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# EVALUACIÓN DE LA INOCUIDAD DE SEMILLAS DE SOJA EXPUESTAS AL GLIFOSATO MEDIANTE ENSAYOS *IN VIVO*

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## RESUMEN

La soja (*Glycine max* (L.) merrill) en el principal cultivo económico del Paraguay, donde se siembra variedades convencionales y/o transgénicas. Los posibles efectos secundarios de la soja transgénica aún están en discusión. El objetivo de este estudio fue evaluar el efecto mutagénico de tres variedades de soja, dos transgénica BRS 245 RR; Nueva Andrea 66R (T1, T2) y una convencional BRS 232, (T3) expuestas/no expuestas a glifosfato en la dieta de ratones utilizando ensayos *in vivo*. Métodos: Los ensayos se realizaron en modelo murino. Se realizaron dos ensayos, primero se alimentaron ratones BALB/C durante 14 días y 42 días con el T1 y T3, luego se alimentaron ratones albinos suizos durante 14 días con el T2 y T3. T1 and T2 treatment were done in two groups: sin glifosato (G0) y con glifosato (G1). A los animales tratados durante 14 días se les realizó

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la prueba de Micronúcleo y a los animales tratados durante 42 días se les realizó la prueba de Micronúcleo y Aberración Cromosómica. Resultados: Ambos ensayos no presentaron efectos mutagénicos en ratones. Se concluye que las variedades de soja evaluadas no presentaron efectos mutagénicos (aneugénicos o clastogénicos) en estas condiciones experimentales.

**Palabras clave:** Aberraciones Cromosómicas-Micronúcleos-Soja-Organismos Genéticamente Modificados.

## ABSTRACT

Soybean (*Glycine max* (L.) merrill) is the main economic crop in Paraguay, where conventional and/or transgenic varieties are planted. Possible side effects of GM soy are still under discussion The aim of this study was to evaluate the mutagenic effect of three varieties of soybean two transgenic BRS 245 RR; *Nueva* Andrea 66R (T1, T2) and a conventional BRS 232 (T3) exposed to Glyphosate in the diet of mice using *in vivo* trials. Methods: tests were carried out in a murine model. Two trials were performed, first feeding BALB/C mice for 14 days and 42 days with T1 and T3, then feeding Swiss albino mice for 14 days with T2 and T3. Treatments were done in two groups: without glyphosate (G0) and with glyphosate (G1). The animals treated for 14 days were tested for Micronucleus and the animals treated for 42 days were tested for Micronucleus and Chromosomal Aberration. Results: Both trials had no mutagenic effects in mice. It is concluded that the soybean varieties evaluated did not present mutagenic effects (aneugenic or clastogenic) under these experimental conditions.

**Keywords:** Chromosome Aberration- Micronucleus –Soybean- Genetically Modified Organism.

## 1. Introduction

Soybean (*Glycine max* (L.) merrill) is a crop native of China, and today is one of the most consumed crops in the world and various derived commercial products are used in food and industries (James, 2011; Azevedo et al., 2010). In Paraguay, the

production of soybeans has been increasing, and it reached a record figure of 9,2 Tons in 2016 to 10.2 Tons in 2018 (CAPPRO, 2023; CAPECO, 2017), and is considered the main agricultural crop in Paraguay (Duarte Sanchez et al., 2021). Seeds of conventional and transgenic soybeans are used for this production, and the use of transgenic varieties has contributed to the economic growth of the country (Morinigo et al., 2018). Glyphosate-resistant transgenic soy has the CP4-5-enolpyruvylshiki-3-phosphate-synthase glyphosate-resistant gene (CP4 EPSPS) (Nair et al., 2002, Nandula, 2019). The adoption of genetically modified soybean seeds increases annually around the world, indicating the excellence of these genotypes for production. Moreover, the possible side effects of transgenic soybeans are still under discussion, authors such as Malatesta (2003) have observed that mice fed with transgenic soybean presented modifications at the nuclear level in hepatocytes, while they did not observe changes at the cytoplasm level. In addition, it was observed that these modifications were potentially reversible (Malatesta et al., 2005), and that the effect produced in treated hepatocytes could be due to the presence of glyphosate residues in the soybean samples (Malatesta et al., 2008a).

In another study, it was observed that animals fed with transgenic soybean presented significant modifications in several characteristics in the nucleus of acinar cells of the pancreas (Malatesta et al., 2003) and in testicular cells (Vecchio et al., 2004). In studies conducted on mice to observe the effects of a transgenic soy diet, found that animals fed a diet containing 14% transgenic soy for 24 months had higher expression of aging markers than animals fed a commercial diet (Malatesta et al., 2008b).

Analysing the possible genotoxic potential of transgenic soy in mice, Azevedo et al., 2010 observed that a diet with 20% protected bone marrow cells from the effects of a mutagenic agent, whereas other genotoxicity studies on mice and *Tradescantia spp* have shown that glyphosate has statistically significant genotoxic effects (Prasad et al., 2009; Alvarez-Moya et al., 2011).

Toxicological genetics aims to analyse and establish the genotoxic risk potential of physical, chemical and/or biological agents that may alter the integrity of the genetic heritage of organisms. The Micronucleus (MN) (Smichd et al., 1975) and

Chromosome Aberration (AC) tests are used to evaluate the potential risk of damage to the genome at chromosomal level (clastogenic or aneugenic) that a chemical or biological agent may have and this test is characterized by their relative low cost, simple and reproducible (Gao et al., 2017; Premkumar and Bowlus, 2003; Preston et al., 1987).

In this research work we have collected two trials with transgenic and conventional soybeans exposed to glyphosate by applying genotoxicity tests to bone marrow cells from soya-fed mice, in order to determine a toxic potential.

## 2. Methodology

2.1. ***Reagents***: Cyclophosphamide-CP (Sigma), fetal bovine serum (PAA), colchicine (Sigma) balanced diet for animals, ethanol, methanol, glacial acetic acid, glyphosate (Roundup®).

2.2. ***Animals:*** a total of 75 animals' female and male of strain BALB/c and 30 animals' female and male of strain 30 Swiss albinos (*Mus musculus*) were used. At the beginning of each assay, the animals were between 7 and 10 weeks of age and had an average weight between 19 and 23 g. Animals (BALB/c) were acquired and maintained in the Central Animal Health Service of the National Animal Health Service (SENACSA) and Swiss Albinos were acquired from the Vivarium of the Research Institute of Health Sciences (*Instituto de Investigaciones en Ciencias de la Salud*, IICS-UNA) and maintained in the Biotechnology Laboratory (*Laboratorio de Biotecnología*, CEMIT-UNA), 2 or 3 animals *per* box. The animals used in this study were kept under the same conditions in bed of autoclaved pine shavings. Water was provided *ad libitum*.

### 2.3. Ethical considerations

The ethical consideration of experimentation with animals defined by the Organization for Economic Cooperation and Development were followed (OECD, 2013; OECD, 2007), recommendations of the Guide of International Principles for Biomedical Research involving animals, prepared by the Council for International Organizations of Medical Sciences (CIOMS, 2007), were followed.

The minimum number of animals possible was used and all precautions were taken to avoid unnecessary suffering and the knowledge acquired in the "Laboratory Animals Course-2013" offered by the University of Buenos Aires (Ar.). The research protocol was approved by the management of CEMIT-UNA before being submitted for funding.

2.4. **Plant material**:

S*oybean cultivars*, to obtain samples of transgenic soybeans, BRS 245 RR (T1) (transgenic) was grown out in the experimental field of the Faculty of Agricultural Sciences (*Facultad de Ciencias Agrarias* FCA-UNA) and Nueva Andrea 66 RR (T2) (transgenic) was grown in a particular field. Additionally, in each trial, the variety BRS 232 (T3) (conventional) was grown.

2.5. **Harvest soybean**:

For the two trials three plots were cultivated in 10mx4m each one. Two transgenic varieties (BRS 245RR; Nueva Andrea 66) and one conventional variety (BRS 232) considered as control. For the first trial, two plots of BRS 245RR soybean were prepared. For the second trial, two plots of Nueva Andrea 66 soybean were prepared. In both trials, the plots were cultivated one with glyphosate and the other without glyphosate. The dose of glyphosate used was 3.2 kg /ha. The last plot corresponds to BRS 232.

2.6. **Preparation of experimental diets**

*2.6.1.* ***Trial 1. Experimental diets*** **using BRS 232 and BRS 245RR soybeans**:

For the first trial, the industrial pellets were prepared by mixing 15% of soybeans of each variety with the balanced diet of animals using the pelletizing machine from the *Cooperativa Colonias Unidas* (Itapúa, Paraguay). Pellet A; 15% soybeans BRS 245 RR (without glyphosate G0); Pellet B: 15% soybeans BRS 245 RR, (with 3.2 kg / ha of glyphosate G1); Pellet C: 15% soybean BRS 232; Groups CTL- (negative control); CP (positive control).

*2.6.2.* ***Trial 2. Experimental diets*** **with BRS 232 and Nueva Andrea 66 RR**: For the second trial, the pellets were prepared in the Biotechnology Laboratory (*Laboratorio de Biotecnología*, CEMIT-UNA). The artisanal pellets were prepared by mixing 20 % soybean, 20% culinary starch and 60% of balanced diet with proteins and water. The pellets were dried in the oven dried at 28 ° C, for 72 h. Pellet E: 20% soybean New Andrea 66RR (without glyphosate G0); Pellet F: 20% soybean New Andrea 66 RR (with 3.2 kg/ha of glyphosate G1); Pellet G: 20% soybean BRS 232. Groups CTL- (negative control) and CP (positive control) were fed with Pellet I (Balanced, starch and water).

2.7. ***In vivo assays***

2.7.1. ***Micronucleus assay (MN)***: Briefly, the animals were sacrificed by cervical rupture, the femurs were removed and the bone marrow was extracted with fetal bovine serum (FBS) and maintained at 37°C. The material was homogenized, transferred to a conical tube, and then centrifuged at 1000 rpm for 5 minutes. The supernatant was discarded and the samples were prepared with the remaining cells. The material was fixed 24 hours later in absolute methanol for 5 minutes. The samples were stained with 4% Giemsa and analyzed in an immersion optical microscope (100x). The polychromatic erythrocytes were counted, including those with micronuclei (MNs). Cyclophosphamide (50 mg/bw) was used as a positive control.

2.7.2. ***Chromosome Aberration*** **(*CA*)**: Briefly, the animals were sacrificed by cervical dislocation, the femurs were removed and the bone marrow was washed with hypotonic KCl solution, at 37 °C. The cell sample was centrifuged, the supernatant discarded, the sample was washed with fixing solution (Ethanol-glacial acetic acid, 3: 1) for three times, the supernatant was discarded and the samples were prepared by dropping 1-2 drops on clean and kept in cold water slide. The samples were stained with 4% Giemsa. One hundred metaphase cells were analyzed per animal treated and the samples were analyzed in immersion optical microscopy (100 x). The treatment with Colchicine (5mg/BW) is done 24 hours before the sacrifice of the animals, to obtain metaphase cells.

2.8. ***Statistical analysis***: The statistical significance of the Micronucleus frequency was evaluated with ANOVA and unilateral Dunnett's post hoc test. Statistical significance of the Chromosome Aberration was assessed with Kruskal-Wallis and post hoc Mann-Whitney pairwise test (significance of sequential Bonferroni).

## 3. Results and discussion

### *3.1. Evaluation of the toxic potential of the experimental diets with 15% BRS 245 RR (T1) or BRS 232 (T3) soybeans*

#### *3.1.1 MN test*

Risk assessment of herbicide-tolerant soybeans still under discussion (Miyazaki et al., 2019). Genotoxicity assays are used to evaluate the genotoxic potential of plant extracts (Lee et al., 2018, Silva et al., 2016), the safety of transgenic soy proteins (Papineni et al., 2017) or food (Sales et al., 2016). This study was carried out to evaluate the effects of transgenic soybean son cells from treated (fed) mice. For this purpose, conventional and transgenic soybeans were cultivated in the experimental field of FCA-UNA and a private field in the harvests corresponding to the year 2013. Transgenic soybean was obtained, BRS 245RR, and a variety of conventional soybean, BRS 232.

The plots (samples) of transgenic soybeans obtained in the field of the FCA-UNA were treated with up to 100% glyphosate (3.2 Kg/ha). The guidelines of the OECD (2013) and Adetutu et al., (2004) were followed. For this test, 25 animals were used, five for each experimental group and the negative and positive control groups (50 mg CP/Kg BW). Female BALB/c mice of the four experimental groups were fed with the pellets obtained for 14 days, while the animals of the negative and positive control groups were fed with the balanced meal used to prepare the pellets of the experimental groups.

**Table 1** shows the results obtained after feeding BALB/c mice with the different pellets, prepared with 15% conventional soybean or transgenic soybean. The cancer drug Cyclophosphamide (Shruthi and Vijayalaxmi, 2016; Berno et al., 2016) was used as a positive control of all the assays and injected intraperitoneal 24 hours before the animals were sacrifice.

**Table 1***:* Frequency of Micronucleated Polychromatic Erythrocytes (MPE) in bone marrow cells of BALB/c mice treated for 14 days.

| Treatment | MPE (%±SD) | Treatment time | Total animals /treatment (♀) |
|---|---|---|---|
| **CTL-** | 15 (0.3±2.24) | 14 days | 05 |
| **CP** | 63(1.26±4.88) * | 24 hours | 05 |
| **Pellet A** | 7 (0.14±1.14) | 14 days | 05 |
| **Pellet B** | 18 (0.36±1.14) | 14 days | 05 |
| **Pellet C** | 14 (0.28±1.64) | 14 days | 05 |

CTL-: negative control; CP: Cyclophosphamide. Pellet A; 15% soybeans BRS 245 RR (without glyphosate G0); Pellet B: 15% soybeans BRS 245 RR, (with 3.2 kg / ha of glyphosate G1); pellet C: 15% soybean BRS 232. * Statistically significant $p<0.05$.

In this study, a total of 5,000 cells were analysed for each treatment group. Of which an average of 1,000 cells *per* animal. It was observed that there were no significant differences between the frequencies of the Micronucleated polychromatic erythrocyte (MPEs) frequencies when the treatment groups (conventional soybean or transgenic) were compared with the negative control group, or the groups of transgenic soybeans with the conventional soybean group.

Modifications in Micronucleus frequencies indicate the structural effect of chromosomes or a failure of chromosome migration during cell division (Luzhna et al., 2013, Lee et al., 2016, Hatch and Hetzer, 2016). An important difference was observed when we compared the frequencies of MPEs of the negative control group versus the positive control group (CP).

### 3.1.2. Micronucleus test in mice treated for 42 days with BRS 245 RR soybean

For this test, 50 mice BALB/c (25 females and 25 males) were used, the sex is important because the effect could be evaluated depending on the sex (Rojas-Lemus et al., 2014). BALB/c mice of the 4 experimental groups were fed with the pellets obtained,

for 42 days, while the animals of the negative and positive control groups were fed with the balanced meal used to prepare the pellets of the experimental groups. The animals of this test were treated 2 hours before sacrifice with colchicine 4mg / Kg of WB and, from each animal, the Micronucleus test was applied to one femur and the Chromosome Aberrations test was applied to the other femur.

Table 2 shows results of frequency of Micronucleated polychromatic erythrocytes (MPE) and the Standard Deviation (SD). As result the frequencies were negative in all of groups of treatment and no external signs of toxicity were observed during the treatment.

**Table 2**: Frequency of Micronucleated Polychromatic Erythrocytes (MPE) in bone marrow cells of male and females BALB/c mice treated for 42 days.

| Treatment | MPEs | | Treatment duration | Total animals /treatment |
|---|---|---|---|---|
| | Females | Males | | |
| **CTL-** | 9 (0.18 ±0.84) | 20 (0,2±1,22) | 42 days | 10 (5♀, 5♂) |
| **Pellet A** | 10 (0.2 ±0.71) | 10 (0,21±2,95) | 42 days | 10 (5♀, 5♂) |
| **Pellet B** | 5 (0.10 ±0.71) | 10 (0,2±0,71) | 42 days | 10 (5♀, 5♂) |
| **Pellet C** | 5 (0.10 ±0.71) | 11 (0,22±1,48) | 42 days | 10 (5♀, 5♂) |
| **CP** | 35 * (0.70 ±2.55) | 31* (0,74±2,86) | 24 h | 10 (5♀, 5♂) |

CTL-: negative control; CP: Cyclophosphamide. Pellet A; 15% soybeans BRS 245 RR (without glyphosate G0); Pellet B: 15% soybeans BRS 245 RR, (with 3.2 kg / ha of glyphosate G1); Pellet C: 15% soybean BRS 232. * Statistically significant $p<0.05$.

Evaluation of BRS 245RR and BRS 232 soybeans. Same as the previous test, the guidelines of the OECD and Adetutu *et al.*, (2004) were followed. Changes in MN frequencies were analyzed in bone marrow cells of mice fed for 14 days with BRS 245RR soybean samples. Modifications in MN frequencies indicate the structural effect of chromosomes or a failure of chromosome migration during cell division (Luzhna *et al.*, 2013, Lee *et al.*, 2016, Hatch and Hetzer, 2016). In this analysis, 1,000 cells *per* treated animal were counted and in the animals of the negative control group the percentage was

0.3, which is in accordance with the range of data found in the literature (Slapšytė *et al.*, 2013). The MN frequencies found in the experimental groups were statistically not significant, when compared with the frequencies of the negative control (**Table 2**).

**3.1.3 Chromosomal Aberration assay in cells from mice treated for 42 days with BRS 232 or BRS 245 RR soybean**

For that test, the same Micronucleus test animals were analysed. The animals were treated for 42 days. Eight animals were analysed *per* treatment group (4 females and 4 males) and 100 cells counted per animal. The guidelines of OECD (2007) were followed. **Table 3** shows the frequency of cells with Chromosomal Aberrations (ACs) in bone marrow cells of mice, also the frequencies of ACs of the negative and positive control groups observed. The frequency of ACs found in the soybean groups was not significant. No external signs of toxicity were observed during the treatment time**.**

**Table 3**: Frequency of CA in bone marrow cells of male and females BALB/c mice treated for 42 days.

| Treatment | CA types | | | Total cells with CA | Treatment time | Animals |
|---|---|---|---|---|---|---|
| | Break | Fragment | Gap | | | |
| CTL- | 1 | 3 | 4 | 4 | 42 days | 8 (4♀, 4♂) |
| CP | 14 | 57 | 6 | 55* | 24 h | 8 (4♀, 4♂) |
| Pellet A | 2 | 4 | 3 | 6 | 42 days | 8 (4♀, 4♂) |
| Pellet B | 2 | 4 | 1 | 5 | 42 days | 8 (4♀, 4♂) |
| Pellet C | 5 | 2 | 3 | 7 | 42 days | 8 (4♀, 4♂) |

CTL-: negative control; CP: Cyclophosphamide. Pellet A; 15% soybeans BRS 245 RR (without glyphosate G0); Pellet B: 15% soybeans BRS 245 RR, (with 3.2 kg/ha of glyphosate G1); pellet C: 15% soybean BRS 232. * Statistically significant $p<0.05$.

Effect of the samples on mice was also evaluated in a 42-day trial, where the frequencies in the experimental groups were similar to the frequencies of the negative control group. In parallel to the MN test, the same animals were tested for changes in the frequencies of CA using a femur for each test.

The results in the experimental groups compared with the negative control were statistically not significant. Whereas, the result in the positive control group was statistically significant in a visual evaluation, the animals were in good condition, with mobility, without external signs of having been affected by the treatment. Therefore, after the clinical evaluation, this result is debatable and more studies must be performed to reach a conclusion.

### 3.2 Evaluation of the toxic potential in the experimental diets with 20% soybean New Andrea 66 RR (T2) and BRS 232(T3)

Another evaluation of transgenic soybean, using a Nueva Andrea 66 RR variety was prepared. Swiss albino mice (3 females and 3 males) fed with pellets prepared with soy samples obtained in the particular field. The total soybean present in each type of pellet was 20%. The animals were treated for 14 days. **Table 4** shows the frequencies of Micronucleated Polychromatic Erythrocytes found in the bone marrow cells of the treated animals. In the group of transgenic soy without glyphosate 5,000 cells were analyzed because one animal died before the end of treatment. SD is not presented due to the low number of MNs found.

**Table 4**: Frequency of Micronucleated Polychromatic Erythrocytes (MPE) in bone marrow cells of male and female Swiss albino mice treated Nueva Andrea RR66 for 14 days.

| l | MNPCEs (%) | Treatment time | N animals |
|---|---|---|---|
| CTL- | 2 (0,03) | 14 days | 06 (3♀, 3♂) |
| CP | 54*(0.9) | 24 h | 06(3♀, 3♂) |
| Pellet E | 0 (0) | 14 days | 06(3♀, 3♂) |
| Pellet F | 7 (0.11) | 14 days | 06(3♀, 3♂) |
| Pellet G | 4 (0.06) | 14 days | 06(3♀, 3♂) |

CTL-: negative control; CP: Cyclophosphamide. Pellet E: 20% soybean New Andrea 66RR (without glyphosate G0); Pellet F: 20% soybean New Andrea 66 RR (with 3.2 kg/ha of glyphosate G1); Pellet G: 20% soybean BRS 232. PCE: Polychromatic Erythrocytes. * statistically significant $p<0.05$.

Considering the results obtained in the evaluation of the BRS 245 RR soybean, we decided to evaluate another variety of transgenic soybean, increasing the percentage of it in the pellet. This test carried out on the Swiss albino strain due to the availability of the animals. In this study, modifications in Micronucleus frequencies analyzed in bone marrow cells from mice fed with the samples obtained. All results obtained in these studies not observed changes in the frequencies of MNs that statistically significant when compared with the frequencies found in the control group or between GM soybean treatment groups. In a visual evaluation, the animals were in good condition, with mobility, without external signs of affected by the treatment. In the treatments carried out for the micronucleus test the number of animals per treatment was 5 animals according to (Henderson et al., 2023; Sánchez-de-la-Rosa et al., 2022). For the chromosomal aberrations (Kour et al., 2017; Lovinskaya et al., 2016) a greater number of animals were used per treatment group, considering that animals may die during the process (Lin et al., 2022b). In this research, the sex of the animals was not taken into account, since the final objective of the test was not to compare the effect of the treatment between sexes, but rather to evaluate the activity by group of individuals. The results found in this work coincide with the results reported by Azevedo et al. (2010) and Venancio et al. (2012), that in two different studies have seen that soybean, conventional and transgenic, does not present genotoxic effects in bone marrow cells of mice, besides presenting a protective effect, when animals are treated with a genotoxic previously. In a recent investigation, after our study, Shi et al, (2019), observed that the intake of 20% transgenic soybean did not produce adverse effects in the testicles of mice. we conclude that transgenic soybeans, BRS 245RR varieties and New Andrea 66RR, do not present a toxic potential, under these experimental conditions.

## 4. Conclusion

The results obtained in the tests applied in laboratory animals do not indicate a genotoxic potential of the soybean varieties evaluated, both conventional and transgenic, on bone marrow cells of animals. Consequently, we conclude that transgenic soybeans varieties

BRS 245RR and New Andrea 66RR, and conventional soybean BRS 232, do not present a toxic potential, under these experimental conditions.

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**References**

Adetutu, A., Odunola, O. A., Owoade, O. A., Adeleke, O. A., & Amuda, O. S. (2004). Anticlastogenic effects of *Hibiscus sabdariffa* fruits against sodium arsenite-induced micronuclei formation in erythrocytes in mouse bone marrow. Phytotherapy Research: An International Journal Devoted to Pharmacological and Toxicological Evaluation of Natural Product Derivatives, 18(10), 862-864.

Alvarez-Moya, C., Reynoso Silva, M., Villalobos Arámbula, A. R., Islas Sandoval, A., Castañeda Vasquez, H., & González Montes, R. M. (2011). Evaluation of genetic damage induced by glyphosate isopropylamine salt using Tradescantia bioassays. Genetics and Molecular Biology, 34, 127-130.

Azevedo, L., Dragano, N. R., Sabino, A. P., Resck, M. C. C., de Lima, P. L. A., & Gouvêa, C. M. (2010). In vivo antimutagenic properties of transgenic and conventional soybeans. Journal of Medicinal Food, 13(6), 1402-1408.

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Berno, C. R., de Toledo Rós, B., da Silveira, I. O. M. F., Coelho, H. R., Antoniolli, A. C. M. B., Beatriz, A., ... & Oliveira, R. J. (2016). 4-Aminoantipyrine reduces toxic and genotoxic effects of doxorubicin, cisplatin, and cyclophosphamide in male mice. Mutation Research/genetic Toxicology and Environmental Mutagenesis, 805, 19-24.

CAPECO. https://capeco.org.py/area-de-siembra-produccion-y-rendimiento/. Revisado en diciembre 2023.

CAPPRO 2017. http://cappro.org.py/estadisticas

CIOMS. Council for International Organizations of Medical Sciencies. (2007). International Guiding Principles for Biomedical Research Involving Animals. URL available in: https://cioms.ch/publications/product/international-guiding-principles-for-biomedical- research-involving-animals-2/.

Gao, Y., Li, C., Wang, Y., Liu, Y., Li, G., Fan, X., ... & Lee, A. W. (2017). Nonclinical safety of astilbin: a 4-week oral toxicity study in rats with genotoxicity, chromosomal aberration, and mammalian micronucleus tests. Food and chemical toxicology, 107, 1-9.

Hatch, E. M., & Hetzer, M. W. (2015). Linking micronuclei to chromosome fragmentation. Cell, 161(7), 1502-1504.

Henderson, R. G., Welsh, B. T., Trexler, K. R., Bonn-Miller, M. O., & Lefever, T. W. (2023). Genotoxicity evaluation of cannabidiol. Regulatory Toxicology and Pharmacology, 142, 105425.

James, C. (2007). Global Estatus of Commercialized Biotech/GM Crops. ISAAA Internacional Service for the Acquisition of Agri-Biotech Applications Biref 2007, Number 37. ISAAA, Ithaca, NY.

Kour, J., Ali, M. N., Ganaie, H. A., & Tabassum, N. (2017). Amelioration of the cyclophosphamide induced genotoxic damage in mice by the ethanolic extract of Equisetum arvense. *Toxicology reports*, *4*, 226-233.

Lin, H. Y., Liao, J. W., Chen, R. S., Chang, C. H., Chang, H. W., Chang, S. C., ... & Lin, H. T. (2022). Food safety assessment of commercial genetically modified soybeans in rats. Foods, 11(4), 496.

Lovinskaya, A. V., Kolumbayeva, S. Z., Kolomiets, O. L., & Abilev, S. K. (2016). Genotoxic effects of pesticide fipronil in somatic and generative cells of mice. Russian Journal of Genetics, 52, 491-497.

Luzhna, L., Kathiria, P., & Kovalchuk, O. (2013). Micronuclei in genotoxicity assessment: from genetics to epigenetics and beyond. Frontiers in genetics, 4, 131.

Malatesta, M., Caporaloni, C., Gavaudan, S., Rocchi, M. B., Serafini, S., Tiberi, C., & Gazzanelli, G. (2002). Ultrastructural morphometrical and immunocytochemical analyses of hepatocyte nuclei from mice fed on genetically modified soybean. Cell structure and function, 27(4), 173-180.

Malatesta, M., Baldelli, B., Battistelli, S., Tiberi, C., Manuali, E., & Biggiogera, M. (2005). Reversibility of hepatocyte nuclear modifications in mice fed on genetically modified soybean. European Journal of Histochemistry, 49(3), 237-242.

Malatesta M, Perdoni F, Santin G, Battistelli S, Muller S, Biggiogera M. (2008). Hepatoma Tissue Culture (Htc) Cells As A Model For Investigating The Effects Of Low Concentrations Of Herbicide On Cell Structure And Function. Toxicol In Vitro. Dec;22(8):1853-60.

Mee-Young, L., Seo, C. S., Ha, H., Park, E., Ji-Young, K., & Shin, H. K. (2018). The genotoxicity of an aqueous extract of Gyejibokryeong-hwan. BMC Complementary Medicine and Therapies, 18.

Miyazaki, J., Bauer-Panskus, A., Bøhn, T., Reichenbecher, W., & Then, C. (2019). Insufficient risk assessment of herbicide-tolerant genetically engineered soybeans intended for import into the EU. Environmental Sciences Europe, 31(1), 1-21.

Morínigo Aguayo, J., Achinelli Báez, M., & Barrios Leiva, O. (2018). La soja en el Paraguay. Una aproximación a la cuantificación económica. Periodo 1994-2016. Población y Desarrollo, 24(46), 24-36.

Nair Rs, Fuchs Rl, Schuette Sa. 2002. Current Methods For Assessing Safety Of Genetically Modified Crops As Exemplified By Data On Roundup Ready Soybeans. Toxicol Pathol. Jan-Feb;30(1):117-25.

Nandula, V. K. (2019). Herbicide resistance traits in maize and soybean: current status and future outlook. Plants, 8(9), 337.

OECD (2013), Test No. 474: Mammalian Erythrocyte Micronucleus Test, OECD Guidelines for the Testing of Chemicals, Section 4, OECD Publishing, Paris, https://doi.org/10.1787/9789264264762-en.

OECD (2007), Test No. 475: Mammalian Bone Marrow Chromosomal Aberration Test, OECD Guidelines for the Testing of Chemicals, Section 4, OECD Publishing, Paris, https://doi.org/10.1787/9789264264786-en.

Papineni, S., Murray, J. A., Ricardo, E., Dunville, C. M., Sura, R. K., & Thomas, J. (2017). Evaluation of the safety of a genetically modified DAS-444Ø6-6 soybean meal and hulls in a 90-day dietary toxicity study in rats. Food and chemical toxicology, 109, 245-252.

Prasad, S., Srivastava, S., Singh, M., & Shukla, Y. (2009). Clastogenic effects of glyphosate in bone marrow cells of Swiss albino mice. Journal of toxicology, 2009(1), 308985.

Premkumar, K., & Bowlus, C. L. (2003). Ascorbic acid reduces the frequency of iron induced micronuclei in bone marrow cells of mice. Mutation Research/Genetic Toxicology and Environmental Mutagenesis, 542(1-2), 99-103.

Preston, R. J., Dean, B. J., Galloway, S., Holden, H., McFee, A. F., & Shelby, M. (1987). Mammalian in vivo cytogenetic assays analysis of chromosome aberrations in bone marrow cells. Mutation Research/Genetic Toxicology, 189(2), 157-165.

Rojas-Lemus, M., Altamirano-Lozano, M., & Fortoul, T. I. (2014). Sex differences in blood genotoxic and cytotoxic effects as a consequence of vanadium inhalation: micronucleus assay evaluation. Journal of Applied Toxicology, 34(3), 258-264.

Sánchez, D. D. D., Barreto, R. G., & Chung, C. K. K. (2022). Importancia de la producción de soja en la economía paraguaya en el año 2021. Revista Científica Estudios e Investigaciones, 11(1), 146-154.

Sánchez-De-La-Rosa, S. V., Lazalde-Ramos, B. P., Morales-Velazquez, G., Zúñiga-González, G. M., Gómez-Meda, B. C., Sánchez-Rivera, S. O., ... & Zamora-Perez, A. L. (2022). Evaluation of genotoxic effect and antigenotoxic potential against DNA damage of the aqueous and ethanolic leaf extracts of annona muricata using an in vivo erythrocyte rodent micronucleus assay. BioMed Research International, (1), 9554011.

Sousa Sales, I. M., Sousa Barbosa, J., Silva dos Santos, F. K., Cavalcanti Carneiro da Silva, F., Pinheiro Ferreira, P. M., de Castro e Sousa, J. M., & Peron, A. P. (2017). Assessment of grape, plum and orange synthetic food flavourings using in vivo acute toxicity tests. Food Technology and Biotechnology, 55(1), 131-137.

Schimid, W. The micronucleus test. Mutat. Res., 1975.vol. 31, no. 1, p. 9-15

Silva, R. M., Pereira, L. D., Véras, J. H., do Vale, C. R., Chen-Chen, L., & da Costa Santos, S. (2016). Protective effect and induction of DNA repair by Myrciaria cauliflora seed extract and pedunculagin on cyclophosphamide-induced genotoxicity. Mutation Research/Genetic Toxicology and Environmental Mutagenesis, 810, 40-47.

Shi, Z., Zou, S., Lu, C., Wu, B., Huang, K., Zhao, C., & He, X. (2019). Evaluation of the effects of feeding glyphosate-tolerant soybeans (CP4 EPSPS) on the testis of male Sprague-Dawley rats. GM crops & food, 10(3), 181-190.

Shruthi, S., & Vijayalaxmi, K. K. (2016). Antigenotoxic effects of a polyherbal drug septilin against the genotoxicity of cyclophosphamide in mice. Toxicology reports, 3, 563-571.

Slapšytė, G., Dedonytė, V., Lazutka, J. R., Mierauskienė, J., Morkūnas, V., Kazernavičiūtė, R., ... & Venskutonis, P. R. (2013). Evaluation of the biological activity of naturally occurring 5, 8-dihydroxycoumarin. Molecules, 18(4), 4419-4436.

Venâncio, V. P., Silva, J. P. L., Almeida, A. A., Brigagão, M. R., & Azevedo, L. (2012). Conventional (MG-BR46 Conquista) and transgenic (BRS Valiosa RR) soybeans have no mutagenic effects and may protect against induced-DNA damage in vivo. Nutrition and cancer, 64(5), 725-731.