

Genetic damage in *Goodea atripinnis* (Goodeidae) and persistent organic-compounds in both Chapala and Sayula Lakes, in Mexico

Daño genético en *Goodea atripinnis* (Goodeidae) y compuestos orgánicos persistentes en los Lagos de Chapala y Sayula, en México

Mónica Reynoso Silva,¹ Armando Arévalo Hernández,¹
Alfredo Fera Velasco,² and Carlos Álvarez Moya¹

¹Laboratorio de Mutagenesis, Departamento de Biología Molecular, Centro Universitario de Ciencias Biológicas y Agropecuarias, Universidad de Guadalajara. 45100, Zapopan, Jalisco, México

²Laboratorio de Neurobiología Celular del Departamento de Biología Celular y Molecular, Centro Universitario de Ciencias Biológicas y Agropecuarias, Universidad de Guadalajara. 45100, Zapopan, Jalisco, México
e-mail: calvarez@cucba.udg.mx

Reynoso. S. M., A. A. Hernández, A.F. Velasco and C. Á. Moya. 2014. Genetic damage in *Goodea atripinnis* (Goodeidae) and persistent organic-compounds in both Chapala and Sayula Lakes, in México. *Hidrobiológica* 24 (3): 215-221.

ABSTRACT

POPs are a group of chemicals with genotoxic capacity, found as contaminants in many water bodies. Given the high chemical pollution of Chapala Lake (including POPs), consumption of this water poses a genotoxic risk for people in the Guadalajara metropolitan area. Concentrations of POPs were quantified in water from Chapala Lake, Sayula Lake and in the liver of *Goodea atripinnis* on two seasons of the year: the dry and rainy seasons of 2006. Chromatographic analysis of the water showed very low concentrations of POPs and variations from one season to another. The increase of methoxychlor, 2,4 D and DDT is notable, indicating that they are dragged into the water bodies during the rainy season. POPs were detected in the liver of fish from both lakes; however, 2,4 D in the liver of fish from Chapala (0.02 ppm) is 10 times as concentrated as in Sayula (0.002 ppm). The evaluation of genetic damage by alkaline comet assay in hepatocytes during the dry season showed significant genetic damage ($p < 0.01$) in fish from the two reservoirs, probably due to 2,4 D. In the rainy season there was a significant difference ($p < 0.01$) in the case of Sayula but not for Chapala. Genetic damage in Sayula can be attributed to other substances with a mutagenic capacity different to that of POPs.

Key words: Bioaccumulation, Comet assay, Genetic Damage, *Goodea atripinnis*, POPs.

RESUMEN

Los COP's son un grupo de sustancias químicas con capacidad genotóxica, correspondiente a contaminantes en muchos cuerpos de agua. Dada la alta contaminación química del Lago de Chapala, México (incluyendo compuestos orgánico-persistentes), el consumo de estas aguas, representa un riesgo mutagénico/genotóxico para los habitantes de la Zona Metropolitana de Guadalajara. En este estudio se cuantificaron las concentraciones de COP's del agua del Lago de Chapala y Laguna de Sayula y también del hígado de peces *Goodea atripinnis*, en dos épocas del año: sequía y lluvias del 2006. El análisis cromatográfico del agua de ambos embalses mostró muy bajas concentraciones de COP's y variación en ambas temporadas. Durante la época de lluvia destacó el incremento de metoxicloro, 2,4 D y DDT lo que indica que estos compuestos son arrastrados hasta los cuerpos de agua durante esta temporada. En el hígado de peces de ambos lagos se detectaron COP's, sin embargo, el valor de 2,4 D en los organismos de Chapala fue 10 veces

más concentrado (0.02 ppm) en comparación con los de Sayula (0.002 ppm). La evaluación de daño genético a través de la prueba del cometa alcalino en hepatocitos durante la época de secas, mostró daño genético significativo ($p < 0.01$) en los peces de ambos embalses, debido probablemente al 2,4 D. En la época de lluvias se encontró daño genético significativo ($p < 0.01$) en peces de Sayula pero no para peces de Chapala. El daño genético encontrado en organismos provenientes de Sayula se atribuyó a otras sustancias con capacidad mutagénica diferentes a los COP's.

Palabras clave: Bioacumulación, COP's, daño genético, *Goodea atripinnis*, prueba cometa.

INTRODUCTION

Chapala is the biggest lake in Mexico, and it receives a large number of chemical pollutants from industries located along the Lerma-Santiago basin (Alvarez & Hernández, 2007; Gómez, 2007) and these waters are a complex environmental mixture of well-known toxicants along with an increasing number of emerging contaminants (Pollack *et al.* 2003; Mayo *et al.*, 2006). Sayula's Lake is near Chapala Lake and it is sited between several regions of Jalisco state (Barba & Guitrón, 2007) and it also receives chemical pollutants from agricultural activities, brought in by many rivers. It has similar conditions than Chapala Lake regarding human activities.

It is known that a number of chemicals present are highly persistent and have mutagenic properties (Waters *et al.*, 1991; Waters *et al.*, 1999). The most dangerous persistent organic pollutants (POPs) are: aldrin, dieldrin, chlordane, lindane, dichlorodiphenyltrichloroethane (DDT), heptachlor, mirex, toxaphene, polychlorinated biphenyls (PCBs), hexachlorobenzene, dioxins and furans (Álvarez & Hernández, 2007). In México the main organochlorine pesticides that contaminate groundwater and surface waters are: DDT, hexachlorohexane (HCH), lindane, chlordane, heptachlor, methoxychlor, toxaphene, aldrin and dieldrin (Martínez, 1994). Jalisco, México ranks second in the use of detrimental chemicals, and neighboring states also use these substances, so the risk of exposure to mixtures of pesticides in freshwater bodies is very high (Jiménez, 2001). POPs remain in the environment for years and are deposited in fatty tissues of animals where concentrations can increase up to 70,000 times the environmental levels (bioaccumulation) (Nagel, 1993; Guillette *et al.*, 1994; Pietrapiana *et al.*, 2002.) Zhou *et al.* (1999) found that bioaccumulation of organochlorine compounds in fish tissues are related to their diet. Sun *et al.* (2002) reported an increase in the concentration of POPs in the water during the dry season, which decreases during the rainy season.

POPs cause diseases in both humans and animals: cancer, damage to the nervous system, reproductive disorders, breaches in the immune system, birth defects and genetic damage and can eventually cause death (Dunier *et al.*, 1991; Guillette *et al.*, 1994; Guillette & Uribe, 2001; Gurge *et al.*, 2001). POPs also have synergistic effects that enhance their action potency 160 to 1,600 times, which makes them extremely dangerous (Lombardi, 1998; Borden & Primental, 1980; Shaw, 1970).

Several tests have been developed for evaluating DNA alterations in aquatic animals, these are based on potentially pre-mutagenic lesions such as DNA adducts; base modifications and DNA strand breaks (Ohe *et al.*, 2004). The analysis of DNA alterations in aquatic organisms has been shown to be a highly suitable method for evaluating the genotoxic contamination of environments, being able to detect exposure to low concentrations of contaminants in a wide range of species.

To assess the integrity of genetic material various tests are performed (Zúñiga *et al.*, 2001). One of the most commonly used is the single cell gel electrophoresis (comet assay) developed by (Singh *et al.*, 1988), was first applied to ecotoxicology about 15 years ago and it has become one of the most popular tests for detecting strand breaks in aquatic animals through *in vivo*, *in vitro* and *in situ* exposures (Ohe *et al.*, 2004). This method uses, among other cell types, fish erythrocytes (Kammann *et al.* 2001; Alink *et al.*, 1980; Prein *et al.*, 1978).

At present there is no information about the concentration of POPs in the water of Chapala and Sayula Lakes, nor reports on bioaccumulation and genetic damage in native organisms. *Goodea atripinnis* (Jordan, 1880) is present in both water bodies (Barba & Guitrón, 2007) and it belongs to the Goodeidae family (Jordan 1880). It can be used as a biomarker of genetic damage due to its high tolerance to environmental conditions (Miller & Smith, 1986). Due to the fact that 90% of the waters of Guadalajara metropolitan area are provided by the Chapala Lake (Mayo *et al.*, 2006), the studies for detecting the genotoxic risks associated with the pollution and consumption of this water are very relevant because it is known that many companies settled along the Lerma-Chapala discharged chemical pollutants to the river. Therefore, this study assessed the concentration of POPs in the water of both Chapala and Sayula lakes, as well as in the liver of fish during the dry and rainy seasons of 2006. Simultaneously, we quantified the genetic damage in hepatocytes in these fishes.

MATERIALS AND METHODS

Sampling. Three water samples from Lake Chapala and Lake Sayula were collected from the surface area (POPs are hydrophobic and remain on the water surface) of both bodies of water in the same periods and locations where the fishes were collected.

In order to obtain negative controls, 15 juvenile *Goodea atripinnis* were captured (fish 3-4 cm in length were selected) at Lake Joya, a small, unpolluted lake (unpublished data) formed by a tributary storm.

Lindane, aldrin, dieldrin, chlordane, DDT, HCB, 2,4 D (dichlorophenolxyacetic Acid), heptachlor, heptachlor epoxide and methoxychlor, were quantified in water samples and liver tissues using a capillary column with an electron capture detector (ECD) for liquid and solid samples following methods 608 and 8080 (EPA, 1984). Extraction of POPs in water samples was performed with dichloromethane (DCM) (Merck) liquid-liquid. For the analysis they were dehydrated by evaporation and re-dissolved in 5 mL of hexane (Merck) and were finally concentrated to 0.2 mL under a gentle stream of nitrogen which was injected in a one microliter aliquot into the gas chromatograph. As a negative control, distilled water subjected to the same treatment was used. In order to extract the POPs from the liver tissue, the liver was thawed, homogenized, suspended in 5 mL of hexane (Nagel, 1993) and was introduced to the standardized chromatograph with the Sigma basic kit (EPA 608-S organochloride pesticides and PCBs kit analytical standard according to the 608 protocol, EPA, 1985).

Five adult specimens of *Goodea atripinnis* were collected (8-10 cm in length) from Lake Chapala, particularly from Alacranes Island and Petatan (where 'malformed' fishes have been spotted by local fishermen) and five from Lake Sayula (Atoyac, Jalisco, which live in small puddles of water along the dry lake), per season during the dry and rainy seasons of 2006, in the months of March and August. The fishes were transported alive to the laboratory where their livers were extracted. A portion of approximately 50 mg was immediately used to carry out the comet assay for the detection of genetic damage in the liver nuclei (Singh *et al.*, 1988; Belpaeme & Kirsch-Volders 1988; Zhou *et al.*, 1999); another part was frozen at -18°C until POPs were quantified by means of the chromatographic method.

To prepare cell suspension, the fish liver was extracted and washed with a phosphate solution (NaCl 160 mM, Na_2HPO_4 8 mM, NaH_2PO_4 8 mM, EDTA 50 mM, pH 7.0, Sigma). For cell dissociation, the tissue was incubated for one hour in 10 mL phosphate buffer now containing 200 mM of N-t-butyl-alpha-phenylnitron (Sigma). Next, the tissue was homogenized in a chilled mortar and passed through a 55 μm paper filter. The filtrate was centrifuged at 5,000 rpm for 10 minutes at 4°C and the supernatant was removed. The pellet with liver nuclei was resuspended in the first mentioned phosphate solution for later use.

Alkaline comet assay. Two slides per specimen were prepared as described by (Belpaeme & Kirsch-Volders, 1988); to prevent the presence of residues that affected the adhesion of the gel, the slides were covered with 0.5 μL normal melting point (NMP) 1% agarose (Sigma) which was removed after it became solidi-

fied. Then 300 μL of 0.6% low melting point (LMP) agarose was placed on the slide and allowed to solidify (both agaroses were obtained from Sigma). Then 100 mL of 0.5% LMP mixed with 10 μL of the nuclear suspension was placed and allowed to solidify. A fourth layer of 100 μL of LMP 0.5% was added to cover the nuclear content.

To induce nuclear lysis and facilitate DNA unfolding, all the slides were immersed in a lysis buffer (2.5 M NaCl, 100 mM Na_2EDTA , 10 mM Tris-HCl, 1% sodium lauryl sarcosine, 1% Triton X-100, and 10% DMSO, pH 10) for 1 h at 4°C . These were then placed in a horizontal electrophoresis system (BIORAD, Model A6), with a high pH buffer (300 mM NaOH, 1 mM Na_2EDTA , pH 13) for 45 min to allow unwinding of DNA prior to electrophoresis which was carried out for 15 min at 1.0 V/cm with an amperage of approximately 200 mA. The same electrophoresis unit and power supply were used throughout the study (Hartmann *et al.*, 2003). In order to avoid additional DNA damage, all the steps described above were carried out under yellow light. Following electrophoresis, the slides, first gently washed to remove alkali, were then immersed in a neutralization buffer (0.4 M Tris-Base, pH 7.5) for 5 min. Gels were stained with ethidium bromide (Sigma) (100 mL at 20 mg/mL) for 3 min and then rinsed three times with distilled water. The slides were then covered with a coverslip. Fluorescence microscopy (Zeiss, Model Axioskop 40, equipped with a 515-560 nm excitation filter) was used to examine the slides. Cells were observed at 40X magnification, and migration determined by the visual scoring of tail length, according to published protocols (Hartmann *et al.*, 2003). Approximately 50 nuclei per slide and two slides for each experimental point and controls were evaluated.

Statistical analysis. Each experiment was repeated twice. At least 100 comets per specimen were measured to obtain the average migration of electrophoresed DNA. Data were analyzed using "Co Stat" software. We applied the F-ANOVA test for variance analysis and *Dunnett's test* for multiple comparisons and a probability level of 0.05 was used.

RESULTS

Quantification of POPs in the liver of *Goodea atripinnis* in Chapala and Sayula Lakes. Quantification of POPs in water samples from Chapala and Sayula Lakes in rainy season (2006) is presented in Table 1. Data indicate very low concentrations of POPs (NOM 127-SSA1-1994) in both Chapala and Sayula. The concentrations of methoxychlor, 2,4 D and DDT in water from both lakes increased from the dry to rainy season approximately ten fold, but the concentrations of hexachlorobenzene, heptachlor and heptachlor epoxide decreased significantly in the rainy season in Sayula. In Chapala, hexachlorobenzene and heptachlor epoxide remained at the same level, as opposed to heptachlor which decreased in the second season.

Table 1. Concentrations of POPs (parts per million) in water samples from Chapala and Sayula Lakes obtained during the months of March and August, dry and rainy seasons, 2006.

POPs	Chapala		Sayula	
	Dry season	Rainy season	Dry season	Rainy season
Lindane	<0.0023	<0.0027	<0.0030	<0.0024
Aldrin	<0.0004	<0.00003	<0.0005	<0.00007
Dieldrin	<0.0005	<0.00004	<0.0006	<0.00003
Chlordane	<0.0003	<0.0002	<0.0004	<0.0007
DDT	<0.0006	<0.001	<0.0007	<0.003
Hexachlorobenzene	<0.0003	<0.0004	<0.0002	<0.00001
2,4,D	<0.002	<0.05	<0.003	<0.06
Heptachlor	<0.0005	<0.00001	<0.0004	<0.00003
Heptachlor epoxide	<0.0004	<0.0002	<0.0005	<0.00008
Methoxychlor	<0.0021	<0.02	<0.0027	<0.03

Lindane and chlordane, in water from both Sayula and Chapala, remained at the same levels, while aldrin and dieldrin were diluted in the rainy season in both water bodies.

In the dry season (Table 2) there was no difference in the concentrations in fish liver between Sayula and Chapala, with the exception of 2,4 D was ten times higher in Chapala. In the rainy season there was no difference between the concentration of POPs in fish liver from Sayula and Chapala.

Alkaline comet assay. In both dry and rainy seasons about 5% of the nuclei obtained did not show genetic damage. In the dry season the average migration of DNA in liver nucleus of fish from Lake Chapala (Table 3) was higher than that from Sayula and both were higher ($p < 0.01$) than the water body named La Joya. In the

rainy season the average DNA migration in the liver nuclei of fish in Sayula Lake is higher than that from both Chapala Lake and La Joya ($p < 0.01$) but there were no significant differences between Chapala Lake and La Joya.

DISCUSSION

POPs are a group of chemicals with genotoxic potential and cause cancer (Fahringer, 1974; Pool, 1977; Guillette, 1994; Lombardi, 1998; Guruge *et al.*, 2001). They can be found as contaminants in many water bodies (Jimenez, 2001) including groundwater and surface water in México (Martinez, 1994). Jimenez, (2001) reported the risk of exposure to pesticides through freshwater bodies. Given the high chemical pollution of Chapala Lake, which is easily

Table 2. Concentration of POPs (parts per million) in the liver of *Goodea atripinnis* from Chapala and Sayula Lakes in the dry and rainy seasons of 2006.

POPs	Dry season Concentration		Rainy season Concentration	
	Chapala*	Sayula*	Chapala*	Sayula*
Lindane	<0.0023	<0.0025	<0.001	<0.002
Aldrin	<0.0004	<0.0002	<0.00005	<0.00006
Dieldrin	<0.0005	<0.0006	<0.00006	<0.00008
Chlordane	<0.0003	<0.0003	<0.0007	<0.0002
DDT	<0.0006	<0.0008	<0.003	<0.008
Hexachlorobenzene	<0.00001	<0.00005	<0.00006	<0.00009
2,4,D	<0.02	<0.002	<0.05	<0.05
Heptachlor	<0.0005	<0.0008	<0.00007	<0.00007
Heptachlor epoxide	<0.0004	<0.0004	<0.00008	<0.00004
Methoxychlor	<0.0022	<0.0023	<0.02	<0.02

* Studied fishes N = 5.

Table 3. Average migration length (μm) of DNA of fish hepatic tissue from Chapala and Sayula Lakes, collected during the dry and rainy seasons of 2006.

	Dry season	Rainy season
Lake Chapala	32.05 \pm 12.013*	25.5493 \pm 4.636
Lake Sayula	28.75 \pm 5.430*	27.45 \pm 4.633*
La Joya	20.175 \pm 4.636	20.175 \pm 4.636

*Significant difference with regard to Lake La Joya specimens ($p < 0.01$).

consumption of this water poses a risk mutagenic/genotoxic for people in the Guadalajara metropolitan area.

The evaluation of concentrations of DDT, 2,4 D hexachlorobenzene, heptachlor, heptachlor epoxide, lindane, chlordane, methoxychlor, aldrin and dieldrin, in the water of Chapala and Sayula Lakes (Table 1) did actually show the presence of these POPs, although in low levels, while 2,4 D and methoxychlor are present in relatively higher concentrations. Our data are consistent with those reported by Martinez (1994), who reported the presence of these pesticides in different states of the Mexican Republic and which confirm that these chemicals were used in Jalisco and neighboring states. The increase in concentration of 2,4 D DDT and methoxychlor (Table 1) from dry to rainy season, both in Sayula and Chapala, indicates that they are dragged during the rainy season as noted above (Buck, 1982; Cooke, 1982). The decrease in concentration of heptachlor, aldrin and dieldrin from dry season to rainy season in the same reservoirs indicates that they are present in residual form and are diluted with the entry of rainwater. It has been confirmed that they are no longer used for agricultural purposes (unpublished data) and that their existence is explained by the fact that they remain for 25 to 40 years in the environment due to their high persistence (Pietrapiana *et al.*, 2002). Heptachlor epoxide decreased during the rainy season only in Sayula indicating dilution and persistence. The same concentrations of lindane and chlordane from dry to rainy season at both bodies of water are probably due to a concentration near the limit of resolution of our chromatography equipment.

POPs can bio-accumulate thousands of times compared to environmental conditions. Detection of greater genetic damage in hepatic nuclei of this fish compared to negative witnesses showed the presence of environmental genotoxics in the water of basin Lerma-Chapala-Santiago. We used this tissue because of the high POPs bioaccumulation that occurs on it.

The analysis of POPs in the liver of *Goodea atripinnis* in Sayula and Chapala from dry season to rainy season (Table 2) showed that concentrations of POPs were similar in dry season with the exception of 2,4 D which had a concentration 10 times higher (0.02

ppm) in Chapala than in Sayula (0.002 ppm) indicating a similar presence to previously reported cases (Guillette *et al.*, 1994).

Concentrations of POPs in fish from Chapala and Sayula in rainy season showed no differences; however, the season had a strong influence in Chapala: in the rainy season, aldrin, dieldrin, heptachlor and heptachlor epoxide seem diluted while methoxychlor, DDT and 2,4 D increased during the rainy season and followed a similar direction to that reported by (Sun *et al.*, 2002). The concentrations of these substances in Sayula are similar to those of Chapala. This behavior is similar to that found in POPs from (Table 1).

ACA detects the breakage of DNA in mammalian cells and in some plants (Belpaeme & Kirsch-Volders, 1998; Nacci *et al.*, 1996) and is frequently used because it is quick, simple and sensitive. In this work the breakage of DNA was detected. The average migration of liver nucleus tails of *Goodea atripinnis* in Lake Sayula and Chapala Lake compared with La Joya in the dry season showed statistically significant differences ($p < 0.01$). A similar behavior was observed for the rainy season in Sayula but not in Chapala. This indicates the presence of agents with genotoxic activity; the relatively high concentration of the potent mutagen 2,4 D (0.02 ppm) in Chapala Lake could be associated with increased genetic damage in this location: as observed, levels of 2,4 D are identical in Chapala and Sayula in rainy season, which could explain too the higher damage of fish in Sayula. At the rainy season there are also statistically significant differences between hepatocytes in fish from Sayula and La Joya, which does not occur in Chapala. On this occasion the presence of 2,4 D was identical at both sites indicating the presence of one or more genotoxic agents not considered in this study. It is clear that consuming untreated water from Chapala Lake represents a genotoxic risk to this population as in other parts of the world (Ivanova *et al.*, 2008; Liu *et al.*, 2009; Kerger *et al.*, 2009) and a risk for local flora and fauna. Unfortunately, even treating water does not guarantee the elimination of certain mutagens (Zhu *et al.*, 2008; Mielli *et al.*, 2009). Lately there has been an increase in the incidence of cancer in the population of the metropolitan area of Guadalajara (Secretaría de Salud Jalisco, 2001). Water consumption is a risk factor, among others, because the urban population is exposed to many chemicals that can induce cancer. In conclusion, there is a presence of POPs in the water of Chapala and Sayula Lakes, of which the concentrations of 2,4 D and DDT are noteworthy, but below the established levels by the Official Mexican Norm. These compounds are also found in the liver of the *Goodea atripinnis* fish. Probably there is not bioaccumulation of 2,4 D in fish from both Chapala and Sayula Lakes. Apparently these and other unidentified compounds found on the water could be responsible for genetic damage in liver cells of this fish but is very difficult to attribute to a single persistent pollutant a particular effect. Due to limitations in this study is necessary to use a more sensitive method.

ACKNOWLEDGEMENTS

The authors wish to thank the Programa de Investigación Ambiental del Fondo Sectorial CONACYT-SEMARNAT and Universidad de Guadalajara.

REFERENCES

- ALINK, G. M., E. M. H. FREDERIX-WOLTERS, M. A. VAN DER GAAG, J. F. F. VAN DER KERKHOFF & C. L. M. POELS. 1980. Induction of sister-chromatid exchanges in fish exposed to Rhine water. *Mutation Research* 78: 369-374.
- ÁLVAREZ, M. C. & A. HERNÁNDEZ. 2007. COPS y su Bioacumulación. In: Álvarez, M. C. (Ed.). *Lago de Chapala, contaminación y riesgo genético*. Universidad de Guadalajara, pp. 59-77.
- BARBA, C. G. & L. GUITRON. 2007. Laguna de Sayula: Aspectos que permiten comparar la contaminación entre esta laguna y el Lago de Chapala. In: Álvarez, M. C. (Ed.). *Lago de Chapala, contaminación y riesgo genético*. Universidad de Guadalajara, pp. 25-35.
- BELPAEME, K. C. & M. KIRSCH-VOLDER. 1988. Development and validation of the *in vivo* alkaline comet assay for detecting genomic damage in marine flatfish. *Mutation Research* 415: 167-184.
- BORDEN, L. & T. PRIMENTAL. 1980. Possible effects of dieldrin and other pesticides on human chromosomes *in vivo* and *in vitro*. *Toxicology Research. Project Directory* 5: 11.
- BUCK, B. J. 1982. "DDT moratorium in Arizona. Residues in soil and alfalfa after 12 year". *Bulletin of Environmental Contamination and Toxicology* 13: 61-72.
- COOKE, A. 1982. Distribution and breakdown of DDT in anorchar soil. *Pesticide Science* 13: 545-551.
- DUNIER, M., A. K. SIWICKI & A. DAMAEL. 1991. Effects of organophosphorus insecticides: effects of trichlorofon and dichlorvos on the immune response of carp (*Cyprinus carpio*). III. *In vitro* effects of lymphocyte proliferation and phagocytosis and *in vivo* effects on humoral response. *Ecotoxicology Environmental Safety* 27: 324-327.
- EPA (ENVIRONMENTAL PROTECTION AGENCY). 1984. *Method Study 18 Method 608-Organochlorine Pesticides and PCBs*, "EPA 600/4-84-061, National Technical Information Service, PB84-211358, Springfield, Virginia, 22161. USA. Junio:1-32.
- EPA (ENVIRONMENTAL PROTECTION AGENCY). 1985. *Verification of PBC spill cleanup by sampling and analysis*. Environmental Agency, Office of toxic Substances, U.S Government Printing Office, Washington, D.C. USA. Agosto: 1-53.
- FAHRINGER, R. 1974. Comparative Mutagenicity Studies with Pesticides in Chemical Carcinogenesis Essays. *IARC Scientific Publication* 10: 161-181.
- GÓMEZ, S. G. 2007. Características y problemas ambientales en Chapala: In: Alvarez, M. C. (Ed.). *Lago de Chapala Contaminación y Riesgo Genético*. Universidad de Guadalajara, pp.19-25.
- GUILLETTE, L. & C. URIBE. 2001. Alteraciones en el sistema reproductor de *Alligator, Mississippiensis* por contaminantes ambientales. *Boletín de la Sociedad Mexicana de Herpetología* 9: 1-11.
- GUILLETTE, L. J. 1994. Endocrine disrupting environmental contaminants and reproduction: lessons from the study of wildlife. In: Popkin D. R. & L. J. Peddl (eds.). *Women's Health Today: Perspectives on Current Research and Clinical Practice*. Parthenon Publication, pp. 201-207.
- GURUGE, K. S., M. WATANABE, H. TANAKA & S. TANABE. 2001. Accumulation status of persistent organochlorines in albatrosses from the North Pacific and the Southern Ocean. *Environmental Pollution* 114 (3): 389-398.
- HARTMANN, A., E. AGURELL, C. BEEVER, S. BRENDLER-SCHWAAB, B. BURLINSON, P. CLAY, A. COLLINS, A. SMITH, G. SPEIT, V. & V. THYBAUD. 2003. Recommendations for conducting the *in vivo* alkaline comet assay. *Mutagenesis* 18: 45-51.
- IVANOVA, E., T. STAYKOVA & I. VELCHEVA. 2008. Cytotoxicity and genotoxicity of heavy metal and cyanide-contaminated water in some regions for production and processing of ore in Bulgaria. *Bulgarian Journal of Agricultural Science* 14 (2): 262-268.
- JIMÉNEZ, C. B. 2001. *La Contaminación Ambiental en México (Causas, Efectos y Tecnología Apropriada)*. Editorial Limusa, México. 60 p.
- KAMMANN, U., M. BUNKE, H. STEINHART & N. THEOBALD. 2001. A permanent Fish cell line (EPC) for Genotoxicity testing of marine sediment with the Comet Assay. *Mutation Research* 498: 67-77.
- KERGER, B. D., W. J. BUTLER, D. J. PAUSTENBACH, J. D. ZHANG & S. K. LI. 2009. Cancer mortality in chinese population an Alloy plant with chromium smelting operations. *Journal of Toxicology and Environmental Health, Part A Current Issues* 72 (5): 329-344.
- LIU, J. R, H. W. DONG, X. L. TANG, X. R. SUN, X. H. HAN, B. Q. CHEN, C. H. SUN & B. F. YANG. 2009. Genotoxicity of water from the Songhua River, China, in 1994-1995 and 2002-2003: potential risks for human health. *Environmental Pollution* 157 (2): 357-364.
- LOMBARDI, J. 1998. Endocrine Disrupters. In: Lombardi, J. (Ed.). *Comparative Vertebrate Reproduction*. Kluwer Academic Publishers, pp. 164-217.
- MARTÍNEZ, R. E. 1994. *Evaluación de la contaminación industrial y urbana en el Río Santiago, tramo Ocotlán-Guadalajara*. Tesis de Licenciatura en Biología, División de Ciencias Biológicas, Universidad de Guadalajara, México. 53 p.
- MAYO, D. O., S. G. MIRANDA & S. F. ROJAS. 2006. Monitoreo de la calidad del agua en el río Texcoco mediante sensores selectivos de iones. *Agrociencia* 3: 277-287.
- MIELLI, A. C., M. E. MATTÁ, A. NERSESYAN, P. H. SALDIVA & G. A. UMBUZEIRO. 2009. Evaluation of the genotoxicity of treated urban sludge in *Tradescantia* micronucleus assay. *Mutation Research* 672 (1): 51-54.
- MILLER, R. R., & M. L. SMITH. 1986. Origin and geography of the fishes of central Mexico. In: Hocutt, C. H. & E. O. Wiley (Eds.). *The zoogeog-*

- raphy of North American freshwater fishes. John Wiley and Sons, New York, pp. 487-517.
- NACCI, D. E., S. CAYULA & E. JACKIM. 1996. Detection of DNA damage in individual cells from marine organisms using the single cell gel assay. *Aquatic Toxicology* 35 (3-4): 197-210.
- NAGEL, R. 1993. Fish and environmental chemical: A critical evaluation of tests. In: Hanke, T. W. & H. Segner (Eds.). *Ecotoxicology and Eco-physiology*. Kluwer Academic Publishers, pp. 174-176.
- NOM 127-SSA1-1994. Salud ambiental, agua para uso y consumo humano - Límites permisibles de calidad y tratamientos a que debe someterse el agua para su potabilización. <http://legislacion.vlex.com.mx/vid/tratamientos-someterse-potabilizacion-39519594>
- OHE, T., T. WATANABE & K. WAKABAYASHI. 2004. Mutagens in surface waters: a review. *Mutation Research* 567: 109-149.
- PIETRAPIANA, D. M., P. GUIDETTI, C. FALUGI & M. VACCHI. 2002. Evaluating the genotoxic damage and hepatic tissue alterations in demersal fish species: a case study in the Ligurian Sea (NW Mediterranean). *Marine Pollution Bulletin* 44: 238-243.
- POLLACK, N., A. R. CUNNINGHAM & H. S. ROSENKRANTZ. 2003. Environmental persistence of chemicals and their carcinogenic risks to human. *Mutation Research* 528: 81-91.
- POOL, D. C. 1977. In vitro mutagenic activity of fourteen pesticides. *Toxicology Applied Pharmacology* 41: 196-203.
- PREIN, A. E., G. M. THIE, G. M. ALINK, C. L. M. POELS & J. H. KOEMAN. 1978. Cytogenetic changes in fish exposed to water of the river Rhine. *Science Total Environmental* 9: 287-291.
- SECRETARÍA DE SALUD JALISCO. 2001. *Registro Cáncer Jalisco 2001*. Boletín 70 2001. Dirección General de Planeación Estadística. Guadalajara, Febrero 14: 6-63.
- SHAW, M. W. 1970. Human Chromosome damage by chemical agents. *Annals of Review Medical* 21: 409-432.
- SINGH, N. P., M. T. MCCOY, R. R. TICE & E. L. SCHNEIDER. 1988. A simple technique for quantitation of low levels of DNA damage in individual cells. *Experimental Cell Research* 175: 184-191.
- SUN, C., Y. DONG, S. XU, S. YAO, J. DAI, S. HAN & L. WANG. 2002. Trace analysis of dissolved polychlorinated organic compounds in the water of the Yangtse River (Nanjing, China). *Environmental Pollution* 117: 9-14.
- WATERS, M. D., H. F. STACK, N. E. GARRETT & M. A. JACKSON. 1991. The genetic activity profile database. *Environmental Health Perspectives* 96: 41-45.
- WATERS, M. D., H. F. STACK & M. A. JACKSON. 1999. Genetic toxicology data in the evaluation of potential human environmental carcinogens. *Mutation Research* 437: 21-49.
- ZHOU, H. Y., R. Y. CHEUNG & M. H. WONG. 1999. Bioaccumulation of organochlorines in freshwater fish with different feeding modes cultured in treated wastewater. *Water Research* 33: 2747-2756.
- ZHU, Z., W. M. GU, W. AN & J. Y. HU. 2008. Carcinogenic risk assessment of drinking water based on genotoxic activities using SOS/umu test. *Asian Journal of Ecotoxicology* 3 (4): 363-369.
- ZÚÑIGA, G. 2001. Sistemas de detección de daño genético. In: Álvarez, M. C. (Ed.). *Genética, Ambiente y Salud*. Universidad de Guadalajara, pp. 127-150.

Recibido: 1 de marzo de 2011.

Aceptado: 22 de enero de 2013.