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Semblanzas Ictiológicas
María Laura Ballesteros



Hugo L. López
y
Justina Ponte Gómez

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Semblanzas Ictiológicas

María Laura Ballesteros



Machu Pichu, Perú, octubre de 2009

Hugo L. López y Justina Ponte Gómez

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Imagen de Tapa
María Laura Ballesteros en Melbourne, Australia, septiembre de 2011

El tiempo acaso no exista. Es posible que no pase y sólo pasemos nosotros.

Tulio Carella

Cinco minutos bastan para soñar toda una vida, así de relativo es el tiempo.

Mario Benedetti

Semblanzas Ictiológicas

A través de esta serie intentaremos conocer diferentes facetas personales de los integrantes de nuestra “comunidad”.

El cuestionario, además de su principal objetivo, con sus respuestas quizás nos ayude a encontrar entre nosotros puntos en común que vayan más allá de nuestros temas de trabajo y sea un aporte a futuros estudios históricos.

Esperamos que esta iniciativa pueda ser otro nexo entre los ictiólogos de la región, ya que consideramos que el resultado general trascendería nuestras fronteras.

Hugo L. López

Nombre y apellido completos: MARÍA LAURA BALLESTEROS

Lugar de nacimiento: Córdoba, 25/06/1981

Lugar, provincia y país de residencia: Córdoba, provincia de Córdoba, Argentina

Título máximo, Facultad y Universidad: Doctora en Ciencias Biológicas, Facultad de Ciencias Exactas, Físicas y Naturales, Universidad Nacional de Córdoba.

Posición laboral: Investigador Asistente- CONICET

Lugar de trabajo: Instituto de Diversidad y Ecología Animal, IDEA. (CONICET-UNC).

Especialidad o línea de trabajo: ecotoxicología

Correo electrónico: mlballesteros@efn.uncor.edu

Cuestionario

- **Un libro:** *La novena revelación*
- **Una película:** *El secreto de sus Ojos*
- **Un CD :** todos los de Maná
- **Un artista:** Maná
- **Un deporte:** natación
- **Un color:** azul en toda su gama
- **Una comida:** pastas, especialmente tallarines
- **Un animal:** perro.
- **Una palabra:** respeto.
- **Un número:** 25
- **Una imagen:** el mar abierto mirando desde la costa
- **Un lugar:** Mar del Plata
- **Una estación del año:** verano
- **Un nombre:** María Belén
- **Un hombre:** mi viejo, Luis
- **Una mujer:** la Madre Teresa de Calcuta
- **Un personaje de ficción:** Dexter, el de la serie
- **Un superhéroe:** Iron Man
- **Un ictiólogo del pasado:** Raúl A. Ringuelet
- **Un ictiólogo del presente:** Gustavo Haro



Con su familia, Córdoba, enero de 2014

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Desayuno de fin de año, Córdoba, diciembre de 2008
De izquierda a derecha: Valeria Amé, Madalena Monferrán, María Laura Ballesteros, María de los Ángeles Bistoni,
Noelia Guyón, Angelina Roggio y Andrea Hued



Reunión de Fin de Año de la Cátedra de Diversidad Animal II, Córdoba, diciembre de 2012
De izquierda a derecha, atrás: Alejandro Franchini, Mariano Sironi, Angelina Roggio, María Eugenia Haro, Gustavo Haro y Ariel Lucero
Adelante: María de los Ángeles Bistoni, María Laura Ballesteros, Alejo Bonifacio, Andrea Hued e Isabella Lucero

Endosulfan acute toxicity and histomorphological alterations in *Jenynsia multidentata* (Anablepidae, Cyprinodontiformes)

MARÍA L. BALLESTEROS¹, GUILLERMO E. BIANCHI¹, MIRIAM CARRANZA²
 and MARÍA A. BISTONI^{1,2}

¹Cátedra de Diversidad Animal II, Universidad Nacional de Córdoba, Córdoba, Argentina

²Cátedra de Morfología Animal, Universidad Nacional de Córdoba, Córdoba, Argentina

Toxicity tests using adult specimens of *Jenynsia multidentata* were carried out during 96 hours in order to determine the lethal concentration (LC50) of endosulfan. Histological alterations were determined in gills and liver. Gill damage was quantified as secondary lamellae thickness. The 96 hr LC50 values were significantly different between males (0.719 $\mu\text{g}\cdot\text{L}^{-1}$) and females (1.317 $\mu\text{g}\cdot\text{L}^{-1}$). The sex difference was attributed to the dimorphism in the lipid content in females (2.16%) and males (1.79%). Histological alterations in gills included hypertrophy and lifting of the epithelium of the secondary lamellae and aneurisms. These alterations caused a significant increase of the secondary lamellae thickness in treatment versus control fish. Finally, reversible histological alterations (such as hydropic degeneration and dilation of sinusoids) were observed in the liver of exposed fish as well as an irreversible change such as necrosis at the highest concentrations.

Keywords: Fish, endosulfan, histopathology, lipid content, liver, gills, secondary lamella thickness.

Introduction

Although the use of organochlorine pesticides is restricted in many countries, pesticides such as endosulfan (6, 7, 8, 9, 10, 10-hexachloro-1, 5, 5-a, 6, 9, 9 a hexahydro-6, 9-methano-2, 4, 3-benzo-dioxathiepin-3-oxide) are still in use in South America. Endosulfan is stable and, when dissolved in water, it persists between three to seven days depending on water pH (alkaline conditions favor its degradation, whereas acidic conditions slow this process).^[1] The low persistence of endosulfan in water and its relatively low toxicity in mammals and bees have justified its use in agriculture. However, this compound is highly toxic for fish. It accumulates in fatty tissues of aquatic organisms that are continually exposed to sublethal concentrations.^[2] Many studies have shown that body lipid content serves as a protective tissue against the toxic effects of organic compounds as these chemicals are stored in the fat tissue.^[3] A differ-

ence in the toxic response of male and female *Carassius auratus* and *Cyprinus carpio* exposed to lindane has been observed.^[3] These studies showed that females are more resistant than males due to the higher lipid content in females.

A useful method to evaluate the effects of pollutants on freshwater fish is to study the morphological changes in their organs.^[4] For example, the liver is the target organ of chemically induced tissue injuries due to its function as xenobiotic biotransformer and its central role in the circulatory system. When toxic compounds exceed the detoxification level of the organ, they tend to accumulate at high concentrations into the liver and modify its structure.^[5] On the other hand, the respiratory system of fish provides a large surface area for interaction with the aquatic environment. Thus gills are also an important organ to evaluate toxic effects of pollutants in fish. However, data about the toxicity and structural changes of pesticides on native South American freshwater fish are scarce compared to other species. The main goal of the present study was to determine the acute toxicity of endosulfan in male and female *Jenynsia multidentata*. We established a differential toxic response between both sexes through the determination of the body lipid content. We also evaluated the histological alterations in liver and gills caused by endosulfan.

Address correspondence to María de los Ángeles Bistoni: Cátedra de Diversidad Animal II, Facultad de Ciencias Exactas, Físicas y Naturales, Universidad Nacional de Córdoba. Av. Vélez Sársfield 299 - CP: 5000- Córdoba, Argentina; E-mail: mbistoni@com.uncor.edu

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Endosulfan induces changes in spontaneous swimming activity and acetylcholinesterase activity of *Jenynsia multidentata* (Anablepidae, Cyprinodontiformes)

M.L. Ballesteros^a, P.E. Durando^b, M.L. Nores^c, M.P. Díaz^d, M.A. Bistoni^{a,*}, D.A. Wunderlin^e

^aFacultad de Ciencias Exactas, Físicas y Naturales, Cátedra Diversidad Animal II, Universidad Nacional de Córdoba, Av. Vélez Sársfield 299, 5000 Córdoba, Argentina

^bFacultad de Ciencias Exactas, Físicas y Naturales, Departamento de Biología, Cátedra de Fisiología Animal, Universidad Nacional de San Juan, Complejo "Islas Malvinas", Av. José I. de la Roza y Meglioli, Rivadavia, San Juan, Argentina

^cFacultad de Ciencias Médicas, Universidad Nacional de Córdoba—CONICET, Ciudad Universitaria, Córdoba, Argentina

^dFacultad de Ciencias Médicas, Cátedra de Estadística y Bioestadística, Escuela de Nutrición, Universidad Nacional de Córdoba, Pabellón Chile, Ciudad Universitaria, 5000 Córdoba, Argentina

^eFacultad de Ciencias Químicas, Dto. Bioquímica Clínica—CIBICI, Universidad Nacional de Córdoba—CONICET, Haya de la Torre esq. Medina Allende, Ciudad Universitaria, 5000 Córdoba, Argentina

This work reports changes observed in spontaneous swimming activity and AchE activity of *Jenynsia multidentata* exposed to sublethal concentrations of Endosulfan.

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ABSTRACT

We assessed changes in spontaneous swimming activity and acetylcholinesterase (AChE) activity of *Jenynsia multidentata* exposed to Endosulfan (EDS). Females of *J. multidentata* were exposed to 0.072 and 1.4 $\mu\text{g L}^{-1}$ EDS. Average speed and movement percentage were recorded during 48 h. We also exposed females to EDS at five concentrations between 0.072 and 1.4 $\mu\text{g L}^{-1}$ during 24 h, and measured the AChE activity in brain and muscle. At 0.072 $\mu\text{g L}^{-1}$ EDS swimming motility decreased relative to the control group after 45 h, while at 1.4 $\mu\text{g L}^{-1}$ EDS swimming motility decreased after 24 h. AChE activity significantly decreased in muscle when *J. multidentata* were exposed to EDS above 0.072 $\mu\text{g L}^{-1}$, while no significant changes were observed in brain. Thus, changes in swimming activity and AChE activity in muscle are good biomarkers of exposure to EDS in *J. multidentata*.

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1. Introduction

The environment is continuously loaded with foreign organic chemicals (xenobiotics) released by urban communities and industries. In the 20th century, thousands of organic pollutants, such as polychlorinated biphenyls (PCBs), organochlorine pesticides (OCPs), polycyclic aromatic hydrocarbons (PAHs), polychlorinated dibenzofurans (PCDFs) and dibenzo-*p*-dioxins (PCDDs) have been produced and, in part, released into the environment (van der Oost et al., 2003).

Fish are sensitive to the impact of these xenobiotics that can be found in the aquatic environment. Fish also play a major ecological role in the aquatic food-webs because of their function as carrier of

energy from lower to higher trophic levels (Chovanec et al., 2003; van der Oost et al., 2003; Kane et al., 2004). The understanding of fish responses to the uptake of toxicants has high ecological relevance. Prolonged and severe exposure to contaminants may induce a sequence of behavioral, functional and structural changes which impair vital functions such as the ability of fish to feed, avoid predation or reproduce (Little et al., 1990).

The use of biomarkers of exposure to xenobiotics in fish has become relevant in toxicological assessments, since it allows the early detection of negative effects of pollutants, providing information of changes induced at sub-lethal level (Livingstone, 1993).

Changes in behavior are among widely used biomarkers. This is mainly because the behavior integrates many cellular processes, which are essential to the viability of the organism, the population and the community. Therefore, observations of behavioral changes provide with a unique toxicological perspective, linking both biochemical and ecological consequences of environmental

* Corresponding author. Tel.: +54 351 433 2100x221; fax: +54 351 433 2099.
E-mail address: mbistoni@com.uncor.edu (M.A. Bistoni).



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Oxidative stress responses in different organs of *Jenynsia multidentata* exposed to endosulfan

M.L. Ballesteros^a, D.A. Wunderlin^b, M.A. Bistoni^{a,*}
^aUniversidad Nacional de Córdoba, Facultad de Ciencias Exactas Físicas y Naturales, Cátedra Diversidad Animal II, Avda. Vélez Sarsfield 299, 5000 Córdoba, Argentina

^bUniversidad Nacional de Córdoba-CONICET, Facultad de Ciencias Químicas, Dto. Bioquímica Clínica-CIBICI, Haya de la Torre y Medina Allende, Ciudad Universitaria, 5000 Córdoba, Argentina

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Abstract

We evaluate antioxidant responses of *Jenynsia multidentata* experimentally exposed to sublethal concentrations of endosulfan (EDS). The main goal was to determine differences in the response between different organs to assess which one was more severely affected. Thus, we exposed females of *J. multidentata* to EDS during 24 h, measuring the activity of GST, GR, GPx, CAT and LPO in brain, gills, liver, intestine and muscle of both exposed fish and controls.

GST activity was inhibited in gills, liver, intestine and muscle of exposed fish but was induced in brain. GR and GPx activities were increased in brain and gills at 0.014 and 0.288 $\mu\text{g L}^{-1}$, respectively. GPx activity was inhibited in liver and muscle at all studied concentrations whereas inhibition was observed in the intestine above 0.288 $\mu\text{g L}^{-1}$. Exposure to 1.4 $\mu\text{g L}^{-1}$ EDS caused CAT inhibition and increase of LPO levels in liver. LPO was also increased in brain at almost all concentrations tested. We find that the brain was the most sensitive organ to oxidative damage. Thus, *J. multidentata* could be used as a suitable bioindicator of exposure to EDS measuring activities of antioxidant enzymes in brain and liver as biomarkers.

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Keywords: Antioxidant enzymes; Oxidative stress; Endosulfan; Fish; Lipid peroxidation

1. Introduction

The widespread use of pesticides has resulted in the pollution of many aquatic habitats worldwide. Pesticides enter to the aquatic systems by different routes, including: direct application, urban and industrial discharges, surface runoff from non-point sources, including agricultural soil, aerosol, particulate deposition and rainfall, etc. (Sharma, 1990).

Among different pollutants, organochlorine pesticides require special attention because of their high stability and toxicity to the aquatic organisms. Endosulfan (EDS) is an organochlorine insecticide belonging to the cyclodiene group. EDS is persistent in soils (60 days for alpha and 800 days for beta isomers) (Stewart and Cairns, 1974). EDS is partially soluble in water (60–100 $\mu\text{g L}^{-1}$) (ATDSR,

2000) where persists from 3 to 15 days (Eichelberger and Litchenberg, 1971). The low persistence of EDS in water and its relatively low toxicity to mammals and bees have justified its use in agriculture (Ghadiri et al., 1995). However, this compound is highly toxic for fish (Naqvi and Vaishnavi, 1993). It accumulates in fatty tissues of aquatic organisms that are continuously exposed to sublethal concentrations (Jonsson and Toledo, 1993). Most organochlorine insecticides are banned in many countries. However, EDS is still in use in Argentina (Miglioranza et al., 2003), where Baudino et al. (2003) found concentrations ranging from 0.97 to 2 $\mu\text{g L}^{-1}$ for alpha and beta isomers in surface and ground water. According to EPA, EDS concentrations above 0.22 $\mu\text{g L}^{-1}$ (acute) and 0.056 $\mu\text{g L}^{-1}$ (chronic) have an adverse impact on the health of aquatic organisms (Mersie et al., 2003).

Negative effects of EDS on fish have been evaluated in several studies, including biochemical (Sharma, 1988,

*Corresponding author. Fax: +54 351 4332099.

E-mail address: mbistoni@com.uncor.edu (M.A. Bistoni).

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Uptake, tissue distribution and metabolism of the insecticide endosulfan in *Jenynsia multidentata* (Anablepidae, Cyprinodontiformes)

M.L. Ballesteros^{a,b,c,*}, M. Gonzalez^{b,c}, D.A. Wunderlin^{c,d}, M.A. Bistoni^a, K.S.B. Miglioranza^{b,c}^aUniversidad Nacional de Córdoba, Facultad de Ciencias Exactas Físicas y Naturales, Cátedra Diversidad Animal II, Av. Vélez Sársfield 299, 5000 Córdoba, Argentina^bUniversidad Nacional de Mar del Plata, Facultad de Ciencias Exactas y Naturales, Laboratorio de Ecotoxicología, Funes 3350, 7600 Mar del Plata, Argentina^cConsejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Argentina^dUniversidad Nacional de Córdoba-CONICET, Facultad de Ciencias Químicas, Dto. Química Orgánica-CIBICI, Haya de la Torre y Medina Allende, Ciudad Universitaria, 5000 Córdoba, Argentina

Endosulfan is accumulated in organs of *J. multidentata* as well as biotransformed to endosulfan sulfate, which relative abundance points out the time from exposure.

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ABSTRACT

The study reports the accumulation, distribution and metabolism of technical endosulfan in *Jenynsia multidentata*. Adult females were exposed to acute sublethal concentrations (0.072, 0.288 and 1.4 $\mu\text{g L}^{-1}$). After 24 h, fish were sacrificed and gills, liver, brain, intestine and muscle were removed. Results show that both isomers of technical-grade endosulfan (α - and β -) are accumulated in fish tissues and biotransformation to endosulfan sulfate occurs at all concentrations tested. Significant differences in endosulfan accumulation were only found at 1.4 $\mu\text{g L}^{-1}$ but not between the lowest concentrations. However a similar distribution pattern was observed at all exposure levels where liver, intestine and brain had the highest levels of α -, β -endosulfan and endosulfan sulfate. Moreover, liver and brain showed the highest endosulfan sulfate: α -endosulfan ratios due to high biotransformation capacity. *J. multidentata* demonstrated to be a sensitive species under exposure to technical endosulfan and, therefore, could be used to assess aquatic pollution.

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1. Introduction

Organochlorine pesticides (OCPs) are persistent and ubiquitous pollutants. After their release to the environment, OCPs are subject to transport and transformation processes that, in addition to environmental parameters and physicochemical properties regulate their distribution and concentration in water, soil, sediments and biota (van der Oost et al., 2003). The high molecular weight and the low water solubility of most OCPs lead to their bioaccumulation in the biota, mainly in fatty tissues, and biomagnification in the food chain as well (Newman and Unger, 2003).

Endosulfan belongs to the cyclodiene group, and represents the last OCPs under current use in many countries. Technical-grade endosulfan is a mixture of α - and β -endosulfan, being the ratio of 7:3 the most frequent used form (ATDSR, 2000). It is relatively soluble in water with a log K_{ow} of 3.83 and 3.52 for α - and β -isomers, respectively (ATDSR, 2000). It is a broad-spectrum non-

systemic insecticide that, due to its low toxicity to bees and mammals, is extensively used to control a wide variety of pests in agriculture, horticulture and public health programs. However it is extremely toxic to fish species (Naqvi and Vaishnavi, 1993). Several works reported adverse effects of endosulfan on fish, i.e. Pandey et al. (2001) found that GST activity was induced in liver, gills and kidney of *C. punctatus* exposed to 5 $\mu\text{g L}^{-1}$ endosulfan for 24 h, while Cengiz and Ünlü (2001) found histological changes in liver and gut of *Gambusia affinis* exposed to 1–5 $\mu\text{g L}^{-1}$ of technical-grade endosulfan (Thiodan®) during 7 days. Moreover, in previous works, we found changes in biotransformation enzymes and swimming activity when *J. multidentata* was exposed to 0.072 and 1.4 $\mu\text{g L}^{-1}$ of technical-grade endosulfan (Galgofan®) during 24 and 48 h (Ballesteros et al., 2009a, 2009b). Because of their toxicity, persistence and global distribution the Stockholm Convention is currently considering the inclusion of endosulfan into the list of Persistent Organic Pollutants (POPs) (UNEP, 2010; Weber et al., 2010). However, endosulfan is still used in Argentina for fruits, vegetables and soybean crops, being their residues found in the environment (Gonzalez et al., 2005; INTA, 2004; Miglioranza et al., 2003a; Ondarza et al., 2011).

* Corresponding author.

E-mail address: mlballesteros@efn.uncor.edu (M.L. Ballesteros).

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Multimatrix measurement of persistent organic pollutants in Mar Chiquita, a continental saline shallow lake



M.L. Ballesteros^a, K.S.B. Miglioranza^{b,*}, M. Gonzalez^b, G. Fillmann^c, D.A. Wunderlin^d, M.A. Bistoni^a

^a Instituto de Diversidad y Ecología Animal (CONICET-UNC), Universidad Nacional de Córdoba, Facultad de Ciencias Exactas Físicas y Naturales, Av. Vélez Sarsfield 299 (5000), Córdoba, Argentina

^b Instituto de Investigaciones Marinas y Costeras (CONICET-UNMdP), Universidad Nacional de Mar del Plata, Facultad de Ciencias Exactas y Naturales, Laboratorio de Ecotoxicología y Contaminación Ambiental, Funes 3350 (7600), Mar del Plata, Argentina

^c Laboratório de Microcontaminantes Orgânicos e Ecotoxicologia Aquática, Universidade Federal do Rio Grande, Rio Grande, RS, Brazil

^d Instituto de Ciencia y Tecnología de Alimentos Córdoba (ICYTAC), CONICET and Facultad de Ciencias Químicas, Dpto. de Química Orgánica, Facultad de Ciencias Químicas, Universidad Nacional de Córdoba, Bv Dr Juan Filloy s/n, Ciudad Universitaria, 5000-Córdoba, Argentina

HIGHLIGHTS

- OCPs, PCBs and PBDEs were found in water, sediments, SPM, and fish.
- Endosulfan was the main pesticide found in all matrices.
- γ -HCH and endosulfans overpassed quality guidelines and represent a risk to aquatic biota.
- PCB levels overpassed the AID for human consumption being a risk for human health.

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ABSTRACT

RAMSAR sites are determined by specific characteristics of the environment in terms of ecological productivity as well services for human development, but they are also one of the most threatened ecosystems. Thus, the objective of this work was to evaluate the dynamic of Persistent Organic Pollutants (POPs) in different biotic and abiotic matrices of the RAMSAR site (wetlands with international importance), Mar Chiquita Lake. Sampling was performed according to land use (agricultural, urban, and industrial) at two stations: Laguna del Plata and Campo Mare. POPs were analyzed in superficial water (Sw), suspended particulate material (SPM), bottom sediment (Bs) and fish tissues (*Odontesthes bonariensis*). Organochlorine pesticides (OCPs), polychlorinated biphenyls (PCBs) and polybrominated diphenyl ethers (PBDEs) were analyzed by GC-ECD. HCHs, Endosulfans, DDTs, PCBs and PBDEs were found in all matrices at both stations. The high persistence and transport processes are responsible for the occurrence of HCHs, DDTs and PCBs in Bs, SPM and fish tissues, even many years after their prohibition. PBDEs showed lower levels according to the scarcity of punctual sources in the area. Endosulfan showed variable amounts in agreement with application periods since this pesticide was used until a few years ago in this area. Finally, PCB levels overpassed the acceptable daily intake for human consumption being a risk for human health. Thus, the present report confirms the occurrence of POPs in Mar Chiquita lake, alerting on the contribution of agricultural and urban pollutants in a RAMSAR site. Current results also raise concerns on biomagnification processes through the food web.

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1. Introduction

Organochlorine pesticides (OCPs), polychlorinated biphenyls (PCBs) and polybrominated diphenyl ethers (PBDEs) represent persistent, bioaccumulative and toxic compounds of global concern (UNEP, 2011). The production and intensive agricultural or industrial use of these compounds during decades have led to widespread contamination of the environment. Most of these substances have been restricted and

forbidden in most countries since the late 1970s, but some developing countries are still using them because of their low costs and versatility in the industry, agriculture, and public health (Loganathan and Kannan, 1994). In Argentina, most of the OCPs and PCBs uses have been banned since 1998 and 2005, respectively, although their residues are still found in aquatic environments. Particularly, technical grade and active ingredients of endosulfan were included in the Persistent Organic Pollutants (POPs) list on April 2011 (UNEP, 2011). This insecticide was highly used in agriculture during the last 15 decades in Argentina, until it was recently phased out in July 2013. So, endosulfan residues were found in several of the aquatic ecosystems associated to environmental matrices (Colombo et al.,

* Corresponding author. Tel.: +54 223 4752426x455; fax: +54 223 47118.
E-mail address: kmiglior@mdp.edu.ar (K.S.B. Miglioranza).



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Museo de La Plata
Facultad de Ciencias Naturales y Museo, UNLP
Paseo del Bosque s/n, 1900 La Plata, Argentina

Directores

Dr. Hugo L. López

hlopez@fcnym.unlp.edu.ar

Dr. Jorge V. Crisci

crisci@fcnym.unlp.edu.ar

Versión electrónica, diseño y composición

Justina Ponte Gómez

División Zoología Vertebrados

Museo de La Plata

FCNyM, UNLP

jpg_47@yahoo.com.mx

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