	0.04^{NS}
	Ile/Val	0.40	0.36	0.38				
	Val/Val	0.28	0.10	0.19	0.48	0.28	0.38	
GPX-1 Pro198Leu	Pro/Pro	0.35	0.38	0.36	0.59	0.68	0.63	0.03^{NS}
	Pro/Leu	0.48	0.6	0.54				
	Leu/Leu	0.17	0.02	0.1	0.41	0.32	0.37	
XRCC1 Arg399Gln	Arg/Arg	0.07	0.01	0.04	0.46	0.02	0.25	0.01^{NS}
	Arg/Gln	0.79	0.01	0.41				
	Gln/Gln	0.14	0.98	0.55	0.54	0.98	0.75	

HWE, Hardy-Weinberg equilibrium of all study population; NS, no significant difference.

Table 2 Statistical analysis of case and control individuals.

Genes	Genotype	Cases (n=92) No. (%)	No. (%) of control (n=90)	OR	95% CI	p-Value
GSTP1 Ile105Val	Ile/Ile	29 (32)	49 (54)	1.0 (reference)		
	Ile/Val	37 (40)	32 (36)	1.95	1.0–3.8	0.07^{NS}
	Val/Val	26 (28)	9 (10)	4.88	2.0–11.8	$<0.001^{a}$
	Ile/Val+Val/Val	63 (58)	41 (37)	2.6	1.4–4.8	$<0.05^{a}$
GPX-1 Pro198Leu	Pro/Pro	32 (35)	34 (38)	1.0 (reference)		
	Pro/Leu	44 (48)	54 (60)	0.87	0.5–1.6	0.77^{NS}
	Leu/Leu	16 (17)	2 (2)	8.5	1.8–39.9	$<0.05^{a}$
	Pro/Leu+Leu/Leu	60 (55)	56 (50)	1.14	0.6–2.1	0.79^{NS}
XRCC1 Arg399Gln	Arg/Arg	6 (7)	1 (1)	1.0 (reference)		
	Arg/Gln	73 (79)	1 (1)	12.2	0.7–219.8	0.4^{NS}
	Gln/Gln	13 (14)	88 (98)	0.03	0.003–0.2	$<0.001^{a}$
	Arg/Gln+Gln/Gln	86 (79)	89 (80)	0.2	0.02–1.4	0.1^{NS}

[a]Significant difference. NS, no significant difference.

water consumed by the population comes mainly from natural sources, such as rivers, marshes or springs that are highly prone to be polluted by chemical substances.

Concerning nutritional status, 2 years after the last aerial spraying with an herbicide mix, we observed that the global malnutrition status of children aged between 6 and 17 years decreased from 10.3% to 3%, whereas the risk of slight malnutrition diminished from 36.3% to 23.2%. As for chronic malnutrition, we observed that this percentage decreased from 29% to 28%, and acute malnutrition diminished from 1.87% to 1%, in comparison with the studies carried out by Acción Ecológica in 2006 (31). Likewise, the body mass index in adults demonstrated no malnutrition in adults over 18 years old; yet, with a tendency to obesity in women (29.7%) and in men (7.8%). This information clearly indicates that during the aerial spraying, the population had nutritional problems due to the broad spectrum herbicides that caused harm in the agricultural products essential for the population feeding, whereas the analyses obtained 2 years after the last aerial spraying confirmed improvement in the general nutritional status of the population.

Regarding family health, we noticed that the percentage of abortions rose during the aerial spraying with an herbicide mix with glyphosate, whereas child mortality decreased. According to the data compiled in the communities bordering Colombia, 5% of child mortality was caused by health complications due to exposure to the aerial spraying with an herbicide mix. Of the interviewed families, 84.7% had an ill relative during the spraying who presented the following symptoms: respiratory, digestive, ophthalmological problems, cephalea, or skin conditions. Regarding the psychological study, one of the most important impacts developed by the aerial spraying was fear. Fear is a feeling that has lasted until now, and 7.7% of the interviewed subjects manifested their fear as nightmares, abnormal behavior, developmental disorders, and stuttering. In the psychological study consisting of drawings made by the children, the pictures reflected sensitivity, creativity, expression capability, adaptation to environmental demands, and in turn, anguish, caution, and paranoid tendencies, where the need for protection and safety was evident.

Genetic assessment consisted of the analysis of DNA damage through the presence of chromosomal aberrations or

Table 3 Chromosomal fragmentation and karyotypes.

Individuals (n=92)	62	2	14	1	2	1	7	1	1	1
Percentage	0	1	1.2	1.4	1.5	1.9	2	2.4	2.5	2.8
Karyotype	46, XX			n=92				100%		

DNA variation through the presence of polymorphisms in the GSTP1, XRCC1, and GPX-1 genes in women of different ages who present a major susceptibility to hepatic toxins due to the variety of physiological processes. In 2006, DNA damage in 24 Ecuadorian individuals exposed to the aerial spraying with an herbicide mix with glyphosate was assessed by means of the comet assay technique, which has a high use in studies with genotoxic substances, such as hydrocarbons, X-rays, and pesticides (32–34). The results showed that DNA in the exposed individuals was highly damaged (comet length=35.5 μm), in comparison with the control group (comet length=25.94 μm). Thus, the results suggest that the individuals exposed to the broadspectrum herbicide suffered a genotoxic effect (35). Two years after the last aerial spraying, none of the studied population had any type of chromosomal alteration, being their normal karyotype (46, XX), and the percentage of chromosomal fragility was within normal parameters. Regarding genetics, the GSTP1 gene encodes proteins that are believed to function in xenobiotic metabolism and play the role as regulator of apoptosis (36–38). We observed a higher frequency of the valine allele in exposed individuals (0.48) than in healthy ones (0.28). Glutathione peroxidase (GPX-1), one of the most important antioxidant enzymes in humans, is responsible for the detoxification of hydrogen peroxide and is part of the enzymatic antioxidant defense system preventing oxidative DNA damage (38). A Pro198Leu polymorphism has been associated with the risk of developing lung, breast, and bladder cancer (23, 25, 39, 40). We observed a higher frequency of the leucine allele in exposed individuals (0.41) than in healthy ones (0.32). Those individuals presenting the GSTP1 Val/Val and GPX-1 Leu/Leu variables may have a higher risk of acquiring problems in the detoxification functions as in the case of the Ecuadorian population with bladder cancer (25). The protein encoded by the XRCC1 gene is involved in the maintenance of the structural integrity of DNA in the face of damage arising from environmental abuse, as well as from normal metabolic processes (41). The Arg allele was found mainly in the population exposed to the glyphosate. The OR test determined no significant risk in the population bearing the Arg399Gln polymorphism. The genetic analyses, carried out during the aerial spraying with an herbicide mix containing glyphosate, showed that the population had suffered DNA fragmentation (35), whereas the cytogenetic assessment executed 2 years after the last aerial spraying with the same herbicide proved that the studied population had no chromosomal alterations.

Several research studies related to glyphosate exposure have been conducted in Colombia by Bolognesi et al. (8), Sanin et al. (21), and Solomon et al. (22), which state that the studied populations have low genotoxic risk associated with glyphosate. Regarding our study, we obtained results showing no chromosomal alterations in the analyzed individuals. Nevertheless, the aerial spraying had a socially and psychologically negative impact on the Ecuadorian communities. Carrying out studies in the short and long term is very important for taking control of population health and for monitoring possible disease development in the coming future.

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