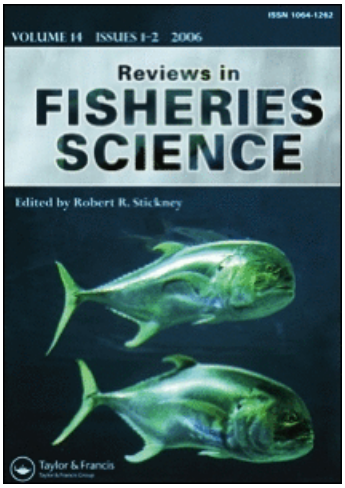

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Evaluation of Genotoxic Effects of Xenobiotics in Fishes Using Comet Assay—A Review

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Comet assay is a useful tool on the evaluation of DNA damage, and it can estimate the genetic risk followed by xenobiotic exposures. This technique has been extensively used to detect DNA lesions in a large number of tissues because its application requires viable cells that are not necessarily mitotically active. It has been recognized that the use of aquatic organisms as sentinels is of fundamental importance on environmental biomonitoring studies, and the DNA strand breaks in these organisms are fast and sensible indicators of pollutant exposure. Additionally, these bioassays detect single or associated toxic effects of these contaminants and reduce the influence of different environmental variability. The current literature presents a large number of in vitro and in vivo analyses using Comet Assay with fishes from Brazilian fauna; however, the use of endemic species is preferential considering the importance of standardization of the methodology that is employed to detect the DNA damage.

Keywords comet assay, fish, biomonitoring, genotoxic assay

INTRODUCTION

The Comet Assay—single cell gel electrophoresis (SCGE)—is a potential tool emerged for the evaluation of the mutagenicity of chemicals. This assay is a sensitive, reliable, and rapid method for detecting DNA single/double strand breaks and alkali-labile sites in individual eukaryotic cells. Besides, this methodology is applied in cancer research, genotoxicity evaluation, and chemoprevention studies.

Rydberg and Johanson (1978) introduced a method for detection of strand breaks in agarose-embedded single cells under alkaline conditions (pH $\geq$ 12). The amount of single-relative to double-stranded DNA was measured by staining with acridine orange that emits green and red light, respectively.

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In 1984 Östling and Johanson described a modified version of gel-embedded cells with electrophoresis at pH = 9.5. When cells in this micro electrophoresis assay were γ-irradiated, damaged DNA stretched toward the anode while DNA with few strand breaks remained circular, giving the appearance of a comet that originated the name of this technique by Olive et al. (1990). The neutral conditions of Comet Assay were sensible to detect DNA single-strand breaks; however, Singh et al. (1988) introduced the alkaline conditions of electrophoresis at pH $>$ 13. With this modification, the methodology made possible the detection of single- and double-strand breaks, alkali-labile sites, and uncompleted DNA repair sites.

The SCGE measures primary DNA strand breaks in individual cells (Singh et al., 1988) with simplicity and low cost. This simplicity and the sensibility of this assay are well appropriated to the biomonitoring of chronic levels of exposure using viable cells in different tissues targets (Belpaeme et al., 1998). The use of viable cells that are not necessarily undergoing mitosis makes Comet Assay an important tool on genetic toxicology and biomonitoring studies.

Table 1 Comet Assay in biomonitoring studies using fish species as bioindicators

Organism	Cell type	Localization	Contaminant	Reference
Leuciscus cephalus	Erythrocytes	Rhône River (France)	Metals	Devaux et al., 1998
Pholis gunnellus	Erythrocytes	Firth of Forth (Scotland)	Metals, hydrocarbons, and organochlorates	Bombail et al., 2001
Tilapia rendalli	Erythrocytes	Igapó II Lake (Brazil)	Domestic and industrial drain	Lemos et al., 2005
Oncorhynchus mykiss	Erythrocytes	Biobio River (Chile)	Polycyclic aromatic hydrocarbons (HPA)	Inzunza et al., 2005
Geophagus brasiliensis	Erythrocytes and kidney cells	Santa Catarina (Brazil)	Domestic drain	Benincá, 2006
Astyanax sp. *B* and *A. altiparanae*	Erythrocytes, hepatocytes, and kidney cells	Paraná (Brazil)	Agrotoxics	Ramsdorf, 2007
Salmo trutta fario	Erythrocytes	Morcille River (France)	Mixture of vineyard pesticides	Bony et al., 2008

The principle of this technique consists into submit cell suspensions embedded in agarose to lyses (this treatment removes cellular and nuclear membranes and proteins), which turns the gel-embedded DNA in the form of nucleoids. Following alkaline treatment and electrophoresis, DNA migrates toward the anode in a way that is dependent on the number of lesions in the nucleoids. The extent of migration is visualized in a fluorescence microscope after staining of the DNA where intact nucleoids are circular and damaged nucleoids look like a comet (Olive et al., 1990). Therefore, the extension of DNA migration reflects the extent of DNA damage (Fairbairn et al., 1995).

There is a large number of possibilities to evaluate and measure the extension of DNA damage obtained in Comet Assay. Kobayashi et al. (1995) suggest the evaluation of the damage by the relation between the nucleus ratio and the extent of comet tails formed during DNA migration (classified from 0—no DNA damage to 3—maximum level of DNA damage). This analysis can be performed visually or by the use of special softwares.

According to Bauch et al. (1999), the interest in Comet Assay as a tool for detection of DNA damage and DNA repair increased significantly since 1984; however, the question concerning the protocol for this methodology was confuse in literature. There were at least three different protocols: Östling and Johanson (1984), Singh et al. (1988), Olive et al. (1990) and, more recently, Speit and Hartmann (1999); however, it is of common sense in literature that the alkaline version of Comet Assay is always preferential, given its sensibility to detect a large number of DNA damage.

De Boeck et al. (2000) made the alert about the experimental variability in this assay, especially in the electrophoresis step. When a large number of samples are obtained simultaneously, different electrophoreses are required. Therefore, it is necessary to include a standard pool of cells in each electrophoresis, which ensures the homogeneity of the procedure along the time.

ECOLOGICAL BIOMONITORING

The impact of toxic materials on the integrity and functioning of cellular DNA has been investigated in a large number of organisms in environmental conditions (Bombail et al., 2001). The DNA strand breaks reflect the exposure to environmental pollutants in a rapid and sensible manner (Martinez and Cólus, 2002).

Monitoring xenobiotic agents in aquatic environment using resident species can benefit environmental quality as well as human health. The localization and determination of pollutant concentrations can ensure the life of these species and an environmental health that is of importance for human feeding, supplying, and leisure (Nicareta, 2004).

Biomonitoring is a promising tool for identification of pollutants that modify human and environmental health, mostly with organisms exposed to these pollutants (bioindicators) using biological system assays (biomarkers) (Da Silva et al., 2003).

Although its primary application is in detecting genotoxicity in mammalian cells (Rydberg and Johanson, 1978), Comet Assay is now widely used for detection of genotoxic agents in aquatic environments, as clearly demonstrated by Sasaki et al. (1997) using the mollusk species *Patunopecten yessoensis* and *Tapes japonica*.

A considerable number of biomonitoring studies using Comet Assay in fish species were published in the last 10 years. Some are presented in Table 1.

In Brazil, biomonitoring studies in aquatic species are increasing in the last years. The most important detected environmental contamination in lakes and fountains is in the South region, which used *Tilapia rendali* (Lemos et al., 2005), *Geophagus brasiliensis* (Benincá, 2006), and *Astyanax* sp.*B* and*A. Altiparanae* (Ramsdorf, 2007) as bioindicators.

GENOTOXICITY ASSAYS

Bioassays allow the study of toxic effects of some contaminants in an isolated or associated way, which decrease significantly the influence of environmental variables. The extrapolation of the results of the environmental context helps us in understanding the factors that affect the environment and human health (Ramsdorf, 2007).

The use of aquatic organisms, mostly endemic species, is of fundamental importance in biomonitoring studies. However, its

Table 2 Use of Comet Assay for detection of genotoxic agents in aquatic environments

Organism	Cell type	Contaminant	Reference
Oncorhynchus mykiss and *Ictalurus punctatus*	Erythrocytes, hepatocytes, and kidney cells	Aflatoxin B_1	Abd-Allah et al., 1999
Hoplias malabaricus	Erythrocytes	Leed	Cestari et al., 2004
Hoplias malabaricus	Erythrocytes	TBT and Leed	Ferraro et al., 2004
Hoplias malabaricus	Erythrocytes	Metilmercury	Lopes-Poleza, 2004
Oncorhynchus mykiss	Fibroblasts RTG-2	Nitrocompounds and benzo(a)pyrene	Nehls and Segner, 2005
Centropomus parallelus	Erythrocytes	$\beta-$naftoflavon	Di Paolo, 2006
Eigenmannia virescens	Erythrocytes	Benzene	Bücker et al., 2006
Prochilodus lineatus	Erythrocytes	Diesel oil	Vanzella, 2006
Oreochromis niloticus	Erythrocytes	Chromo	Matsumoto et al., 2006
Gambusia affinis	Erythrocytes	Residual hydrocarbons	Caliani et al., 2008
Channa punctatus	Lymphocytes, gill and kidney cells	Organophosphate pesticide	Ali and Kumar, 2008

use requires standard protocols for detection of DNA damage (Ferraro et al., 2004).

The literature reports *in vitro* and *in vivo* Comet Assay in fish species. For *in vivo* experiments, the test substance is injected or mixed with water or food (Cotelle and Ferard, 1999). Table 2 shows several studies using Comet Assay for detection of genotoxic agents in aquatic environments.

In 1999, Abd-Allah et al. published a pioneer study using Comet Assay in two fish species, the rainbow trout *Oncorthyncus mykiss* (sensitive) and the channel catfish *Ictalurus punctatus* (resistant), where they demonstrated the genotoxic effects of Aflatoxin-B.

The SCGE has been widely used in peripheral blood erythrocytes because this cell type is easily handled once cellular dissociation is not required (Belpaeme et al., 1998).

In Brazil, a large number of studies employed the freshwater fish *Hoplias malabaricus* for genotoxicity assays (Cestari et al., 2004; Ferraro et al., 2004; Lopes-Poleza, 2004).

The use of chromosomal aberrations and SCGE to evaluate the genotoxic effects of $Pb(NO_3)_2$ demonstrated that *H. malabaricus* is an important bioindicator to clastogenicity estimation of xenobiotics (Cestari et al., 2004). Lopes-Poleza (2004) evaluated the genotoxic effects of methyl mercury (CH3Hg+) using chromosomal aberration (kidney), micronuclei, and Comet Assay in erythrocytes. The results showed that mercury accumulates preferentially in target tissues than in blood and that assays using peripheral blood erythrocytes were not sensible enough to demonstrate the genotoxic effects of this metal.

Ferraro et al. (2004) also evaluated the mutagenic potential of tributyltin (TBT) and inorganic lead (PbII) using Comet Assay on micronuclei and chromosomal aberrations. Lead exhibited high mutagenic effects in all assays; however, TBT demonstrated clastogenic properties but was not positive for Comet Assay, at least in the cell types analyzed.

Other fish species from Brazilian fauna were used in the analysis of genotoxic potential of the β-isoflavone (BNF) by Comet Assay in exposed erythrocytes from *Centropomus parallelus* (Di Paolo, 2006). The results showed a pattern of increased DNA damage in exposed erythrocytes than in controls; however, this tendency was not statistically significant.

Bücker et al. (2006) studied the mutagenicity and genotoxicity of benzene exposure (50 ppm) in the electric fish species *Eingenmannia virescens* from Brazilian Amazon using Comet Assay and micronucleus tests (MNs). Although the results from MNs were not positive in the erythrocytes analyzed, the results from Comet Assay suggested a slight increase in the number of cells with higher classes of DNA damage according to exposure time, denoting a time-dependent effect. These results suggested that Comet Assay is more sensible than MNs.

In the South region in Brazil, Vanzella (2006) used the Comet Assay and MNs in the fish species *Prochilodus leneatus* to evaluate the genotoxicity of the soluble fraction of commercial diesel oil (FSD). The animals were exposed to 50% dilution in water in acute (6, 24, 96 hr) or sub-chronic (15 days) ways. The results clearly showed the presence of aneugenic and clastogenic compounds in the FSD tested. Consequently, these two methodologies together in this study were suitable and advantageous.

CONCLUSIONS

Some characteristics of Comet Assay are significantly advantageous when compared to other methodologies in the detection of DNA damage: it can be performed in a fast way with low costs and complexity and does not require cells undergoing mitotic division; however, the cells might be viable; the number of cells analyzed for detection of DNA damage is relatively short when compared to other methodologies. On the other hand, the standardization of this methodology in different laboratories, cell types, and diversity of environments represents some difficulty that should to be taken into account. Thus, studies of genotoxicity in target tissues from organisms in aquatic environments are of fundamental importance for the standardization of this methodology as a routine practice.

REFERENCES

Abd-Allah, G., R. El-Fayoumi, M. Smith, R. Heckmann, and K. O'Neill. A comparative evaluation of aflatoxin B1 genotoxicity in fish models using the comet assay. *Mutat. Res.*, **446:** 181–188 (1999).

Ali, D., and S. Kumar. Long-term genotoxic effect of monocrotophos in different tissues of freshwater fish *Channa punctatus* (Bloch) using alkaline single cell gel electrophoresis. *Sci. Total Environ.*, **405:** 345–350 (2008).

Bauch, T., W. Böcker, U. Mallek, W. Müller, and C. Streffer. Optimization and standardization of the "Comet Assay" for analyzing the repair of DNA damage in cells. *Strahlenthund Onkol.*, **175:** 333–340 (1999).

Belpaeme, K., K. Cooreman, and M. Kirsch-Volders. Development and validation of the *in vivo* alkaline comet assay for detecting genomic damage in marine flatfish. *Mut. Res.*, **415:** 167–184 (1998).

Benincá, C. Biomonitoramento das lagoas estuarinas do Camacho—Jaguaruna (SC) e Santa Marta—Laguna (SC); Utilizando *Geophagus brasiliensis* (Cichlidae). Dissertation, University of Paraná (2006).

Bombail, V., A. Dennis, E. Gordon, and J. Batty. Application of the Comet and Micronucleus Assays to butterfish (*Pholis gunnellus*) erythrocytes from the Firth of Forth, Scotland. *Chemosphere*, **44:** 383–392 (2001).

Bony, S., C. Gillet, A. Bouchez, C. Margoum, and A. Devaux. Genotoxic pressure of vineyard pesticides in fish: Field and mesocosm surveys. *Aquatic Toxicol.*, **89:** 197–203 (2008).

Bücker, A., W. Carvalho, and J. Alves-Gomes. Avaliação da mutagênese e genotoxicidade em *Eigenmannia virescens* (Teleostei: Gymnotiformes) expostos ao benzeno. *Acta Amazônica*, **36:** 357–364 (2006).

Caliani, I., S. Porcelloni, G. Mori, G. Frenzilli, M. Ferraro, L. Marsili, S. Casini, and M. Fossi. Genotoxic effects of produced waters in mosquito fish (*Gambusia affinis*). *Ecotoxicology*, **18:** 75–80 (2008).

Cestari, M., P. Lemos, C. Ribeiro, J. Costa, E. Pelletier, M. Ferraro, M. Mantovani, and S. Fenocchio. Genetic damage induced by trophic doses of lead in the neotropical fish *Hoplias malabaricus* as revealed by the comet assay and chromosomal aberrations. *Genet. Mol. Biol.*, **27:** 270–274 (2004).

Cotelle, S., and J. Ferard. Comet assay in genetic ecotoxicology: A review. *Environ. Molec. Mutagen.*, **34:** 246–255 (1999).

Da Silva, J., V. Heuser, and V. Andrade. Biomonitoramento ambiental. *Genética Toxicológica*, 167–178 (2003).

De Boeck, M., N. Touil, G. De Visscher, P. Vande, and M. Kirsch-Volders. Validation and implementation of an internal standard in comet assay analysis. *Mutat. Res.*, **469:** 181–197 (2000).

Devaux, A., P. Flammarion, V. Bernardon, J. Garric, and G. Monod. Monitoring of the chemical pollution of the river Rhone through measurement of DNA damage and cytochrome P450 1A induction in chub (*Leuciscus cephalus*). *Mar. Environ. Res.*, **46:** 257–262 (1998).

Di Paolo, C. Aplicação do Ensaio Cometa a estudo de danos ao DNA de robalos, *Centropomus parallelus* (Poey, 1860) expostos à β-naftoflavona. Dissertation, University of São Paulo (2006).

Fairbairn, D., P. Olive, and K. O'Neil. The comet assay: A comprehensive review. *Mutat. Res.*, **339:** 37–59 (1995).

Ferraro, M., A. Fenocchio, M. Mantovani, C. Ribeiro, and M. Cestari. Mutagenic effects of tributyltin and inorganic lead (Pb II) on the fish *H. malabaricus* as evaluated using the comet assay and the piscine micronucleus and chromosome aberration tests. *Genet. Mol. Biol.*, **27:** 103–107 (2004).

Inzunza, B., R. Orrego, M. Peñalosa, J. Gavilán, and R. Barra. Analysis of CYP4501A1, PAHs metabolites in bile, and genotoxic damage in *Oncorhynchus mykiss* exposed to Biobío River sediments, Central Chile. *Ecotoxicol. Environ. Safety*, **65:** 242–251 (2006).

Kobayashi, H., C. Suguyama, Y. Morikawa, M. Hayashi, and T. Sofuni. A comparison between manual microscopic analysis and computerized image analysis in the single cell gel electrophoresis. *MMS Comm.*, **3:** 103–115 (1995).

Lemos, N., A. Dias, A. Silva-Souza, and M. Mantovani. Evaluation of environmental waters using the comet assay in *Tilapia rendalli*. *Environ. Toxicol. Pharmacol.*, **19:** 197–201 (2005).

Lopes-Poleza, S. Avaliação do efeito do metilmercúrio (CH3Hg+) em *Hoplias malabaricus* através da freqüência de Aberrações Cromossômicas e dos ensaios Cometa e Micronúcleo. Dissertation. University of Paraná (2004).

Martinez, C., and I. Cólus. Biomarcadores em peixes neotropicais para o monitoramento da poluição aquática na bacia do rio Tibagi. **In:** Moacyr, E., E. Bianchini, O. Shibatta, J. Pimenta. *A Bacia do Rio Tibagi,* cap. 29, pp. 551–557 (2002).

Matsumoto, S., M. Mantovani, M. Malaguttii, A. Dias, I. Fonseca, and M. Marin-Morales. Genotoxicity and mutagenicity of water contaminated with tannery effluents, as evaluated by the micronucleus test and comet assay using the fish *Oreochromis niloticus* and chromosome aberrations in onion root tips. *Genet. Mol. Biol.*, **29:** 148–158 (2006).

Nehls, S., and H. Segner. Comet assay with the fish cell line rainbow trout gonad-2 for *in vitro* genotoxicity testing of xenobiotics and surface waters. *Environ. Toxicol. Chem.*, **24:** 2078–2087 (2005).

Nicareta, L. Biomarcadores para a detecção de efeitos subletais causados pela deltametrina em *Ancistrus multispinnis*. Dissertation, University of Paraná (2004).

Olive, P., J. Banath, and R. Durand. Heterogeneity in radiation-induced DNA damage and repair in tumor and normal cells measured using the "comet assay." *Radiat. Res.*, **122:** 86–94 (1990).

Östling, O., and K. Johanson. Microelectrophoretic study of radiation-induced DNA damages in individual mammalian cells. *Biochem. Biophys. Res. Commun.*, **123:** 291–298 (1984).

Ramsdorf, W. Utilização de duas espécies de *Astyanax* (*Astyanax* sp. *B* e *A. altiparanae*) como bioindicadores de região contaminada por agrotóxico (Fazenda Cangüiri, UFPR). Dissertation, University of Paraná (2007).

Rydberg, B., and K. Johanson. Estimation of DNA strand breaks in single mammalian cells. **In:** *DNA Repair Mechanisms* (P. C. Hanawalt, E. C. Friedberg, and C. F. Fox, Eds., pp. 465–468). New York: Academic Press (1978).

Sasaki, Y., F. Izumiyama, E. Nishidate, S. Ishibashi, S. Tsuda, N. Matsusaka, N. Asano, K. Saotomo, T. Sofuni, and M. Hayashi. Detection of genotoxicity of polluted sea water using shellfish and the alkaline single-cell gel electrophoresis (SCE) assay: A preliminary study. *Mut. Res.*, **393:** 133–139 (1997).

Singh, N., M. McCoy, R. Tice, and E. Schneider. A simple technique for quantification of low levels of DNA damage in individual cells. *Exp. Cell Res.*, **175:** 184–191 (1988).

Speit, G., and A. Hartmann. The comet assay (single-cell gel test). **In:** *Methods in Molecular Biology, volume 113, DNA Repair Protocols: Eukaryotic Systems* (D. S. Henderson, Ed., pp. 203–212). Totowa, NY: Humana Press (1999).

Vanzella, T. Efeitos genotóxicos e mutagênicos da fração solúvel do óleo diesel em uma espécie de peixe neotropical. Dissertation, University of Londrina (2006).