CROCODILE SPECIALIST GROUP

Student Research Assistance Scheme (SRAS)

FINAL REPORT

Project title: DNA oxidative damage in *Caiman latirostris* exposed to pesticides formulations under semi-natural conditions

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INTRODUCTION

Human activities continuously introduce several amounts of agrochemicals into the environment, especially associated to transgenic crops, independently of their persistence, bioaccumulation and toxicity (Vera Candioti, et al, 2013).

Caiman latirostris is an important reptile species representative of the wetlands of the northeastern region of Argentina, one of the main region of agricultural activity in our country. This overlapping of areas means that the natural populations of *C. latirostris* are constantly exposed to pesticides of different nature such as Cypermethrin, Glyphosate and Chlorpyrifos, which are widely used in transgenic soybean crops in our country (Lacelli, 2016). Due to their biological and ecological characteristics, these organisms have been used as biological monitors and are considered sentinels of environmental pollution (Poletta et al, 2008; Poletta et al, 2009).

Several xenobiotics, and among them pesticides, can produce reactive oxygen species (ROS). The augment of ROS levels can cause oxidative modification of the nucleic acid bases, with serious consequences at genomic instability (Valavanidis et al, 2006; Collins, 2009; Azqueta et al, 2009).

The modified Comet Assay (CA), which incorporates the bacterial endonucleases Formamidopyrimidine DNA glycosylase (FPG) and Endonuclease III (ENDO III), increases the sensitivity of the assay and, more importantly, their specificity (Smith et al, 2006; Azqueta et al, 2009), allowing to detect the generation of oxidative stress on DNA, acting as a mechanism of pesticides toxicity (Poletta et al, 2016). Their application on peripheral blood represents an important advantage, as we can obtained samples without causing any damage to the animals (Poletta et al, 2016).

Taking into account that *C. latirostris* is an environmental and economic resource very representative of Argentina and its natural populations can be severely affected by the application of pesticides, it is important to incorporate biomarkers of oxidative stress that, in combination with other types of biomarkers used in our research group, constitutes an important tool for environmental monitoring programs.

OBJETIVE

The aim of this project was to evaluate the potential DNA oxidative damage in juveniles of *Caiman latirostris* exposed to different pesticides formulations and a mixture of them in semi-naturals conditions, using the modified CA with the enzymes FPG and ENDO III.

MATERIALS AND METHODS

We used juveniles of *C. latirostris* between 7 and 8 months old, hatched from eggs harvested from different nests in the Natural Managed Reserve "El Fisco".

Animals were randomly distributed into 5 enclosures, with 20 animals each (N=100): **1.** a negative control (NC) treated with potable water; **2.** a group exposed to glyphosate formulation (RU - Roundup®Full II- 2 %); **3.** a group exposed to cypermethrin formulation (CYP - Atanor® - 0.12%); **4.** a group exposed to chlorpyrifos formulation (CPF - Lorsban* - 0.8%) and **5.** a group exposed to a mixtures of this three agrochemicals.

The experiment was carried out, in the warm season (December to February), during three months. The enclosures were built with outdoor at the "Proyecto Yacaré" facilities with an approximate size of 2m in base diameter and 1.5 m in height, with the upper part totally closed with shade clothe to avoid direct sun incidence and keep predators out. The distance between them were enough to avoid cross contamination.

Concentration of pesticides formulations was equivalent to those recommended for their application in soybean crops. Two applications were made using a backpack sprayer, spraying the total surface from a height of 0.5 meter and before the applications the caiman were taken out from the enclosures to avoid direct spraying on them.

At the end of the experiment, blood samples (0.5 ml) were taken from the spinal vein of all animals with heparinized syringes and 25G x 5/8" needles to apply the modified CA.

Animals were measured in snout-vent length (SVL), total length (TL) and weighed at the beginning and at the end of the experiment to determine growth in each experimental group.

Fig. 1: Enclosure used in the experiment.

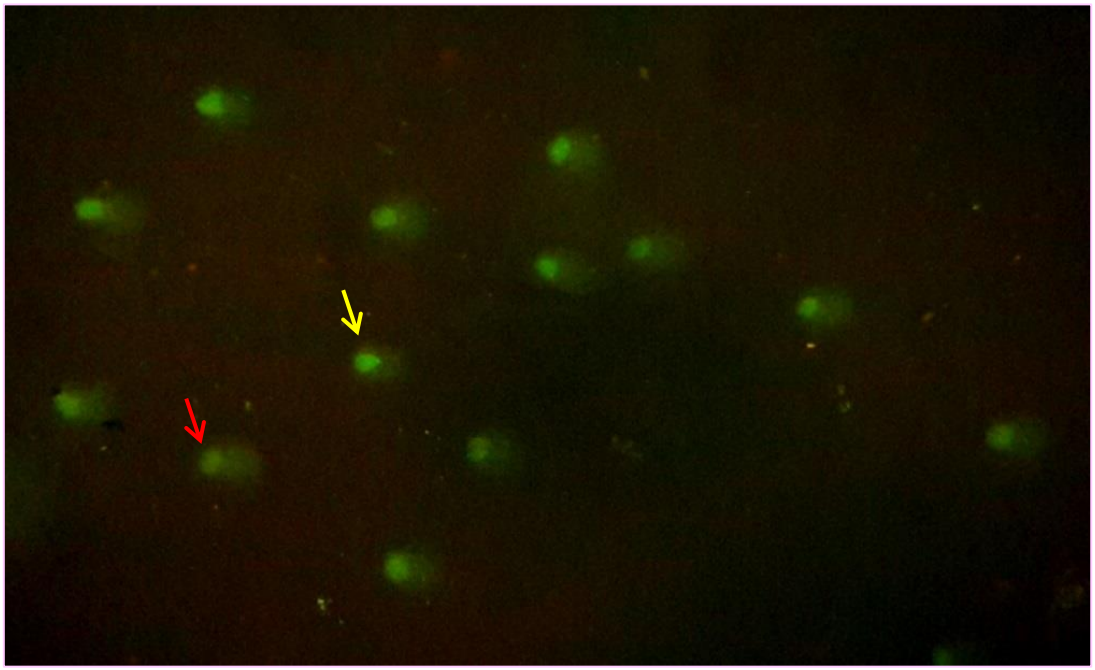

Fig. 2: Comet images of different damage classes. Class 2: low damage (yellow arrow) and class 3: high damage (red arrow), according to tail size and intensity. Staining: AO (400X)

RESULTS AND DISCUSSION

Results demonstrated oxidation of purines and pyrimidines respect to the NC, indicating oxidative DNA damage in M (p=0.0166), RU (p=0.0307), CPF (p=0.0138) and CYP (p=0.0263). This result can be attributed to the overproduction of reactive oxygen species produced by pesticides, that impact directly on DNA, producing oxidative damage to the molecule. This can lead to multiple deficiencies in the affected animals.

TREATMENT	FPG SITES	ENDO III SITES
NC	11.2	12.9
CPF	43,2*	51,1*
RU	42,88*	50,5*
CYP	48,57*	72,86*
M	66,1*	74,7*

TABLE I: Means of the treatments applied in *C. latirostris* animals respect to the NC ($p < 0.05$)

On the other hand, no effects were observed in length or weight of the caimans exposed to any pesticide formulation or complex mixtures tested in any of the experiments, compared with the NC ($p>0.05$ in all analysis performed).

The incorporation of biomarkers of oxidative stress, in combination with other markers of genotoxicity, immunotoxicity and developmental alterations is extremely important to evaluate the impact of different pesticides and mixtures as environmental stress factors of human origin, considering the implication this would have on the conservation and management of *C. latirostris*.

Furthermore, the application of oxidative stress biomarkers in peripheral blood represents an important advantage as we can obtain samples without causing any damage to the animals, which means all there parameters can be applied to wild caiman population under risk of exposure.

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