Asian Jr. of Microbiol. Biotech. Env. Sc. Vol. 23, No. (1) : 2021 : 1-7
© Global Science Publications
ISSN-0972-3005

ENVIRONMENT CHEMISTRY: COMPARATIVE STUDIES AND SUBLETHAL ECOTOXICITY OF NEW ANTIFUNGALS ON *DAPHNIA MAGNA* AS MODEL ORGANISM

RENO ULISES[1,2], MACHUCA L. MARCELA[2,3], REGALDO LUCIANA[1,2], MURGUÍA M. CÉSAR[2,4]* AND GAGNETEN A. MARIA[1]

[1] Laboratorio de Ecotoxicología, Facultad de Humanidades y Ciencias, Universidad Nacional del Litoral, (3000) Santa Fe, Argentina
[2] Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Godoy Cruz 2290 (C1425FQB) Buenos Aires, Argentina
[3] Instituto de Investigaciones Científicas (IDIC), Facultad de Ingeniería y Tecnología, Universidad de la Cuenca del Plata (UCP), Corrientes 57, (3450) Goya, Corrientes, Argentina
[4] Laboratorio de Química Aplicada, Facultad de Bioquímica y Ciencias Biológicas, Universidad Nacional del Litoral, (3000) Santa Fe, Argentina

(Received 23 September, 2020; Accepted 5 November, 2020)

Key words: *Gemini surfactants, Full life cycle tests, Daphnia magna, Sublethal ecotoxicity*

Abstract – The objective of this work was to compare through chronic tests, the sublethal effects of two compounds: the antifungal commercial Pellital Bio-F36 (TCMTB), and a synthetic eco-friendly gemini (Gem-3e) compound in *Daphnia magna*. Both compounds are used in the industry as antifungals for the preservation of woods and leathers. The attributes of life history recorded as endpoints were: survival; growth; fecundity and net reproductive rate (Ro). The net reproductive rate (Ro) was =0 in the bioassays conducted with $1x10^{-4}$, $5x10^{-5}$ and $2.5x10^{-5}$ mg L^{-1} of TCMTB. This value is indicative of no reproductive events, condition that would indicate a population decline and possible local extinction. For the synthetic compound type-gemini (Gem-3e) only in the lowest concentration Ro was >1. Changes occurred in the life cycle of *D. magna* exposed to both compounds, but the concentrations tested with the commercial formulation was between 2100 and 2403 times lower than those of the Gem-3e compound.

INTRODUCTION

One of the twelve principles of green chemistry emphasizes the need to synthesize safer chemicals. These chemicals should be capable of performing their desired function but would be designed to elicit minimal toxicity. In support of this effort, recent studies have explored the relationships between chemical properties and acute or chronic toxicity as measured through standardized OECD and EPA protocols. However, in last decades, ecological risk assessment was driven by the negative –usually mortality- effects of a contaminant on the biota and the mechanism underpinning the effect –through data on life history parameters - is often given lower priority (Cairns and Pratt, 1989) In this study, we examined the potential utility and effectiveness of full life cycle tests to evaluate aquatic toxicity of a common industrial chemical together with a new synthesized one. The bioassays were performed with *Daphnia magna* (Cladocera) towards the likelihood of encountering *N*-acetylated gemini compounds with better environmental performance than commercially available ones. This way, we hope to contribute to the establishment of US EPA thresholds of chronic toxicity to standardized cladoceran models.

The universe of fungi is an extremely complex community containing various types of substrates, microhabitats and interacting species. These organisms possess a wide range of activities such as human or plant pathogens, as producers of many metabolites of importance for humanity, and as decomposers. Aught to a specialized enzyme system they can decompose the cellulose and wood, causing serious economic losses. Thus, it can be stated that there are virtually no natural organic compounds that cannot be used as nutrient source for the fungi causing the decline of various types of organic materials such as paper, textiles, wood and leathers (Menger and Keiper, 2000).

Thus, the damage caused by these organisms is reflected in the increased use of biocide compounds. In turn, after use, the majority of antifungals is discarded in industrial effluents or sewage and, ultimately, can reach rivers, lakes and oceans. A rise in the resistance of some species of fungus to different fungicides has been observed (Orlita, 2004)

Toxicity problems, which add to the related phenomena of resistance, maintain in force the need for further development of new antifungal drugs that provide significant advantages over existing ones. Given this scenario, the production of amphiphile molecules with new and interesting properties has increased in the last decade in the field of applied organic chemistry through the aforementioned goals of green chemistry.

Gemini surfactant is the family of surfactant molecules possessing a long hydrocarbon chain, an ionic group, a rigid or flexible spacer, a second ionic group, and another hydrocarbon tail. These surfactants usually have better surface-active properties than corresponding conventional surfactants of equal chain length. Gemini compounds are used as promising surfactants in industrial detergency and have shown efficiency in skin care, antibacterial property, metal-encapped porphyrazine and vesicle formation, construction of high porosity materials, among others (Menger *et al.*, 2000; Hait and Moulik, 2003; Woch *et al.*, 2018).

Moreover, these compounds have an excellent biodegradability and low toxicity so being friendly to the environment. All these relevant properties have attracted our attention because in a previous work a series of new *N*-acetylated non-ionic and cationic gemini surfactants were synthesized. Their antifungal potency, surface properties and the acute toxicity of the molecule with better performance, *N,N*-Bis [2-(3-dodecyldimethylammonio-2-hydroxypropoxy)ethyl] acetamide dichloride -called gemini (Gem-3e)- was also studied through acute bioassays using the microalgae *Chlorella vulgaris* and the cladoceran *D. magna* as biological models. The toxicity was compared to the obtained for a commercially available reference compound: Pellital Bio-F36, 2-(thiocyanomethylthio)-benzothiazole (TCMTB), an active agent used to preserve leather, very efficient due to its high penetrating power (Machuca *et al.*, 2015).

Chlorella vulgaris bioassays showed that with TCMTB, the effective concentration (EC_{50}) was 2.172 x 10^{-4} mgL^{-1} and with the Gem-3e compound EC_{50} was 0.1765 mg L^{-1} (three orders of magnitude higher than the commercial product). *D. magna* acute bioassays showed that with TCMTB 24 and 48 h lethal concentration (LC_{50}) were 0.00034 mg L^{-1} and 0.0001 mg L^{-1} respectively. Conversely, with Gem-3e the values were 1.234 mg L^{-1} and 0.474 mg L^{-1} respectively –between 3.6 and 4.7 times higher, that is to say less toxic- than the commercial one (Machuca *et al.,* 2015).

However, lethal effects –that can be known through acute tests - are often not enough to ensure that a certain compound is not harmful to the environment. In this line, full life-cycle tests are recommended in risk assessment particularly where the mode of action of a contaminant is unclear (Ingersoll and MacDonald, 1999; Reno *et al.*, 2018). This is often the case of compounds of new synthesis, such as gemini compounds. Full life-cycle tests expose animals from hatching to reproductive maturity and, therefore, incorporate aspects of embryonic development and reproductive and growth parameters. For this reason, it is important to check the sublethal toxicity to aquatic organisms to ensure the safety of the new synthesized compound. The application of ecofriendly chemical compounds, allows to get closer to cleaner production, with the aim to prevent pollution by replacing the hard-toxic chemical processes that negatively impact the environment with others that are less polluting.

The purpose of this work was to compare the chronic effects of the compounds previously tested through acute tests: the commercially, TCMTB and the Gem-3e selecting *D. magna* as biological model. The objective was to confirm and reinforce or reject the results previously obtained through acute tests, and to assess if the latter compound is environmentally a safer chemical -e.g. is less toxic- than commercial one, taking in consideration key life history traits of the selected species.

MATERIALS AND METHODS

General procedure

All chemicals for the synthesis of gemini compound Gem-3e were reagent grade commercial materials and used without further purification (Merck & Co., Nueva Jersey, USA). Gem-3e was synthesized by the reaction of N,N-dimethyldodecylamine, diglycidyl ether and tetrabutyl ammonium bromide (TBABr) in absolute ethanol (30 °C, 18 h) following a procedure previously reported (Murguía *et al.*, 2008). TCMTB was used as a commercially available reference compound (commercial solution, 30 % w/w). Fourier-transform infrared spectroscopy (FTIR) spectra were recorded on a Shimadzu 8201 PC spectrophotometer; ^{1}H-NMR and ^{13}C-NMR spectra on a Bruker AV-300 spectrometer, using D_2O and $CDCl_3$ as solvent. Mass Spectra (MS) were performed on a waters UPLC-MS SQ2 Single Quadrupole Detector. High-resolution mass spectrometric measurements (HRMS) were conducted at the Mass Spectrometry facility of the University of Buenos Aires, Argentina. Gas chromatographic (GC) analyses were performed on a DANI GC equipped with a methyl-phenyl silicone capillary column (30 m x 0.32 mm, 0.25 μm film thickness) and FID (flame ionization detector). Column chromatography (CC) was performed on silica gel (70–230 mesh ASTM). Isolated and authenticated compounds were used as internal standards (ST) to perform quantitative GC analyses.

The purity and chemical structure of the synthesized compound were checked by thin layer chromatography (TLC), high resolution mass spectrometry (HRMS), and nuclear magnetic resonance (NMR) spectra. All samples and structures for TCMTB and for the synthetized compound Gem-3e were quantified and confirmed by liquid chromatography (UPLC-MS), spectroscopy (FTIR, ^{1}H and ^{13}C NMR) and HRMS methods. All analytical methods indicated high levels of purity for TCMTB (30 %) and for the Gem-3e (100 %) (Figure 1).

Bioassays

Chronic tests with *Daphnia magna*

A stock solution of 1×10^{-2} mg L^{-1} was prepared for TCMTB and for compound Gem-3e, respectively. Then, for each compound a series of dilutions were prepared to study their effects over *D. magna* specimens. The concentrations of the stock solutions and all the dilutions for the tests were adjusted and quantified by Ultra-performance liquid chromatography coupled to a mass spectrometer (UPLC-MS).

Chronic toxicity tests were performed with *D. magna* exposed to concentrations of TCMTB and for the Gem-3e. As a criterion for selecting concentrations to be used, in this work they were considered taking as reference the LC_{50}-48 h values, reported by Machuca *et al.*, 2015.

Full life cycle tests were conducted to compare the sublethal effects of TCMTB and Gem-3e compound on *D. magna,* following the protocol proposed by the OECD, 2012. The specimens were exposed to six and five concentrations of each compound: 1×10^{-4}; 5×10^{-5}; 2.5×10^{-5}; 1.25×10^{-5}; 6.25×10^{-6}; 3.125×10^{-6} mg L^{-1} and 0.12; 0.06; 0.03; 0.015; 0.0075 mg L^{-1} for TCMTB and Gem-3e, respectively. A neonate (<24-h-old) of *D. magna* was transferred into a 50 mL beaker containing 30 mL of culture medium (APHA, 1998) in treatments and in control (without toxic), exposing them to sublethal concentrations of both compounds, with 10 replicates each. Temperature and photoperiod were maintained constant at (21±1) °C and 16 light:8 darkness. Animals were fed three times a week with 40 μL per wheel of a suspension of *C. vulgaris* (absorbance = 1.5 λ, 650 nm). pH values and dissolved oxygen concentrations were recorded at the beginning and at the end of each assay, taking into account the limits established by APHA (1998). As endpoints, the survival (number of living and dead organisms, growth (number of molts produced), fecundity (number of neonates released) were recorded three times per week. In addition, were determined the age of first reproduction and the net reproductive rate (Ro) according to the following formula proposed by Pianka, (1982):

$$Ro = \sum lx.mx$$

where Ro: net reproductive rate, lx: survival at age x, and mx: fertility at age x.

Statistical analysis

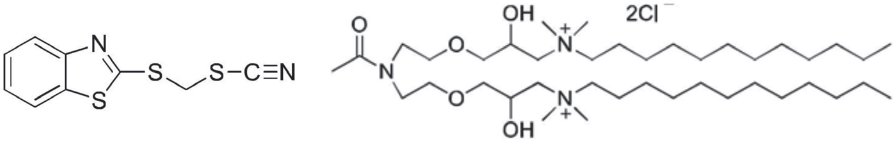

Fig. 1. Chemical structure of 2-(thiocyanomethylthio)-benzothiazole (TCMTB) and *N*, *N*-Bis[2-(3-dodecyldimethylammonio-2-hydroxypropoxy) ethyl] acetamide dichloride (Gem-3e).

In order to quantify possible significant differences in each of the mentioned endpoints between control and treatments with both compounds (TCMTB and Gem-3e), one factor ANOVA was carried out, followed by a Tukey-Kramer Multiple Comparisons post Test at a 95% confidence level. Prior to each analysis, the normality (Kolmogorov -Smirnov's test) of the data obtained was verified. Kruskal-Wallis Test and Dunnet's Multiple Comparisons post Test were done to evaluate the possible significant differences in age of first reproduction between control and treatments. Statistical analyses were carried out using the package InfoStat (2004).

RESULTS

An antifungal molecule with high performance, Gem-3e was tested to evaluate its sublethal ecotoxicity. Our interest was to generate knowledge and experience applied to the synthesis of new eco-friendly gemini compounds, with possible application in leather preservation. In this direction, efforts to study new structures capable of generating disruption on the membranes of prokaryotic and eukaryotic organisms have been successful (Menger and Littau, 1993; Murguía and Grau, 2001; Murguía *et al.*, 2005; Murguía *et al.*, 2008)

Chronic assays showed that the commercial compound (TCMTB), significantly affected the analyzed life history traits: survival (%), fecundity (number of neonates released) and growth (number of molts release), at concentrations that were between 2100 and 2403 lower than those tested for Gem-3e (Table 1).

On the other hand, in Table 1 and Figure 2, it can be seen that the most sensitive life history attribute for the two compounds was fertility. Only at the lowest concentrations (3.125×10^{-6} and 0.0075 mg L^{-1} of TCMTB and Gem-3e, respectively) were no statistically significant differences observed with the control.

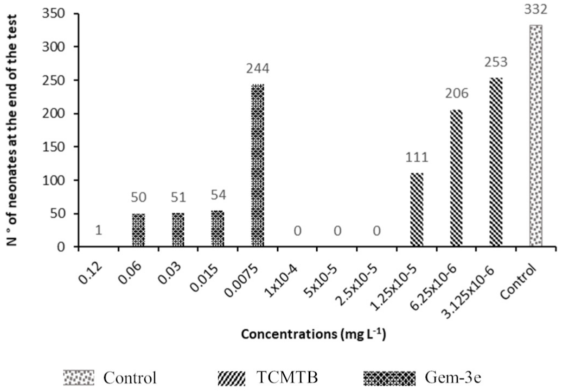

Fig. 2. Number of neonates produced at the end of the 21-day chronic test, for each evaluated concentration of TCMTB, Gem-3e and control (without toxic).

The net reproductive rate (Ro) was = 0 in the *D. magna* bioassays conducted with 1×10^{-4}, 5×10^{-5} and 2.5×10^{-5} mg L^{-1} of TCMTB. This value is indicative of no reproductive events. Conversely, in the rest of the concentrations tested, Ro was > 1. For the Gem-3e, only in the lowest concentration (0.0075 mg L^{-1}) Ro was > 1. These results must be understood in the scope of the range of the concentrations of both compounds tested.

DISCUSSION

These results show: 1) the environmental risk due to the use of highly toxic compounds in the industry, such as the TCMTB; 2) the need to develop less toxic inputs to minimize the environmental impact of production, in leather tanning.

Considering the marked differences in the concentrations tested of both compounds, it can be concluded that both compounds are toxic to *D. magna*, being the commercial one much more toxic. TCMTB acts on the inhibition of the electron transport chain in mitochondria (Fernández-Alba *et al.*, 2002), which could explain the effects reported in this work.

Table 1. Comparison in survival, fecundity and gowth between the control (without toxic) and all the concentrations tested with TCMTB- (left) and Gem-3e (right). n.s.: $p>0.05$; * $p<0.05$.

	TCMTB (mg L^{-1})				Gem-3e (mg L^{-1})		
	Survival	Fecundity	Growth		Survival	Fecundity	Growth
Control vs 3.125×10^{-6}	n.s.	n.s.	n.s.	Control vs 0.0075	n.s.	n.s.	n.s.
Control vs 6.25×10^{-6}	n.s.	*	n.s.	Control vs 0.015	n.s.	*	n.s.
Control vs 1.25×10^{-5}	n.s.	*	n.s.	Control vs 0.03	n.s.	*	n.s.
Control vs 2.5×10^{-5}	*	*	*	Control vs 0.06	*	*	n.s.
Control vs 5×10^{-5}	*	*	*	Control vs 0.12	*	*	*
Control vs 1×10^{-4}	*	*	*				

On the other hand, the concentrations evaluated in this work are environmentally relevant, since Turquet *et al.* (2010), reported up to 2.5x10^{-5} mg L^{-1} of TCMTB in aquatic environments. For this reason, the results reported in this work on the effects of TCMTB on *D. magna* could give an approximation of what is happening in aquatic environments where this compound is present.

Regarding the variables analyzed in this work, the importance of carrying out ecotoxicological evaluations are highlighted, where the response variable is taken as life history attributes (survival, growth and fecundity) and integrative population parameters such as Ro.

This integrative approach would allow the development of risk assessments more suitable for an integral management of products used in leather tanning and other industrial processes. Reno *et al.* 2018, reported the importance of using integrated parameters to carry out more accurate environmental assessments, which allow contributing to environmental management, through the development of guide levels for the protection of aquatic biota.

In addition, this study and results published by other authors (Guilhermino *et al.*, 2000. Kergaravat *et al.*, 2018), provide good evidence of the applicability of using cladoceran tests as prescreening methods. As Ingersoll and Mac Donald, (1999), stressed many years ago, full life cycle tests allow a contaminant to be defined as a developmental or reproductive toxicant. Ten years before, Depledge, (1989) pointed out that in a holistic approach to ecosystem management, understanding how a toxicant exerts its effect, is essential to identify the target species or groups most at risk. Far from being out of use, this approach has recently been utilized to predict sublethal effects of pesticides, nanomaterials (Lapresta-Fernandez *et al.*, 2012; Reno *et al.*, 2015. Wieczerzak *et al.*, 2016; Reno *et al.*, 2016) and establish thresholds of ecotoxicological concern for various organic chemical compounds (Sanchez-Hernandez *et al.* 2018; Machado and Soares, 2019). These promising results highlight that the interaction of chemistry and ecotoxicology would allow knowing the global behavior of chemicals in natural environments from an integrative perspective, as was assessed for some emerging contaminants as quinolones by Kergaravat *et al.*, (2018).

On the other hand, in this work it was shown that fecundity was the most sensitive attribute, since no reproductive events were recorded in the higher concentrations. In this sense, Nawrocki *et al.*, 2005, evaluated the chronic toxicity of TCMTB, on *Ceriodaphnia dubia* for 7 days, reporting effects on fecundity at concentrations higher than those evaluated in this work (between 1x10^{-2} and 5 x10^{-3} mg L^{-1}).

The results obtained in this work contribute to the ecophysiological theory, in the sense that survival is the most important feature to conserve of life history to achieve this objective, organisms can adopt *trade-offs*, which implies diminishing other biological functions, such as mobility, sexual activity, fecundity and even growth, in order to survive stressful events (Dodson and Hanazato, 1995).

Environmental contamination by antifungals exposes non-target organisms to the urgency of responding quickly and efficiently to events of stress, been forced to balance different energy demands. In this sense, the need to eliminate a toxic substance can break the balance between the different components of the energy budget, causing changes in population dynamics. According to Sibly and Calow, (1989), it can be established a compromise between the ability to survive the toxic, the growth rate and the fecundity. On the other hand, Calow and Sibly, (1990) and Stearns, (1993) reported that specimens generally do not provide resources to all the functions that can be involved in a stress situation. However, such imbalances can have relevant ecological consequences at the population, community and ecosystem levels (Fleeger *et al*., 2003; Diamond and Harrad, 2009).

CONCLUSION

The results obtained in the present work bring an update of the toxicity of antifungal compounds commonly used in the treatment of woods and leathers and remark the relevance of the development of novel avenues for the synthesis of ecofriendly molecules Gem-3e, with lower toxicity for people and the environment (Murguía *et al.*, 2019). The application of ecofriendly chemical compounds, Gem-3e compound, in different industrial processes, allows to get closer to cleaner production, with the aim to prevent pollution by replacing the hard-toxic chemical processes that negatively impact the environment with others that are less polluting. It is important to keep in mind that the release of pollutants to the environment (including the accidental release) is an indication of inefficient production. Therefore, the development of preventive strategies involves an increase of economic competitiveness and environmental quality, which are directly related in a circular strategy: by improving, one improves the other and this is one of its greatest advantages over corrective eco-strategies.

ACKNOWLEDGEMENTS

This work was supported by Agencia Nacional de Promoción Científica y Tecnológica (ANPCyT) (Project code: PICT 2016-4607), Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET) (Project code: PIP 2015-024) and Universidad Nacional del Litoral (UNL) (Project code: CAID 2016-107). Authors thank for their financial support and contribution to the development of this work.

REFERENCES

American Public Health Association (APHA). 1998. *Standard Methods for the Examination of Water and Wastewater.* 20th eds., Washington D.C. United States. Ap. 8010G

Cairns, J. and Pratt, J.R. 1989. The scientific basis of bioassays. In: Munawar M., Dixon G., Mayfield C.I., Reynoldson T., Sadar M.H. (eds) *Environmental Bioassay Techniques and their Application.* Developments in Hydrobiology. Vol. 54. Springer, Dordrecht

Calow, P. and Sibly, R.M. 1990. A physiological basis of population processes: ecotoxicological implications. *Functional Ecology*. 4 : 283–288.

Depledge, M.H. 1989. Observations on the feeding behaviour of *Gaetice depressus* (De Haan) (Grapsidae: Varuninae) with special reference to suspension feeding. *Marine Biology*. 100 : 253-259.

Diamond, M.S. and Harrad, S.J. 2009. The chemicals that will not go away: implications for human exposure to reservoirs of POPs. In: S.J. Harrad (Ed.) *Persistent Organic Pollutants: Current Issues and Future Challenges*, Wiley, Chichester, UK.,

Dodson, S. and Hanazato, T. 1995. Commentary on effects of anthropogenic and natural organic chemicals on development, swimming behavior, and reproduction of *Daphnia*, a key member of aquatic ecosystems. *Environ Health Persp*. 103 (41) : 7–11.

Fernández-Alba, A.R., Hernando, M.D., Piedra, L., Chisti, Y. 2002. Toxicity evaluation of single and mixed antifouling biocides measured with acute toxicity bioassays. *Anal Chim Acta.* 456 : 303–312.

Fleeger, J.W., Carman, K.R. and Nisbet, R.M. 2003. Indirect effects of contaminants in aquatic ecosystems. Review. *The Science of the Total Environment*. 317 : 207–233.

Guilhermino, L., Diamantino, T., Silva M.C. and Soares A.M.V.M. 2000. Acute Toxicity Test with *Daphnia magna*: An alternative to mammals in the prescreening of chemical toxicity? *Ecotoxicology and Environmental Safety.* 46 (3) : 357–362.

Hait, S.K. and Moulik, S.P. 2003. Gemini surfactants: A distinct class of self-assembling molecules. *Current Science*. 82 (9) : 1101-1111.

InfoStat, 2004. Grupo Infostat, FCA. Universidad Nacional de Córdoba. 1ra ed. Editorial Brujas, Argentina.

Ingersoll, C.G. and MacDonald, D.D. 1999. An assessment of sediment injury in the West Branch of the Grand Calumet River, vol. 1. US Geological Survey, Columbia, MO, MacDonald Environmental Sciences Ltd., Ladysmith, British Columbia, 161 pp

Kergaravat, S.V., Gagneten, A.M. and Hernandez, S.R. 2018. Development of an electrochemical method for quinolones detection: Application to cladoceran ecotoxicity studies. *Microchemical Journal.* 141 : 279–286.

Lapresta-Fernandez, A., Fernandez, A. and Blasco, J. 2012. Nanoecotoxicity effects of engineered silver and gold nanoparticles in aquatic organisms. *Trends in Analytical Chemistry*. 32 : 40-59.

Machado, M.D. and Soares, E.V.2019. Sensitivity of freshwater and marine green algae to three compounds of emerging concern. *J Appl Phycol*. 31 (1) : 399-408.

Machuca, L.M., Reno, U., Plem, S.C., Gagneten, A.M., Murguía, M.C. 2015. *N*-Acetylated Gemini Surfactants: Synthesis, Surface-Active Properties, Antifungal Activity, and Ecotoxicity Bioassays. *Adv Chem Eng Sci.* 5 : 215-224.

Menger, F.M. and Littau, C.A. 1993. Gemini surfactants: A new class of self-assembling molecules. *J Am Chem Soc.* 115 : 10083-10090.

Menger, F.M. and Keiper, J. 2000. Gemini Surfactants. *Angew Chem, Int. Ed*. 39 : 1906-1920.

Menger, F.M., Keiper, J.S. and Azov, V. 2000.Gemini Surfactants with Acetylenic Spacers. *Langmuir*. 16 (5): 2062–2067.

Murguía, M.C. and Grau, R.J. 2001. Synthesis of new pentaerythritol-based gemini surfactants. *Synlett*. 8 : 1229-1232.

Murguía, M.C., Cabrera, M.I., Guastavino, J.F. and Grau, R.J. 2005. New oligomeric surfactants with multiple-ring spacers: synthesis and tensioactive properties. *Colloids and Surfaces A: Physicochemical and Engineering Aspects*. 262 : 1-7.

Murguía, M.C., Machuca, L.M. and Fernandez, M.E. 2019. Cationic gemini compounds with antifungal activity and wood preservation potentiality. *Journal of Industrial and Engineering Chemistry*. 72 : 170-177.

Murguía, M.C., Machuca, L.M., Lurá, M.C., Cabrera, M.I. and Grau, R.J. 2008. Synthesis and properties of novel antifungal gemini compounds derived from *N*-acetyldiethanolamines. *Journal of Surfactants and Detergents*. 11 : 223-230.

Nawrocki, S. T., Drake, K. D., Watson, C. F., Foster, G. D., Maier, K. J. 2005. Comparative Aquatic Toxicity Evaluation of 2-(Thiocyanomethylthio) benzothiazole and Selected Degradation Products Using *Ceriodaphnia dubia. Arch. Environ. Contam. Toxicol*. 48: 344–350.

Organisation for Economic Cooperation and Development (OECD). 2012. Guideline for the Testing of chemicals. 211. Adopted: 2 October 2012. Paris, France.

Orlita, A. 2004. Microbial biodeterioration of leather and its control: a review. *International Biodeterioration & Biodegradation*. 53 : 157–163.

Pianka, E. 1982. *Ecología Evolutiva.* Omega. Barcelona, España, 365.

Reno, U., Gutierrez, M.F., Longo, M., Vidal, E., Regaldo, L., Negro, A., Mariani, M., Zalazar, C. and Gagneten, A.M. 2015. Microcrustaceans: biological models to evaluate a remediation process of glyphosate -based formulations. *Water Air Soil Pollut*. 226 : 349.

Reno, U., Regaldo, L. and Gagneten, A.M. 2016. Efectos subletales de cuatro formulaciones de glifosato sobre *Daphnia magna* y *Ceriodaphnia dubia* (Crustacea, Cladocera). *Natura Neotropicalis*. 47 : 7-20.

Reno, U., Doyle S., Momo, F., Regaldo, L. and Gagneten A.M. 2018. Effects of glyphosate formulations on population dynamics of freshwater microcrustaceans. *Ecotoxicology Special Issue: Pesticides*. 27 (7) : 784-793.

Sanchez-Hernandez, J.C., Ríos, J.M. and Attademo, A.M. 2018. Response of digestive enzymes and esterases of ecotoxicological concern in earthworms exposed to chlorpyrifos-treated soils. *Ecotoxicology.* 27 : 890-899.

Sibly, R.M. and Calow, P. 1989. A life–cycle theory of responses to stress. *Biol. J. Linn. Soc*. 37 : 101–116.

Stearns, S. 1993. *The Evolution of Life Histories.* Oxford University Press. United Kindom, 249.

Turquet, J., Quiniou, F., Delesmon, R. and Durand, G. 2010. ERICOR Evaluation du risque « pesticides » pour les récifs coralliens de La Réunion [ERICOR risk assessment of pesticides to coral reefs de La Reúnion]. La Reunión, Republique Française.

Wieczerzak, M., Kudlak, B. and Namieœnik, J. 2016. Bioassays as one of the green chemistry tools for assessing environmental quality: A review. *Environment International.* 94 : 341-361.

Woch, J., I³owska, Z., Hordyjewicz-Baran, S., Arabasz, B., Kaczmarczyk, R., Grabowski, M., Libera, A., Dworak, A. and Trzebicka, B. 2018. Aqueous solution behaviour and solubilisation properties of octadecyl cationic gemini surfactants and their comparison with their amide gemini analogues. *Soft Matter*. 14 : 75.