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Social, Health and Genetic Diagnoses of the Zone of Influence Affected by Glyphosate Aerial Spraying in the North Ecuadorian Border

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INTRODUCTION

BACKGROUND

- Control of annual, biennial and perennial species of grasses.
- 20,000 tons in Europe and 51,000 tons in USA.

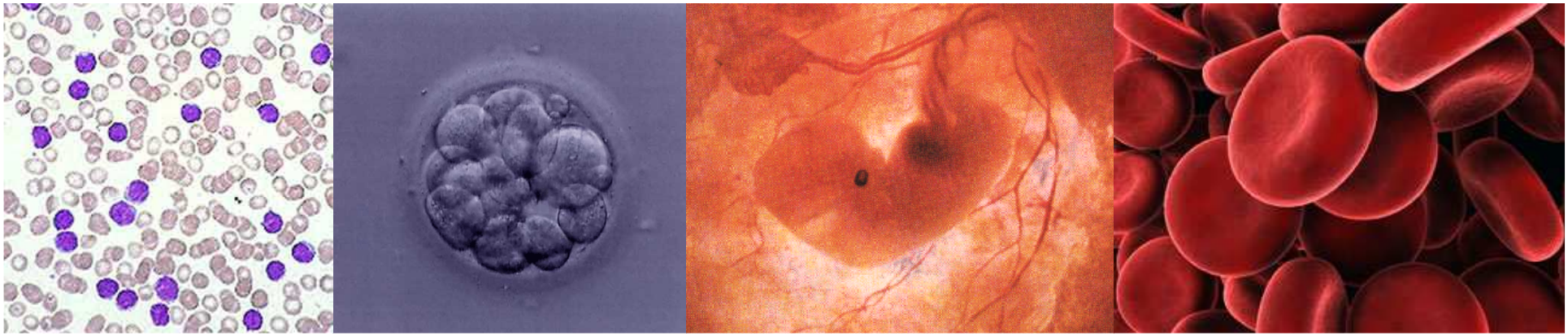


ROUNDUP in a coktel

- **Decrease the surface tension of the solution.**
- **Increase penetration into the tissues.**
- **Isopropylamine salt of glyphosate, surfactant POEA and Cosmoflux 411F.**
- **Symptoms of poisoning depend on the dose and exposure time.**

HUMANS

- Placental and embryonic cells.
- Sexual steroid biosynthesis.
- Human peripheral blood mononuclear cells.
- Risk of non-Hodgkin’s lymphoma.



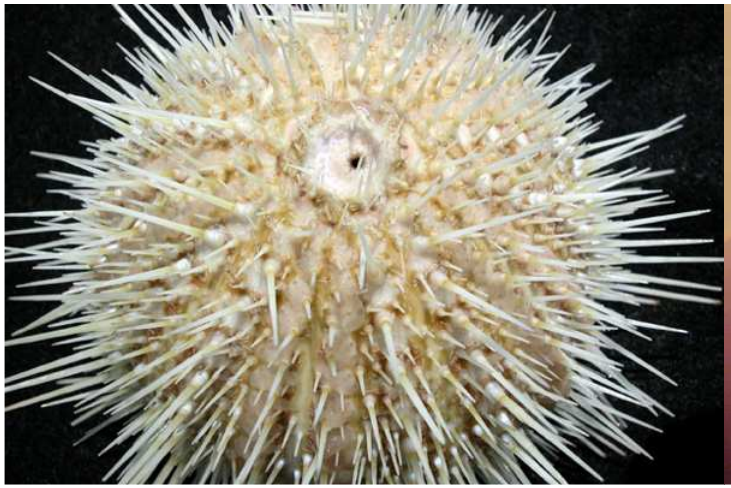
AMPHIBIANS

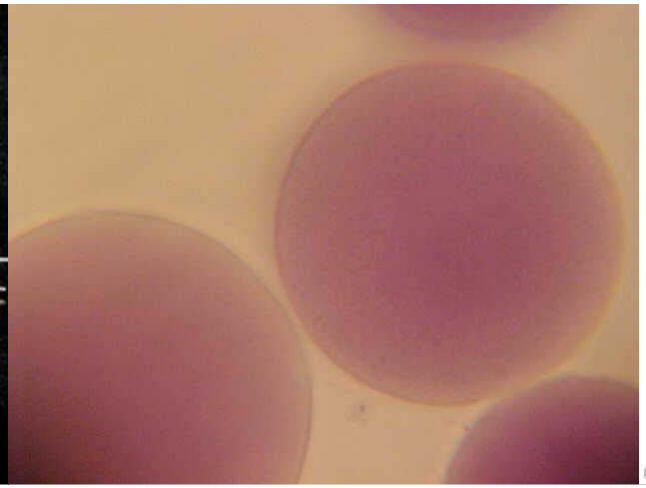
- ***Rana pipiens*** **tadpoles showed decreased snout-vent length, tail damage and gonadal abnormalities.**

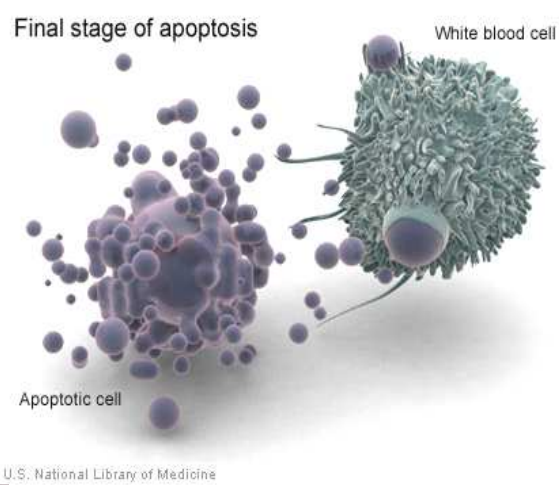
- **World-wide decline of amphibian populations**

SEA URCHIN EGGS

- **This pesticide affected sea urchin eggs development.**
- **DNA damage checkpoint CDK1/ cycline B.**

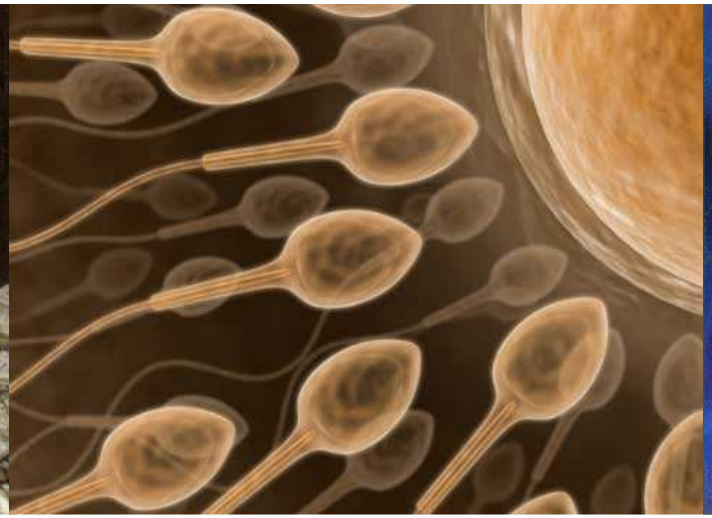


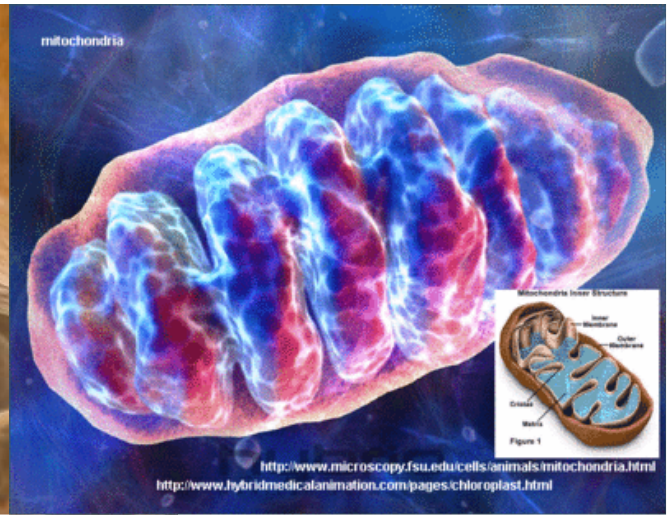




MAMMALS

- In rabbits, decline in body weight, sperm concentration and semen osmolality.
- Mitochondrial DNA damage.
- Formation of altered DNA in the kidneys and liver.



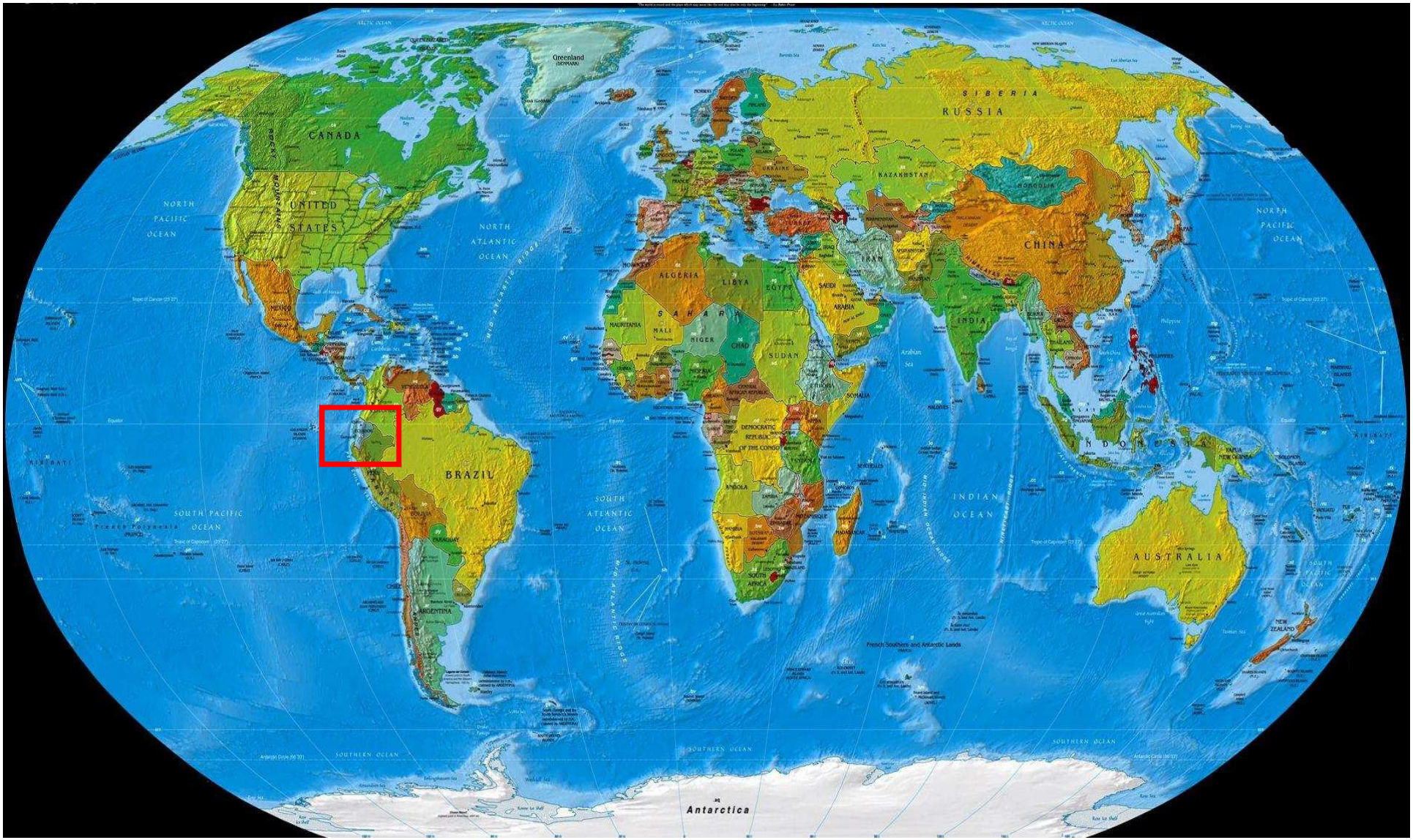


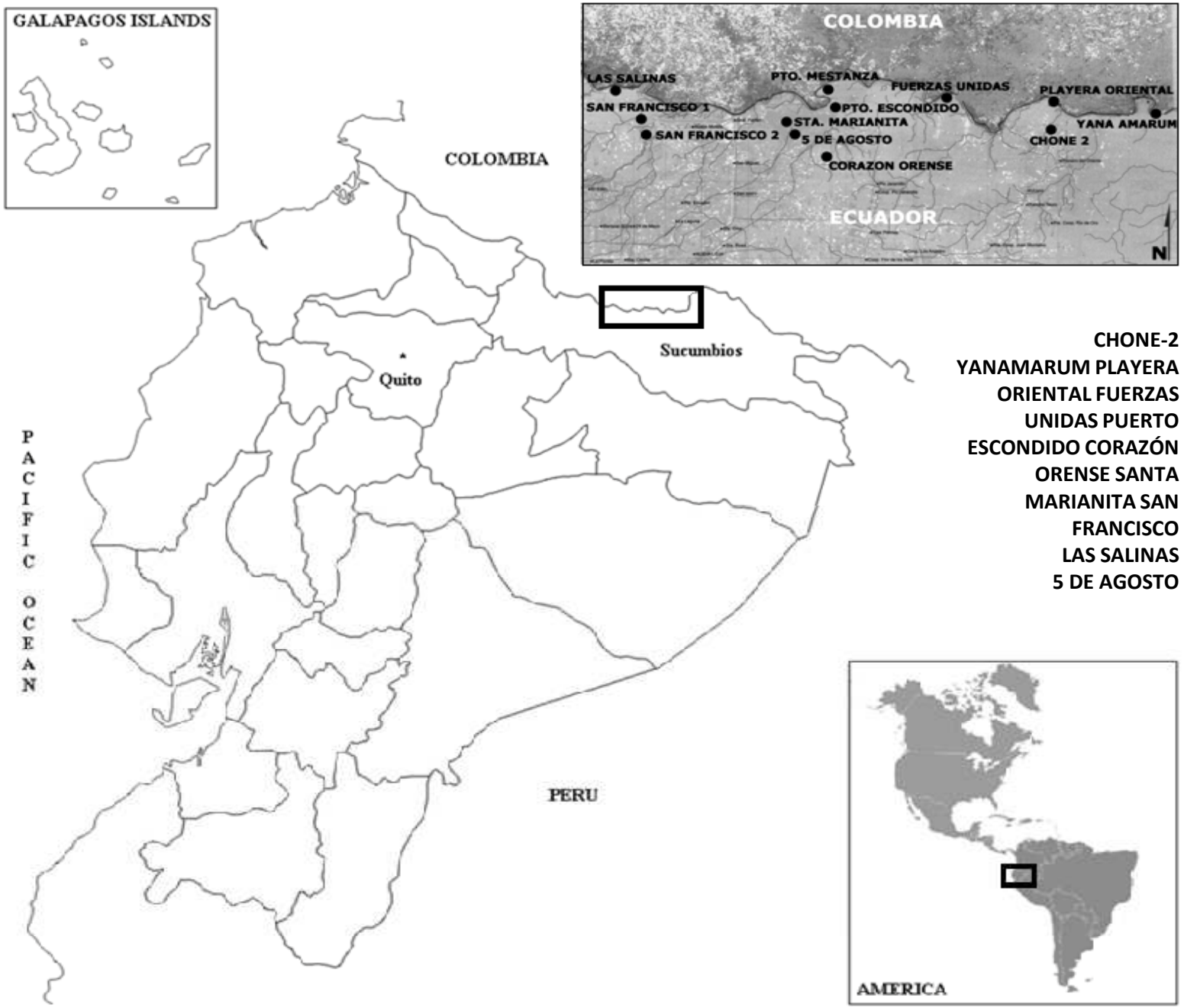
AIM

- **Since 2001, Plan Colombia.**
- **Medical diagnoses were performed.**
- **Chromosomal aberrations were analyzed.**
- **DNA fragmentation and frequency of polymorphisms of GSTP1, XRCC1 and GPX1 genes were calculated.**

MATERIALS AND METHODS

AREA OF STUDY





CHONE-2
YANAMARUM PLAYERA
ORIENTAL FUERZAS
UNIDAS PUERTO
ESCONDIDO CORAZÓN
ORENSE SANTA
MARIANITA SAN
FRANCISCO
LAS SALINAS
5 DE AGOSTO

BIOLOGICAL SAMPLES

- **144 individuals were interviewed.**
- **521 medical diagnoses were obtained.**
- **The origin of the population:**

  53.4% of Amazonian region

  46.6% from other Ecuadorian regions

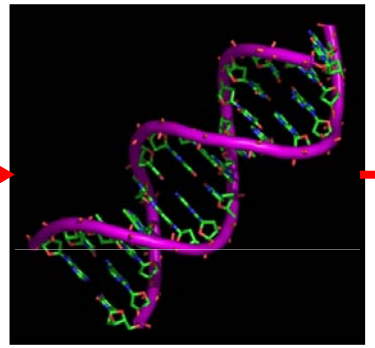
  16.1% are immigrants from Colombia

- **Peripheral blood of 92 exposed and 90 health subjects.**

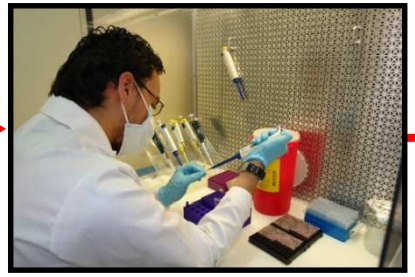
GENOTYPING PCR-RFLP

PERIPHERAL BLOOD

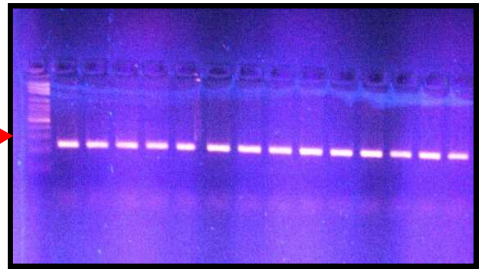
DNA EXTRACTION



PCR



ENZYME DIGESTION

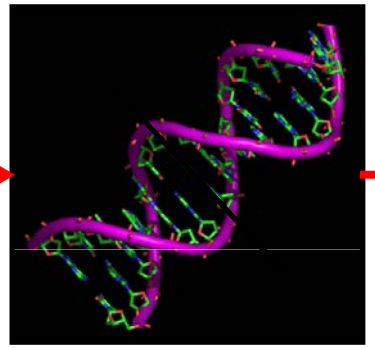


POLYMORPHISM	GENE	FRAGMENT	RESTRICTION ENZYME
Ile105Val	GSTP1	177 bp	Alw261
Arg399Gln	XRCC1	242 bp	MspI
Pro198Leu	GPX1	191 bp	ApaI

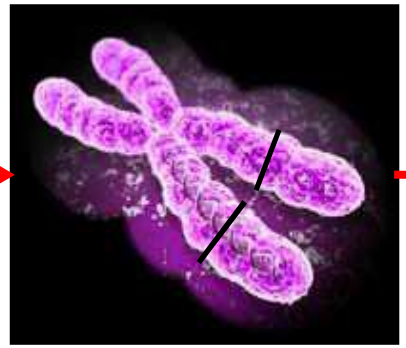
COMET ASSAY

GLYPHOSATE

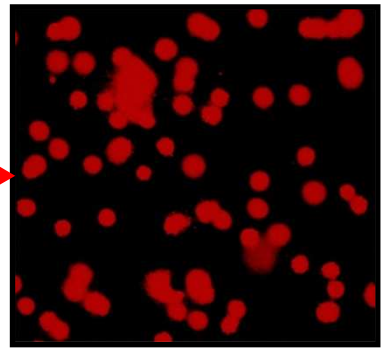
DNA FRAGMENTATION



CHROMOSOMAL BREAK



DNA DEGRADATION



PARAMETERS
1) 100 nucleoids analyzed for each individual.
2) Classified according to the parameter head-to-tail comet length.
3) Determination of the total number on nucleoids according to the level of damage.
4) Mean of the length of the nucleoids measured in µm.

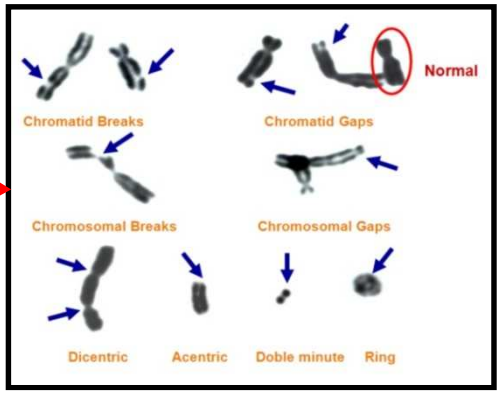
KARYOTYPING

PERIPHERAL BLOOD

LEUCOCYTE CELLS

KARYOTYPING

CHROMOSOMAL ABERRATIONS



PARAMETERS
1) A total of 849 metaphases were analyzed in each group
2) We calculated the percentage of altered damaged metaphases, excluding individuals with zero scored cells.

RESULTS

SOCIAL AND HEALTH

- **Baseline of the population health was realized.**
- **Malnutrition according in children was 3%, with a risk of light malnutrition of 23.2%.**
- **The 11.8% uses glyphosate and 5.37% manifest intoxication.**

SOCIAL AND HEALTH

- **The 32.3% of the families got seriously sick during aerial spraying.**
- **A 7.7% of the interviewed had children with malformations.**
- **The frequency of abortions before the aerial spraying was 8.4%, and after that was 12.7%.**

GENOTYPING – *Ile105Val* GSTP1

Group	Frequency	Individuals	%	Genotypic frequency	Allelic frequency
Exposed (n = 92)	Ile/Ile	29	32	0.32	0.52
	Ile/Val	37	40	0.40	
	Val/Val	26	28	0.28	0.48
Control (n = 90)	Ile/Ile	49	54	0.54	0.72
	Ile/Val	32	36	0.36	
	Val/Val	9	10	0.10	0.28

Ile105Val	Ile/Ile	Ile/Val	Val/Val	χ^2	OR
Exposed	32%	40%	28%	9.7*	2.6, CI 95% 1.4-4.7
Control	54%	36%	10%	*P* < 0.05	*P* < 0.05

GENOTYPING – *Arg399Gln* XRCC1

Group	Frequency	Individuals	%	Genotypic frequency	Allelic frequency
Exposed (n = 92)	Arg/Arg	6	7	0.07	0.46
	Arg/Gln	73	79	0.79	
	Gln/Gln	13	14	0.14	0.54
Control (n = 90)	Arg/Arg	1	1	0.01	0.02
	Arg/Gln	1	1	0.01	
	Gln/Gln	88	98	0.98	0.98

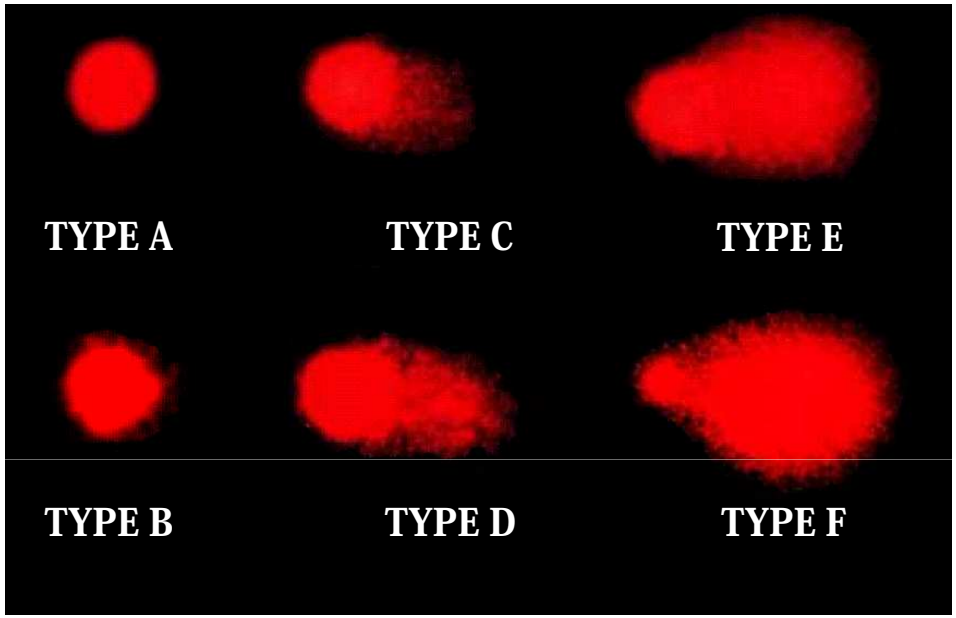
Arg399Gln	Arg/Arg	Arg/Gln	Gln/Gln	χ^2	OR
Exposed	7%	79%	14%	3.6^{NS}	0.2, CI 95% 0.02-1.4
Control	1%	1%	98%	$P > 0.05$	$P > 0.05$

GENOTYPING – *Pro198Leu* GPX1

Group	Frequency	Individuals	%	Genotypic frequency	Allelic frequency
Exposed (n = 92)	Pro/Pro	32	35	0.35	0.59
	Pro/Leu	44	48	0.48	
	Leu/Leu	16	17	0.17	0.41
Control (n = 90)	Pro/Pro	34	38	0.38	0.68
	Pro/Leu	54	60	0.6	
	Leu/Leu	2	2	0.02	0.32

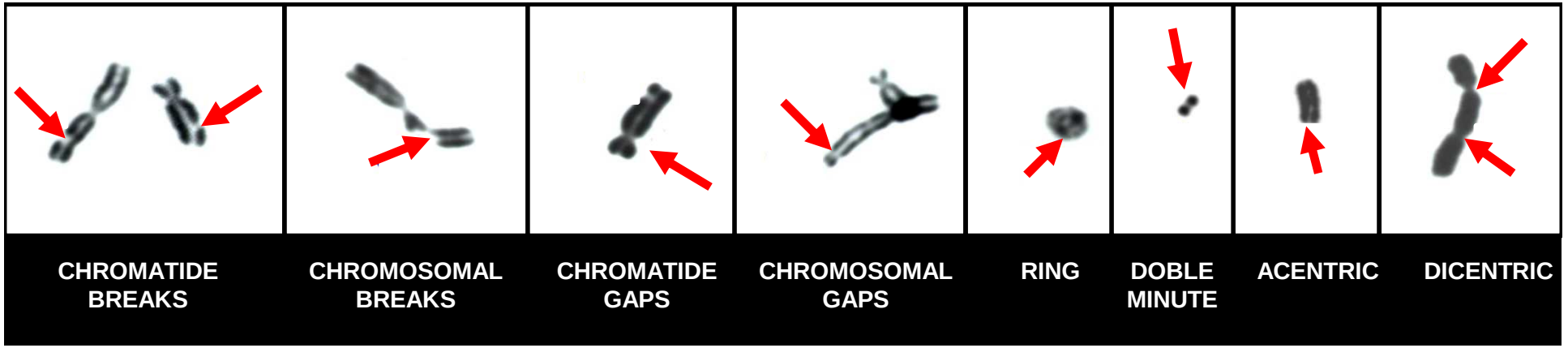
Pro198Leu	Pro/Pro	Pro/Leu	Leu/Leu	χ^2	OR
Exposed	35%	48%	17%	0.2*	1.1, CI 95% 0.6-2.1
Control	38%	60%	2%	$P < 0.05$	$P < 0.05$

COMET ASSAY



TYPE	CATEGORY	LENGHT (µM)	NUCLEOIDS	PERCENTAGE (%)	MEAN	MEDIAN
A	NO DAMAGE	25	300	27	28.5	157
B	LOW LEVEL OF DAMAGE	26 – 30	126	12	11.5	63
C	AVERAGE LEVEL OF DAMAGE	31 – 40	365	33	30.1	165.5
D	HIGH LEVEL OF DAMAGE	41 – 70	295	27	26.8	147.8
E	TOTAL DAMAGE	70	14	1	1.3	7
		TOTAL	1100	100	19.6	108.1

KARYOTYPING



GROUP	INDIVIDUALS	METAPHASE	STRUCTURAL ABERRATIONS				NUMERICAL ABERRATIONS					
			GAPS	%	BREAKS	%	HYPODIPLOID	%	HYPERDIPLOID	%	ENDOREDUPLICATION	%
CONTROL	25	849	1	0.1	0	0	10	1.2	0	0	0	0
EXPOSED	25	849	11	1.3	1	0.1	102	12	4	0.5	1	0.1

CONCLUSIONS

- **Social-economic and political conflict between Ecuador and Colombia.**

- **Colombian government in 2006, used 44% of concentration of Roundup Ultra.**

- **The ranges recommended by USA is 1.6% to 7.7%.**

- **Regarding genetics, the GPX1 is an antioxidant enzyme and function in the detoxification of hydrogen peroxide.**

- **GPX1 prevents oxidative damage of DNA.**

- **Higher frequency of the leucine allele in exposed (0.41) than in healthy ones (0.32).**

- **The χ^2 determined significant difference.**

- **Odds ratio: 1.1 times more susceptibility to detoxification problems and development of some disease.**

- **The GSTP1 gene encodes proteins that are thought to function in xenobiotic metabolism and play role like regulation of apoptosis.**

- **Higher frequency of the valine allele in exposed (0.48) than in healthy ones (0.28).**

- **The χ^2 determined significant difference.**

- **Odds ratio: 2.6 times more susceptibility to mutation and damage as a result of exposure to oxidative stress.**

- **Comet assay: the studied population present high DNA damage.**
- **Long-term prospective studies.**
- **The cytogenetic analysis: structural and numerical abnormalities were present in a greater percentage of affected individuals unlike control individuals.**

- **In social area, one of the most important impacts developed by the aerial spraying was fear.**

- **7.7% of people has manifested as nightmares, abnormal behavior, developmental disorders and stuttering.**

- **In the psychological study:**

  Sensitivity, creativity, expression capability, adaptation.

  Anguish, fragility, caution, paranoid tendencies and surveillance.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THANK YOU!

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