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Toxicidade do herbicida atrazina em Colossoma macropomum

Toxicity of atrazine herbicide in Colossoma macropomum

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RESUMO

O objetivo do presente estudo foi investigar a toxicidade do herbicida GESAPREME, pertencente ao grupo das Triazinas (princípio ativo é atrazina = 500gL^{-1}), em *Colossoma macropomum* juvenis (tambaqui). Para este propósito foram determinados a CL_{50} e a freqüência de micronúcleos (MN) em eritrócitos. A mortalidade de tambaquis aumentou com o aumento nas concentrações do GESAPREME na água. O valor da CL_{50} (96h) foi atingido na concentração de 22 mg L^{-1}. Após 48 horas de exposição ao herbicida, nas concentrações de 20, 22 e 25 mg L^{-1}, notou-se hemorragias por todo o corpo dos animais. O número de micronúcleos nos eritrócitos aumentou significativamente durante a exposição dos peixes ao GESAPREME nas concentrações de 10, 20 e 30 mg L^{-1}, quando comparado ao grupo controle. A ação do herbicida foi claramente capaz de induzir a formação de micronúcleos em *Colossoma macropomum*. Estes resultados sugerem que as células são danificadas em sua integridade pela exposição à atrazina, devido à alta incidência de micronúcleos observada nos eritrócitos. Os agronegócios têm se expandido no Brasil e os riscos de contaminação das águas dos rios e pisciculturas pelos agentes xenobióticos têm aumentado. Assim, a detecção de agrotóxicos e seus efeitos sobre os organismos são estudos importantes, uma vez que eles podem indicar o impacto destes produtos sobre os organismos vivos.

Palavras-chave: CL_{50}, estresse, micronúcleo, tambaqui, xenobiótico

SUMMARY

The present study aimed to investigate the toxicity of the herbicide GESAPREME, belonged to the Triazines group (the active principle is atrazine = 500gL^{-1}), on the juveniles of the fish species *Colossoma macropomum* (tambaqui). For this purpose, the LC_{50} and the frequency of micronuclei (MN) in erythrocytes were determined. Fish mortality increased together with the increment in water GESAPREME concentrations. The value of LC_{50} (96h) was reached in the concentration of 20 mg L^{-1}. After 48h of herbicide exposition, hemorrhages could be noted in the whole body of fish exposed to the concentrations of 20, 22 and 25 mg L^{-1}. The number of micronuclei in erythrocytes increased significantly during the exposition of fish to GESAPREME concentrations of 10, 20 and 30 mg L^{-1}, when compared to the control group. The herbicide action was clearly capable to induce micronuclei formation in the erythrocytes of *Colossoma macropomum*. These results suggest that cells are seriously damaged in its integrity by the exposition to GESAPREME, due to the high micronuclei incidence observed in erythrocytes. Agribusiness has been expanded in Brazil and the risk of water rivers and aquaculture contamination by xenobiotics agents has been increased. Therefore, the detection of pesticide and their effects on the organisms have been became important studies, since they can predict the impact of these products on live organisms.

Keywords: LC_{50}, micronuclei, stress, tambaqui, xenobiotic

INTRODUCTION

The massive use of pesticides has been an important strategy for the increment in production even they are known to be important pollutants and causative of environmental changes. Studies examining several vertebrates have shown that exposure to pesticides can result in detrimental effects on developing nontarget organisms both in natural habitats and in the laboratory (GILBERT & BOLKER 2001; MATSUSHITA et al., 2006; ROHR et al., 2006; LENKOWSKI et al., 2008). Despite of the use of pesticides as an important way for controlling plagues and diseases, which in turn could bring damage and lost for agriculture, on the other hand they also bring the risk of toxicity to several organisms (YOUNES & GALAL-GORCHEV, 2000; WIEGAND et al., 2001; RESGALLA et al., 2002; TOMITA & BEYRUTH, 2002; VEIGA et al., 2002; ARMAS et al., 2005; GARCIA-REYERO & DENSLOW, 2006; ALBINATI et al., 2007; GUIMARÃES et al. 2007).
Biological tests for toxicity and genotoxicity are indispensable for the evaluation of pollutants on living organisms. One of the widely employed methods to evaluate damages caused for xenobiotics substances in living organisms is the Micronuclei Test (MN). This kind of test has been recommended for studies on environmental biomonitoring (CAMPANA et al., 1999; FENECH, 2000; GRISOLIA, 2002; PORTO et al., 2005; BÜCKER et al., 2006). Among the tests employed to investigate genotoxicity, the MN test has been proved to be a sensible indicator of chromossomic damages and has been used successfully in hematopoietic and epithelial tissues of a number of organisms (DE LA SIENRAA et al., 2003; RODRIGUEZ-CEA, 2003). Triazinic herbicides have a broad potential of contamination in different environmental compartments. The GESAPREME 500 CIBA−GEIGY is a selective herbicide indicated for controlling weed in corn, sugar cane and sorghum cultures. The active principle is atrazine (6-chlorine-n2-ethyl-n4-isopropyl-1.3.5-triazine-2.4diamine).
The present study aimed to evaluate the acute toxicity of the herbicide GESAPRIM 500 on the fish species *Colossoma macropomum* (tambaqui). For this purpose, the LC_{50} and the frequency of micronuclei (MN) in fish erythrocytes were determined. *C. macropomum* was chosen as a target fish species because it is widely reared in Brazilian aquaculture and has economical and nutritional importance.

MATERIAL AND METHODS

Specimens of juveniles *C. macropomum* (350 ± 15g) were collected in aquaculture tanks from Araguaína city (Tocantins State, Brazil) and transferred to the laboratory, where fish were kept in 1000L tanks at 25 ± 2ºC, in water constantly renewed and aerated. Fish were maintained at those conditions during 30 days prior the experiments and feed with commercial balanced ration. The water quality during the experimental period did not change among the days nor among the different treatments, and it is whithin certain range as appropriate for freshwater fish. Feeding were not allowed 24 hours prior the beginning of the experiment.
Preliminary tests were performed for determination of the product lethal concentration. From the smallest lethal

concentration obtained in the preliminary tests, the definitive concentrations were calculated. After that, eight animals per group were submitted to five different concentrations (10, 20, 25, 30 and 40 mg/L) of the herbicide GESAPRIM 500 CIBA−GEIGY (the active principle is atrazine = 500g/L) together with the control group, in order to determine the lethal concentration for 50% of tested organisms ($LC_{50\text{-}96h}$). This experimental procedure was performed three times. The concentrations used were calculated using the concentration (mg/L) stated on the product's label. The experiment was performed in 170/L aquaria, in a semi-static system, where water was renewed each 24 hours at the period of 96 hours. At the end of the experiment, eight fish from the groups control, 10, 20 and 30 mg/L were sampled for determination of erythrocytes MN frequency. Blood samples from each fish were taken from the caudal vein by heparinized needles, according to method adapted for using in fish (GRISOLIA & CORDEIRO, 2000). Blood smears were prepared, fixed in absolute ethanol for 10 minutes and stained with the blood dye Giemsa 5% in phosphate buffer (pH 6.0) for 10 minutes. Micronuclei (MN) counting in erythrocytes (2000 cells per animal) were performed under light microscopy (1000X oil-immersion).

Results are expressed as mean ± SEM. Statistical analyses were performed by the software "Instat for Windows". Analysis of variance (ANOVA) was applied in order to verify possible differences between the control and pesticide exposed groups. Comparisons of MN frequency among the different treatments were made by one-way analysis of variance and Tukey test ($P<0.05$). For determination of LC_{50}, data of mortality were analyzed by the statistical software Trimed Sperman Karber (HAMILTON et al., 1977) and linear relationship.

RESULTS AND DISCUSSION

Tambaqui mortality increased together with the increment of the GESAPRIM 500 concentrations in water (Table 1). The value of $LC_{50\text{-}96h}$ was reached in the concentration of 20 mgL^{-1}. Linear relationship between the mortality and the herbicide concentrations indicated a positive correlation and showed a significant difference at $P<0.05$. These results indicate that mortality rate of exposed fish increased concomitantly with the increase in the concentration of GESAPRIM 500 (R^2 = 0.7405, 95% confidence interval). After 48h of herbicide exposition, hemorrhages could be noted in the eyes, lips or even in the whole body of fish exposed at the concentrations of 20, 22 and 25 mgL^{-1}. These concentrations induced loss of equilibrium and lethargic behavior. The frequency of opercular movements and the thickness of the inferior lips were either notably increased. This casual lips development is already well document as an adaptation of fish to certain situations where the increase of the inferior lips surface can facilitate the oxygen uptake by the process called as aquatic surface respiration (ASR). Changes in fish behavior can be used as indicator of acute changes in the environmental chemical composition (SAGLIO et al., 2001). For example, goldfish exposed to a 5 μgL^{-1} atrazine solution affected directly and indirectly fish behavior, altering the chemical perception of natural substances of eco-ethological importance (SAGLIO & TRIJASSE, 1998).

Table 1. Mortality of *Colossoma macropomum* exposed to different herbicide GESAPRIM 500 concentrations. Note the value of $LC_{50\text{-}96h}$ = 22 mgL^{-1} and R^2 = 0.7405

Concentrations(mg/L)	Number of died fish				Total number of fish		Mortality (%)
	24h	48h	72h	96h	Initial	Final	
Control	0	0	0	0	8	8	0
10	0	0	0	0	8	8	0
20	0	0	0	1	8	7	12.5
22	0	1	1	2	8	4	50
25	0	2	5	1	8	0	100
30	5	2	1	0	8	0	100
40	8	0	0	0	8	0	100

Several studies demonstrate the values of CL_{50} and the effects of the atrazine on teleosts fish. The $LC_{50\text{-}96h}$ for trout embryos and larvae exposed to atrazine ranged from 0,87 to 1.11 mg/L (OULMI et al., 1995). In *Tilapia mossambicus*, the atrazine $LC_{50\text{-}96h}$ was 8.8 mg/L (PRASSAD & REDDY, 1994); in *Rhamdia quelen*, it was 10.2 mg/L (KREUTZ et al., 2008) and in *Cyprinus carpio* it was 18.8 mg/L (NESKIVICK et al., 1993). Comparing those data with the present study, *C. macropomum* seems to be more resistant to atrazine than the other species ($LC_{50\text{-}96h}$ = 22mg/L).

The erythrocyte micronucleus test has been used in different fish species in order to monitor aquatic pollutants, which can display mutagenic features (De FLORA et al., 1993).

In order to investigate the micronuclei frequency in *C. macropomum* erythrocytes, only three concentrations were taken, since those concentrations were considered as references for this toxicity assay. Those references can better demonstrate the sensibility of tested organisms to the xenobiotic. The number of micronuclei in *C. macropomum* erythrocytes increased significantly during the exposition of fish to the herbicide concentrations of 10, 20 and 30 mg/L, when compared to the control group in a period of 4 days (Table 2).

Table 2. Micronuclei mean frequency (MN) in *Colossoma macropomum* erythrocytes exposed to different concentrations of the GESAPRIM 500 herbicide. S.E.M. (± standard error mean)

Concentration (mg/L)	Total	Mean	S.E.M.
Control	8	1.2	0.36
10	8	9.5*****	1.15
20	8	7.5*****	0.80
30	8	8.2*****	1.15

***** indicate significant differences from control ($p < 0.0001$).

It was observed the presence of binuclear cells during the MN counting. The formation of those cells occurs prior the process of cellular differentiation, which represents cellular changes. The micronuclei can represent acentric chromosomes, chromosomic fragments or even whole chromosomes that were lost during cellular anaphases and which are easily visible in erythrocytes (HUGHES & HERBERT, 1991; FENECH & CROTT, 2002). Despite micronuclei can appear spontaneously, micronuclei induction is commonly utilized to detect cytogenic damages as a result to the exposition of carcinogenic agents (STRUNJAK et al., 2003).

Exposition to the concentrations of 20 and 30 mg/L of the herbicide in *C. macropomum*, presented lower frequencies of MN when compared with the concentration of 10 mg/L. Probably it is due to the higher turnover of damaged cells associated with the inhibition of the erythropoiesis process.

Changes in hematopoiesis play a key role in the micronucleus test, acting as a possible confounding factor. Several genotoxic agents, at certain concentrations, can interrupt the erythropoiesis process. Therefore, not only the production of erythrocytes ends, but also the micronucleated erythrocytes either, giving a false negative. On the other hand, if blood from experimental animals is sampled too frequently, erythropoiesis is stimulated, resulting in an "inundation" of polychromatic erythrocytes in the peripheral circulation. In this way, the micronucleated erythrocytes previously formed are diluted, with a lowering of their frequency (UDROIU, 2006).

Studies on the micronuclei rates of various fish species showed that they generally peaked between the first and fifth days after treatment (Al-SABTI & METCALFE, 1995; GRISOLIA & CORDEIRO, 2000), but in the most species it takes place after 2 or 3 days (SOLDATOV,1995). The present results show that the herbicide action was clearly capable to induce micronuclei formation in *C. macropomum* during the acute exposition.

Fish are considered to be good bioindicator of aquatic contamination by genotoxic substances (SÁNCHEZ-GALÁN et al., 1998; RUSSO et al., 2004). Despite the use of pesticides has been increased intensely in Brazil, there is no appropriated environmental monitoring in order to identify levels of contamination by xenobiotics. The present study shows that fish species, and specially erythrocytes from peripheral blood, can be accessed and used as a good tool for biomonitoring of environmental conditions.

Because of the accelerated expansion of agribusiness and the potential risk for water rivers and aquaculture contamination by xenobiotics agents, it become important to evaluate the toxicity of herbicides in species considered as "sentry organisms". The detection of pesticide and their possible effects on the organisms are important studies, since it can predict the impact of these products on the animals. The present study demonstrated that the pesticide was toxic for *C. macropomum* and that this fish species can be used as a bioindicator of environmental contamination by xenobiotics. Further research on the effects of sublethal concentrations of selected pesticides is being held in order to investigate bioaccumulation.

REFERENCES

ALBINATI, A.C.L.; MOREIRA, E.L.T.; ALBINATI, R.C.B.; CARVALHO, J.V.; SANTOS, G.B.; LIRA, A.D. Toxicidade aguda do herbicida roundup® para piauçu (*Leporinus macrocephalus*). **Revista Brasileira Saúde Produção Animal**, v.8, n.3, p.184-192, 2007. [Links].

AL-SABTI, K.; METCALFE, C. Fish micronuclei for assessing genotoxicity in water. **Mutation Research**, v.343, p.121-135, 1995. [Links].

ARMAS, E.D.; MONTEIRO, R.T.R.; AMÂNCIO, A.V.; CORREA, R.M.L.; GUERCIO, M.A. Uso de agrotóxicos em cana-de-açúcar na bacia do rio corumbataí e o risco de poluição hídrica. **Química Nova**, v.28, n.6, p.975-982, 2005. [Links].

BÜCKER, A.; CARVALHO, W.; ALVES-GOMES, J.A. Avaliação da mutagênese e genotoxicidade em *Eigenmannia virescens* (Teleostei: Gymnotiformes) expostos ao benzeno. **Acta Amazônica**, v.36, n.3, p.357 - 364, 2006. [Links].

CAMPANA, M.A.; PANZERI, A.M.; MORENO, V.J.; DULOUT, F.N. Genotoxic evaluation of the pyrethroid lambda-cyhalothrin using the micronucleus test in erythrocytes of the fish *Cheirodon interruptus interruptus*. **Mutation Research**, v.438, p.155-161, 1999. [Links].

DE FLORA, S.; VIGARIO, L.; D´AGOSTIN, F.; CAMOIRANO, A.; BAGNASCO, M.; BENNECELLI, C.; MELODIA, F.; ARILLO, A. Multiple biomarkers in fish exposed *in situ* to polluted river water. **Mutation Research**, v.319, p.167-177, 1993. [Links].

DE LA SIENRAA, E.; ARMIENTAB, M.A.; GONSEBATTA, M.E. Potassium dichromate increases the micronucleus frequency in the crayfish *Procambarus clarkia*. **Environmental Pollution**, v.126 p.367-370. 2003. [Links].

FENECH, M. The in vitro micronucleus technique. **Mutation Research**, v.455, p.81-95, 2000. [Links].

FENECH, M.; CROTT, J.W. Micronuclei, nucleoplasmic bridges and nuclear buds induced in folic acid deficient human lymphocytes-evidence for breakage-fusion-bridge cycles in the cytokinesis-block micronucleus assay. **Mutation Research**, v.504, p.131-136, 2002. [Links].

GARCIA-REYERO, N.; DENSLOW, N.D. Applications of genomic technologies to the study of organochlorine pesticide-induced reproductive toxicity in fish. **Journal of Pesticide Science**, v.31, n.3, p.252-262, 2006. [Links].

GILBERT, S.F.; BOLKER, J.A. Homologies of process and modular elements of embryonic construction. **Journal of Experimental Zoology**, v.291, n.1, p.1-12, 2001. [Links].

GRISOLIA, C.K. A comparison between mouse and fish micronucleus test using cyclophosphamide, mitomycin C and various pesticides. **Mutation Research**, v.518, p.145-150, 2002. [Links].

GRISOLIA, C.K.; CORDEIRO, C.M.T. Variability in micronucleus induction with different mutagens applied to several species of fish. **Genetics and Molecular Biology**, v.23, n.1, p.235-239, 2000. [Links].

GUIMARÃES, A.T.B.; SILVA, A.H.C.; BOEGER, W. The effect of trichlorfon on acetylcholinesterase activity and histopathology of cultivated fish *Oreochromis niloticus*. **Ecotoxicology and Environmental Safety**, v.68 p.57–62, 2007. [Links].

HAMILTON, M. A.; RUSSO, R.C.; THURSTON, R.V. Trimed Spearman-Karber method for estimating median lethal concentration in toxicity bioassays. **Environmental Science Technology**, v.7, n.11, p.714-719, 1977. [Links].

HUGHES, J.B.; HEBERT, A.T. Erythrocyte micronuclei in winter flounder (*Pseudopleuronectes americanus*): results of field surveys during 1980-1988 from Virginia to. Nova scotia and in long Island Sound. **Archives Environmental Contamination and Toxicology**, v.20, n.4, p.474-479, 1991. [Links].

KREUTZ, L.C.; BARCELLOS, L.J.G.; SILVA, T.O.; ANZILIERO, D.; MARTINS, D. LORENSON, M.; MARTENINGHE, A; SILVA, L.B. Acute toxicity test of agricultural pesticides on silver catfish (*Rhamdia quelen*) fingerlings. **Ciência Rural**, v.38, n.4. p.1050-1055, 2008. [Links].

LENKOWSKI, J.R.; REED, J.M.; DEININGER, L.; McLAUGHLIN, K.A. Perturbation of organogenesis by the herbicide atrazine in the amphibian *Xenopus laevis*. **Environmental Health Perspectives**, v.116, n.2, p.223-230, 2008. [Links].

MATSUSHITA, S.; YAMASHITA, J.; IWASAWA, T.; TOMITA T.; IKEDA, M. Effects of in ovo exposure to imazalil and atrazine on sexual differentiation in chick gonads. **Poultry Science**, v.85, n.9, p.1641-1647, 2006. [Links].

NESKIVICK, N.K; ELEZOVIĆ, I.; KARAN, V.; POLEKSIĆ, V.; BUDIMIR, M. Acute toxicity of atrazine to carp (*Cyprinus carpio Ll.*). **Ecotoxicology and Enviromental Safety**, v.25, n.2, p.173-182, 1993. [Links].

OULMI, Y.; NEGELE, R.D.; BRAUNBECK, T. Segment specificity of the cythological response in rainbow trout (*Oncorhncus mykiss*) renal tubules following prolonged exposure to sublethal concentrations of atrazine. **Ecotoxicology and Environmental Safety**, v.32, n.1, p.39-50, 1995. [Links].

PORTO, J.I.R.; ARAÚJO, C.S.O.; FELDBERG, E. Mutagenic effects of mercury pollution as revealed by micronucleus test on three Amazonian fish species. **Environmental Research**, v.97, p.287-292, 2005. [Links].

PRASSAD, T.A.V.; REDDY, D.C. Atrazine toxicity on hydromineral balance of fish, *Tilapia mossambicus*. **Ecotoxicology and Environmental Safety**, v.28, p.313-316, 1994. [Links].

RESGALLA, JÚNIOR, C.; NOLDIN, J.A.; SANTOS, A.L.; SATO, G.; EBERHARDT, D.S. Toxicidade aguda de herbicidas e inseticida utilizados na cultura de arroz irrigado sobre juvenis de carpa (*Cyprinus carpio*). **Pesticidas: revista de ecotoxicologia e meio ambiente**, v.12, p.59-68, 2002.[Links].

RODRIGUEZ-CEA, A.; AYLLON, F.; GARCIA-VAZQUEZ, E. Micronucleus test in freshwater fish species: an evaluation of its sensitivity for application in field surveys. **Ecotoxicology and Environmental Safety,** v.56, p.442-448, 2003. [Links].

ROHR, J.R.; SAGER, T.; SESTERHENN, T.M.; PALMER, B.D. Exposure, postexposure, and density-mediated effects of atrazine on amphibians: breaking down net effects into their parts. **Environmental Health Perspectives**, v.114, p.46–50, 2006. [Links].

RUSSO, C.; ROCCO, L.; MORESCALCHI, M.A.; STINGO, V. Assessment of Environmental stress by the micronucleus test and the comet assay on the genome of teleost populations from two natural environments. **Ecotoxicology and Environmental Safety**, v.57, p.168-174, 2004. [Links].

SAGLIO, P.; OLSÉN, H.; BRETAUD, S. Behavioral and olfactory responses to prochloraz, bentazone and nicosulfuron-contaminated flows in goldfish. **Archives Environmental Contamination Toxicology**, v.41, p.192-200, 2001. [Links].

SAGLIO, P.; TRIJASSE, S. Behavioral responses to atrazine and diuron in goldfish. **Archives of Environment Contamination and Toxicology**, v.35, p.484-491, 1988. [Links].

SÁNCHEZ-GALÁN, S.; LINDE, A.R.; IZQUIERDO, J.I.; GARCÍA-VÁSQUEZ, E. Micronuclei and fluctuating asymmetry in brown trout (*Salmo trutta*): complementary methods to biomonitor freshwater ecosystems. **Mutation Research**, n.412, p.219-225, 1998. [Links].

SOLDATOV, A.A. Peculiarities of organization and functioning of the fish red blood system. **Journal Evolution Biochemistry Physiology**, v.41, p.272-281, 1995. [Links].

STRUNJAK-PEROVIC, I.; COZ-RAKOVAC, R.; TOPIC-POPOVIC, N. Micronucleus occurrence in diploid and triploid rainbow trout (*Oncorhynchus mykiss* Walbaum). **Veterinary Medicine**, v.48, p.215-219, 2003.[Links].

TOMITA, R.Y.; BEYRUTH, Z. Toxicologia de agrotóxicos em ambiente aquático. **Biológico**, n.64, p.135-142, 2002. [Links].

UDROIU, I. The micronucleus test in piscine erythrocytes. **Aquatic Toxicology**, v.79, p.201-204, 2006. [Links].

VEIGA, M.L.; RODRIGUES, E.L.; PACHECO, F.J. Histopathologic changes in the kidney tissue of *Prochilodus lineatus* Valenciennes, 1836 (Characiformes, Prochilodontidae) induced by sublethal concentration of trichlorfon exposure. **Brazilian Archives of Biology and Technology**, v.45, n.2, p.171–175, 2002. [Links].

WIEGAND, C.; KRAUSE, E.; STEINBERG, C.; PFLUGMACHER, S. Toxicokinetics of atrazine in embryos of the zebrafish (*Danio rerio*). **Ecotoxicology and Environmental Safety**, v.49, p.199-205, 2001.[Links].

YOUNES, M.; GALAL-GORCHEV, H. Pesticides in drinking water: a case study. **Food and Chemical Toxicology**, v. 38, p.87-90, 2000. [Links].

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