

THE USE OF LYMPHOCYTE CYTOKINESIS-BLOCK MICRONUCLEUS ASSAY TO MONITORING PESTICIDE-EXPOSED FARMWORKERS FROM CASIMIRO DE ABREU/ RJ



<u>Amazonas JC</u><sup>2,4</sup>; Poça, KS<sup>1,2</sup>; Indio-do-Brasil, V<sup>1</sup>; Nunes, RFN<sup>1,2</sup>; Da Silva PVB<sup>1,2</sup>; Oliveira, MAM<sup>1,2</sup>; Siqueira, J<sup>3</sup>; Aguiar, G<sup>3</sup>; Otero U<sup>1</sup>; Nogueira, FAM<sup>1</sup>; Sarpa, M<sup>1,2</sup>.

1 Occupational and Environmental Cancer Branch, CONPREV, Instituto Nacional de Câncer José Alencar Gomes da Silva (INCA), Rio de Janeiro/Brazil. 2 Laboratório de Mutagenese Ambiental, Department of Biochemistry, Federal University of the State of Rio de Janeiro (UNIRIO), Rio de Janeiro/Brazil. 3 Programa de Saúde do Trabalhador, Secretaria Municipal de Saúde de Casimiro de Abreu. 4 National School of Public Health Sergio Arouca (ENSP), Oswaldo Cruz Foundation (Fiocruz) Rio de Janeiro/Brazil.

# INTRODUCTION

Brazil has been the main consumer of pesticides since 2008, and intensive and indiscriminate use has placed the environment and human health at risk. Casimiro de Abreu, located in the coastal plain of the State of Rio de Janeiro, has intense agricultural activity, with a predominance of family food production. According to information provided by the Municipal Health Department pesticides (eg glyphosate, deltamethrin, paraquat, 2,4-D) are used and associated with toxic effects such as hormonal changes, immunological, genotoxic and cancer. The present study aims to evaluate the frequency of micronuclei in rural workers of Casimiro de Abreu and to compare the results to those observed in workers of the city who are not exposed to pesticides.

## RESULTS

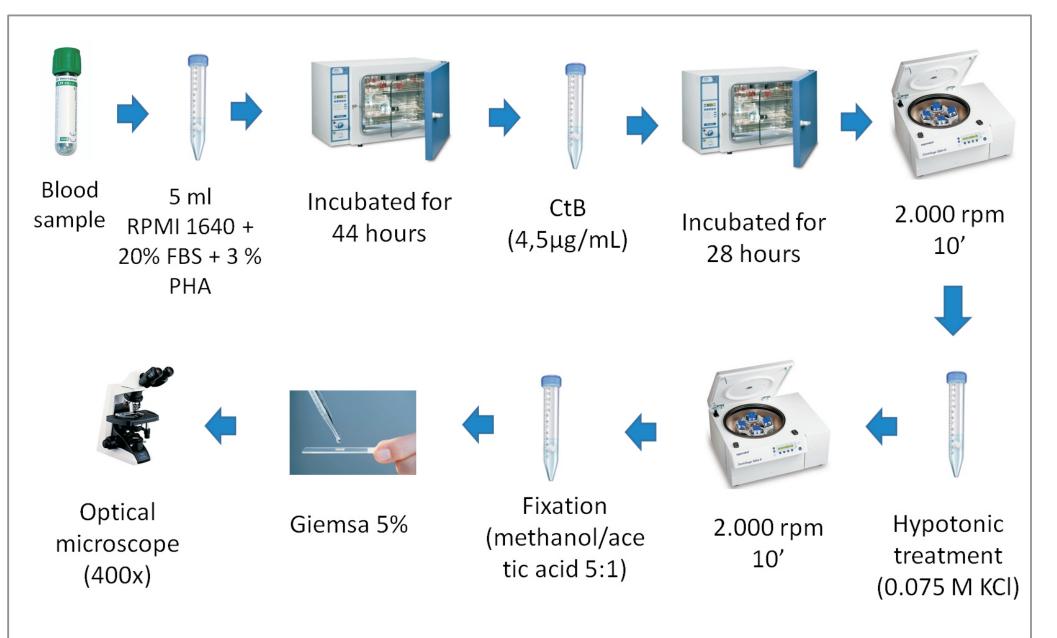
To date, 88 rural workers and 100 control subjects not exposed to pesticides have been recruited.

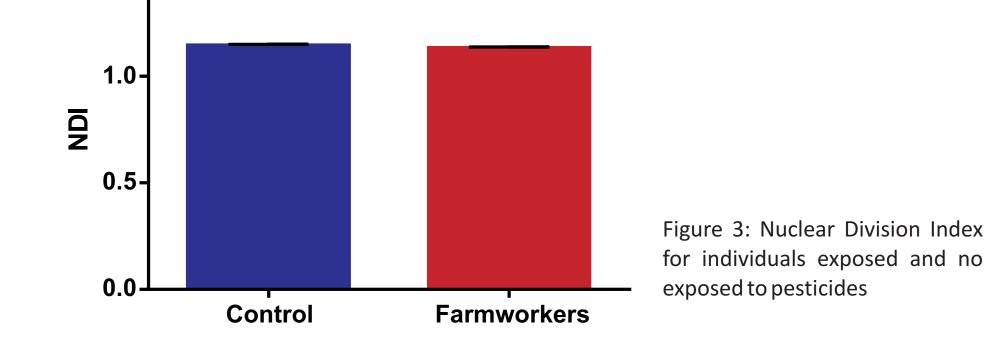
#### **Cytotoxic effects:**

The NDI for farmworkers was  $1.13 \pm 0.1$  (M ± SD, N = 75) and for individuals not exposed to agrochemicals was  $1.14 \pm 0.1$  (N= 100).

# **METHODS**

This is a cross-sectional study with individuals living in the country of Casimiro de Abreu, approved by the INCA Research Ethics Committee (CAAE: 64799217.3.0000.5274). Exposure assessment is performed using the micronucleus assay with cytokinesis block. Whole blood samples (500uL) are incubated for 72 hours (RPMI 1640 medium, 20% fetal bovine serum, 3% phytohemagglutinin) at 37 °C with 5% CO<sub>2</sub>. Cytokinesis is blocked with cytochalasin B (4.5 ug/mL), added 44 hours after the culture has been started. At the end of the culture the samples undergo hypotonic treatment (0.075 M KCI) and fixation (methanol/acetic acid 5:1), when finally stained (Giemsa 5% solution) and analyzed under an optical microscope. The cytotoxic potential is determined by the nuclear division index (NDI) and the genotoxic potential by frequency micronuclei in binucleate lymphocytes.





#### **Genotoxic effects:**

Frequency of binucleated cells containing micronucleus (Figure 4) are determined to all participantes.

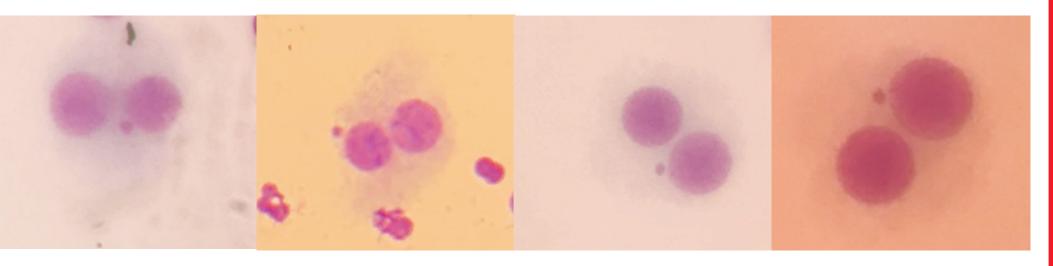
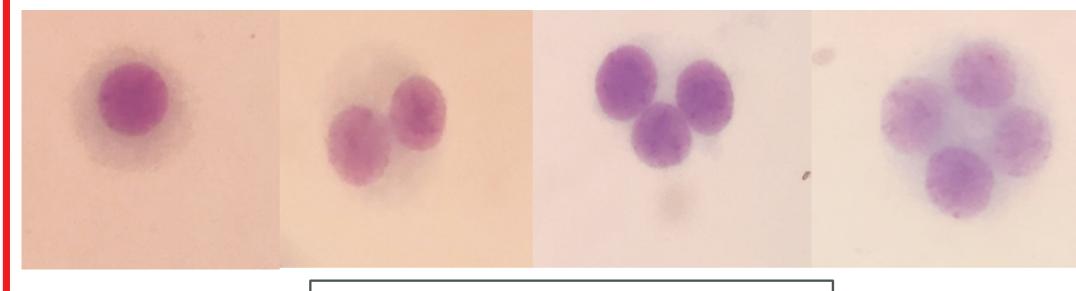


Figure 4: Micronuclei in binucleate lymphocytes

• Evaluation of Micronuclei: Until moment genotoxic potential is available for 11 volunteers (5 exposed and 6 controls), showing that the frequency of binucleate cells containing micronuclei was  $5.50 \pm 2.2$  in those exposed versus  $3.83 \pm 2.1$  in the controls. When evaluating the total micronuclei, the exposed had  $5.9 \pm 2.7$  and the control group  $4.08 \pm 2.5$ .

Figure 1: Methodology of the micronucleus assay with cytokinesis block



$$NDI = \frac{M1 + 2(M2) + 3(M3)}{N}$$

Figure 2: The cytotoxic potential - The nuclear division index (NDI)

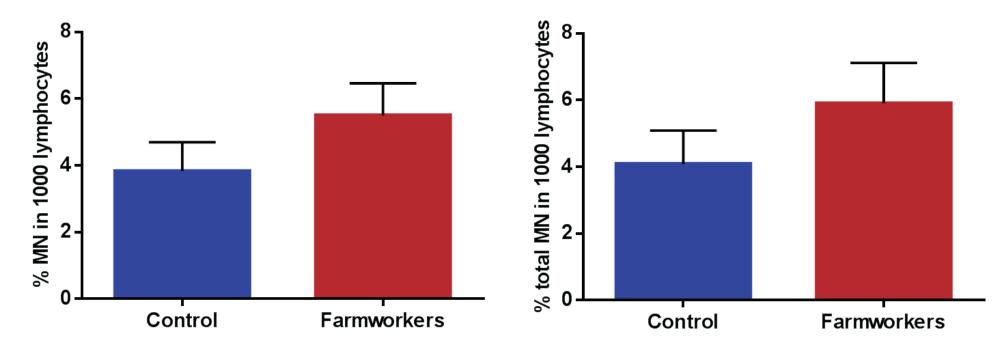


Figure 5: Frequency of genotoxic effects obseved to controls and exposed individuals to pesticides

### CONCLUSION

The results are still partial, but the study intends to identify genetic damages that may contribute to the development of cancer, aiding in decision making, as early as possible.

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