Ecotoxicological examination of some spirohydantoins and their derivatives towards Black Sea Mussel (*Mytilus galloprowincialis*)

Donyo Ganchev, Marin Marinov, Petja Marinova, Stefan Krustev, Milena Zlateva, Nadezhda Atanasova, Angel Nikolov, Neyko Stoyanov

Ecotoxicological examination of some spirohydantoins and their derivatives towards Black Sea Mussel (Mytilus galloprowincialis): *This article represent an investigation conducted in order to be revealed the eventual toxic action of cyclopentanespiro-5-hydantoin, cyclohexanespiro-5-hydantoin, cyclopentanespiro-5-(2,4-dithiohydantoin) and 1-aminocyclohexanecarboxylic acid to Black Sea Mussel (Mytilus galloprowincialis) which is the most economic important shellfish species for the region of Black Sea. Shellfish species are famous for their sensitiveness to presence of toxicants in the environment due to their specific way of life, that's why, the ecotoxicological investigation especially with economic important species like Black Sea Mussel are extremely significant in the area of ecology, ecotoxicology and fishery.*

Key words: *Mytilus galloprowincialis, Spirohydantoins.*

INTRODUCTION

Black sea mussel (*Mytilus galloprowincialis*) is one of the most perspective novel food source with proteins content equal to the cattle meat. The mussels are also important natural bio filter – at 17°C temperature of the water, one mussel is able to filter almost 3 litters sea water per hour. During recent years in Bulgaria is the increasing interest to this animal as profitable breeding culture. However due to the industrial contamination of Black Sea there is serious obstacles in realization of mussels farms.

The goal of this investigation is to be reveal the acute toxicity of some synthetic compounds: cyclopentanespiro-5-hydantoin (CPSH), cyclohexanespiro-5-hydantoin (CHSH), cyclopentanespiro-5-(2,4-dithiohydantoin) (CPSDTH) and 1-aminocyclohexanecarboxylic acid (ACHCA), which are also in the process of screening for biocide activity.

RESULTS AND DISCUTIONS

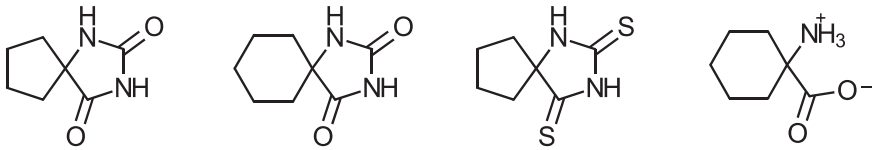
1. Test animals

Commercially available *Mytilus galloprovincialis* from a mussel farm situated in Bulgarian Black Sea coast were purchased. Mussels of similar size 7-8 cm were placed in aquarium filled with natural sea water received from mussel farm under continuously aerated conditions, temperature of 25°C and natural light. The mussels were acclimatized in laboratory conditions for at least 7 days before the beginning of the experiments and were fed with approximately 0.05 g powdered Spirulina every day [1].

2. Synthetic compounds

All used chemicals were purchased from Merck and Sigma-Aldrich. The initial cyclopentanespiro-5-hydantoin (CPSH, Figure 1a) and cyclohexanespiro-5-hydantoin (CHSH, Figure 1b) were synthesized *via* the Bucherer-Lieb method [2]. The cyclopentanespiro-5-(2,4-dithiohydantoin) (CPSDTH, Figure 1c) was synthesized in accordance to Marinov et. al. [3]. The 1-aminocyclohexanecarboxylic acid (ACHCA, Figure 1d) was obtained according to Stoyanov and Marinov [4]. The melting points were determined with a Koffler apparatus and with a digital melting point apparatus SMP 10. The elemental analysis data were obtained with an automatic analyzer Carlo Erba 1106. IR spectra were taken on spectrometers Bruker-113 and Perkin-Elmer FTIR-1600 in KBr discs. NMR spectra were taken on a Bruker DRX-250 spectrometer, operating at 250.13 and 62.90 MHz for ^{1}H and ^{13}C, respectively, and on a Bruker Avance II + 600 MHz spectrometer, operating at 600.130 and 150.903 MHz for ^{1}H and ^{13}C, respectively, using the standard Bruker software. Chemical shifts were referenced to tetramethylsilane (TMS). Measurements were carried out at ambient temperature.

All the products obtained were characterized by physicochemical parameters, IR and NMR spectral data. The results obtained from these analyses are identical with the previously published in the literature [3-5].



CPSH a) CHSH b) CPSDTH c) ACHCA d)

Figure 1

Ten concentrations of the tested compounds were prepared using the natural sea water from aquarium. The saturated concentrations of the compounds in the sea water were as follows: CPSH – 1 %, CHSH – 0.1 %, CPSDTH – 0.025 %, ACHCA – 2 %. The mussels were acclimatized in laboratory conditions for at least 5 days before the beginning of experiments. Healthy mussels were placed in separated mini aquariums filled with test solution with volume 5 litters and mussel density - 10 mussel / aquarium at 12 hours photoperiod daily [6]. The water was changed every 12 h and was spiked with test solutions after each renewal

The general condition of the animals and the mortalities were recorded daily and generally after 96 h (4 days) according to OEDC standard for performing acute toxicity fish tests [7].

3. Mussel mortality

After finishing the test, the general conditions of the tested animals - reaction to stimuli, excretion of mucus, attachment to glass-walls were visually observed including with digital microscope. The percents of mortality (response to current compound) were calculated using Abbot's formula [8].

4. Statistical analisys

On base of mussel mortality LD_{05} (NOEL) and LD_{50}, were calculated for CPSH using R language for statistical computing [9] and R packages DoseFinding [10] with logistic model at 95 % confidence level with AIC coefficient of the model = 75.7166.

Non-linear regression modelling was conducted by R language DoseFinding packages function fitDRModel() in order to be determined values of LD_x (LD_{05} and LD_{50}) which are as follows:

- LD_{05}= 0.17 %
- LD_{50}= 0.57 %

The dose-response curve of CPSH created by R language function plot() with main argument fitDRModel() function is presented on Figure 2.

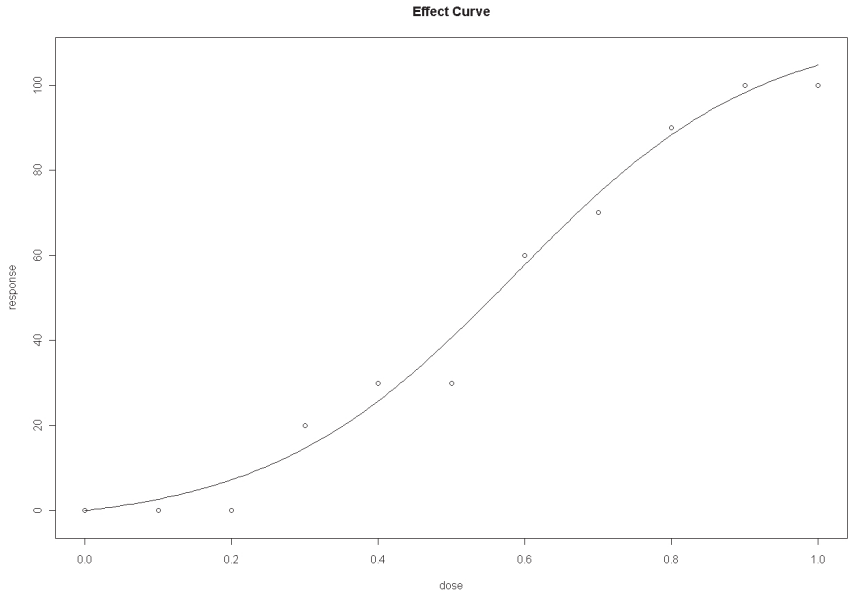


Figure 2. Dose-response curve of CPSH

CONCLUSIONS

In all tested variants except CPSH were not observed any toxic manifestation on mussels. The results from experiment show that in highest possible concentration CHSH, CPSDTH and ACHCA do not cause black sea mussel mortality. However the tested compound CPSH is poetically dangerous for mussels expressing extraordinary toxicity according to them with LD_{50}= 0.57 % (NOEL (LD_{05}) = 0.17 %).

Acknowledgements: Financial support by the Agricultural University – Plovdiv, Bulgaria (Contract 06-12) is gratefully acknowledged. We are grateful also to Mr. G. Marinov, Sofia and Mr. V. Gergov, Burgas, for stimulating discussions.

REFERENCES

[1] Emmanouil,Ch., K.M.Kasiotis, K.Machera. Bioaccumulation of thiram in Mytilus galloprovincialisand its effect on different tissues. Hellenic Plant Protection Journal, 2011, 4, 57-69.

[2] Bucherer,H.T., V.Lieb. Über die Bildung substituierter Hydantoine aus Aldehyden und Ketonen. Synthese von Hydantoinen. J. Prakt. Chem., 1934, 141, 5-43.

[3] Marinov,M., S.Minchev, N.Stoyanov, G.Ivanova, M.Spassova, V.Enchev. Synthesis, spectroscopic characterization and ab initio investigation of thioanalogues of spirohydantoins. Croat. Chem. Acta., 2005, 78, 9-16.

[4] Stoyanov,N., M.Marinov. Two Methods for spirothiohydantoin synthesis. Acta Chim. Slov., 2012, 59 (3), 680-685.

[5] Enchev,V., N.Stoyanov, V.Mateva, J.Popova, M.Kashchieva, B.Aleksiev, M.Mitewa. Copper (II) complexes of spirohydantoins. Synthesis, quantum-chemical, and spectroscopic study. Struct. Chem., 1999, 10 (5), 381-385.

[6] Emmanouil,C., S.Kypriotakis, A.Kungolos, K.Machera. Effects of Thiram or MCPA Acid on Mussel Gill DNA. Discussion Paper Series, 2008 14 (24), 455-468.

[7] OECD № 203 Guideline for testing of chemicals. Fish, acute toxicity test. Adopted by Council on 17.07.1992.

[8] Abbot,S. A method for computing the effectiveness of an insecticide. Journal of Economoc Enthomology. 1925, 18, 367-271.

[9] R Development Core Team (2011). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0. URL http://www.R-project.org/.

[10] Bornkamp,B., J.Pinheiro, F.Bretz (2012). DoseFinding: Planning and Analyzing Dose Finding experiments. R package version 0.6-1. http://CRAN.R-project.org/package=DoseFinding.

About the autors:

Donyo Ganchev, PhD, Faculty of Plant Protection and Agroecology, Agricultural University – Plovdiv, e-mail: donyo@abv.bg.

Marin Marinov, PhD, Faculty of Plant Protection and Agroecology, Agricultural University – Plovdiv, e-mail: m_n_marinov@abv.bg.

Petja Marinova, PhD, Faculty of Chemistry, University of Plovdiv, e-mail: marinova@uni-plovdiv.bg.

Stefan Krustev, Assoc. Prof., PhD, Faculty of Plant Protection and Agroecology, Agricultural University – Plovdiv, e-mail: krust@abv.bg.

Milena Zlateva, student, Faculty of Plant Protection and Agroecology, Agricultural University – Plovdiv, e-mail: a_v_r_i_l@abv.bg.

Nadezhda Atanasova, student, Faculty of Plant Protection and Agroecology, Agricultural University – Plovdiv, e-mail: nadejda1988@abv.bg.

Angel Nikolov, Assoc. Prof., PhD, Faculty of Plant Protection and Agroecology, Agricultural University – Plovdiv, e-mail: nikolaev_au@abv.bg

Neyko Stoyanov, Assoc. Prof., PhD, Department of Chemistry and Chemical Technology, University of Ruse – Branch Razgrad, e-mail: nstoianov@uni-ruse.bg.

The paper is reviewed.