Journal scientific and applied research, vol. 10, 2016
Association Scientific and Applied Research
International Journal

Original Contribution ISSN 1314-6289

TOXICITY RESEARCH OF SOME SPIROHYDANTOIN DERIVATIVES TOWARDS LUMBRICUS TERRESTRIS

Donyo H. Ganchev,[a] Marin N. Marinov[b], Neyko M. Stoyanov[c]

[a] DEPARTMENT OF ECOLOGY AND ENVIRONMENTAL PROTECTION, FACULTY OF PLANT PROTECTION AND AGROECOLOGY, AGRICULTURAL UNIVERSITY – PLOVDIV
[b] DEPARTMENT OF GENERAL CHEMISTRY, FACULTY OF PLANT PROTECTION AND AGROECOLOGY, AGRICULTURAL UNIVERSITY – PLOVDIV
[c] DEPARTMENT OF CHEMISTRY AND CHEMICAL TECHNOLOGIES, "ANGEL KANCHEV" UNIVERSITY OF RUSE, RAZGRAD BRANCH

Abstract: *This paper presents an ecotoxicological examination of cyclopentanespiro-5-hydantoin, cyclopentanespiro-5-(2,4-dithiohydantoin) and 1-aminocyclopentane-1-carboxylic acid towards Lumbricus terrestris – the common earthworms which are extremely important for the ecological stability and fertility of soils.*

Keywords: *Toxicity, Cyclopentanespiro-5-hydantoin, Cyclopentanespiro-5-(2,4-dithiohydantoin), 1-Aminocyclopentane-1-carboxylic acid, Lumbricus terrestris.*

I. Introduction

Ecotoxicological tests with cyclopentanespiro-5-hydantoin and its derivatives towards Black Sea Mussel *(Mytilus galloprowincialis)* [1], *Planorbis planorbis* (Ram's Horn Snail) [2], *Sea lettuce (Ulva lactuca)* [3], some cultural and non-cultural plants [4], wheat [5], *Pseudocrossidium revolutum* [6], Mealy plum aphid (*Hyalopterus pruni*) and *Prunus cerasifera* [7], *Alternaria solani* and tomato plant varieties [8], Oleander aphid (*Aphis nerii*) and *Buddleja davidii* [9], *Spirogyra* sp. [10], oil yielding rose (*Rosa damascena Mill*) [11] have been performed in previous studies of ours. The cyclopentanespiro-5-(2,4-dithiohydantoin) has shown strong insecticidal activity against *Cladius pectinicornis* [12]. Fungicidal activity of cyclopentanespiro-5-hydantoin, cyclopentanespiro-5-(2,4-dithiohydantoin) and 1-aminocyclopentane-1-carboxylic acid towards *Blumeria graminis* f. sp. *tritici* has been established [13].

The current study is a continuation of the ecotoxicological examination of cyclopentanespiro-5-hydantoin, cyclopentanespiro-5-(2,4-dithiohydantoin) and 1-aminocyclopentane-1-carboxylic acid, performed in different directions with the aim to create novel plant protection products. The purpose of this study is to evaluate the effect of the above mentioned synthetic organic compounds towards *Lumbricus terrestris*.

II. Experimental

II.1. Synthetic compounds

All chemicals used were purchased from Merck and Sigma-Aldrich. The cyclopentanespiro-5-hydantoin (Figure 1) was synthesized *via* the Bucherer-Lieb method [14]. The cyclopentanespiro-5-(2,4-dithiohydantoin) (Figure 2) was synthesized in accordance with Marinov et al. [12] by a modified method of those reported by Curphey [15]. The 1-aminocyclopentane-1-carboxylic acid (Figure 3) was obtained in accordance with Stoyanov and Marinov [16].

All products obtained were characterized by physicochemical parameters, IR and NMR spectral data. The results obtained from these analyses are identical with those previously published in the literature [16-19].

The saturated concentrations of the compounds in water were prepared as follows:

- cyclopentanespiro-5-hydantoin – 0.01 %;
- cyclopentanespiro-5-(2,4-dithiohydantoin) – 0.025 %;
- 1-aminocyclopentanecarboxylic acid – 0.1 %.

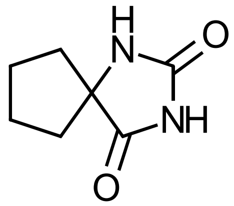


Figure 1. *Structural formula of cyclopentanespiro-5-hydantoin (Systematic name: 1,3-diazaspiro[4.4]nonane-2,4-dione)*

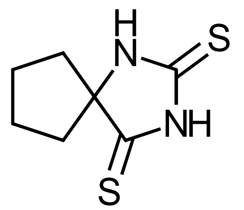


Figure 2. *Structural formula of cyclopentanespiro-5-(2,4-dithiohydantoin) (Systematic name: 1,3-diazaspiro[4.4]nonane-2,4-dithione)*

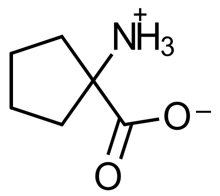


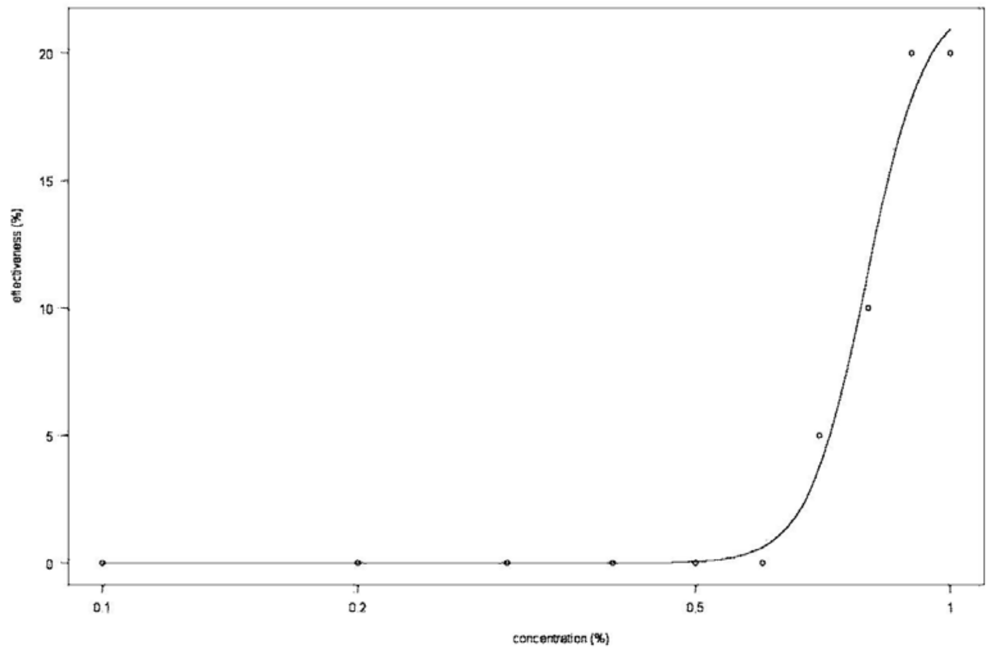
***Figure 3.** Structural formula of 1-aminocyclopentane-1-carboxylic acid*

II.2. Ecotoxicological tests

Conducted experiments were in accordance with test № 207 of the Organisation for Economic Co-operation and Development (OECD) [20] with test species *Lumbricus terrestris* – adult form with an individual weight between 300 and 600 mg. A filter paper test was performed with flat-bottomed glass vials of 8 cm length and 3 cm diameter, lined with filter paper cut slides. In each vial, 1 ml of the corresponding substance in given test concentration was pipetted and evaporated to dryness. In the control variant – 1 ml of distilled water was added. After drying, 1 ml of distilled water was added to each test variant to moisten the filter paper. Each vial was sealed with a cap or plastic film with a small ventilation hole. Each concentration of the given test substance was set in 10 replicates, where each replicate included 1 worm per vial. Tests were performed for 2 days (48 hours) and mortality was recorded on each 24 h – worms were considered as dead when they did not respond to a gentle mechanical stimulation to the front end. Worms were weighted before and after test and weight loss ratio was calculated as a percentage based on the Abbott's formula [21]. Dose-response modeling was performed using R language for statistical computing [22] and drc package [23] for estimation of NOAEC (No Observed Adverse Effect Concentration), LOAEC (Lowest Observed Adverse Effect Concentration) and LC_{50}.

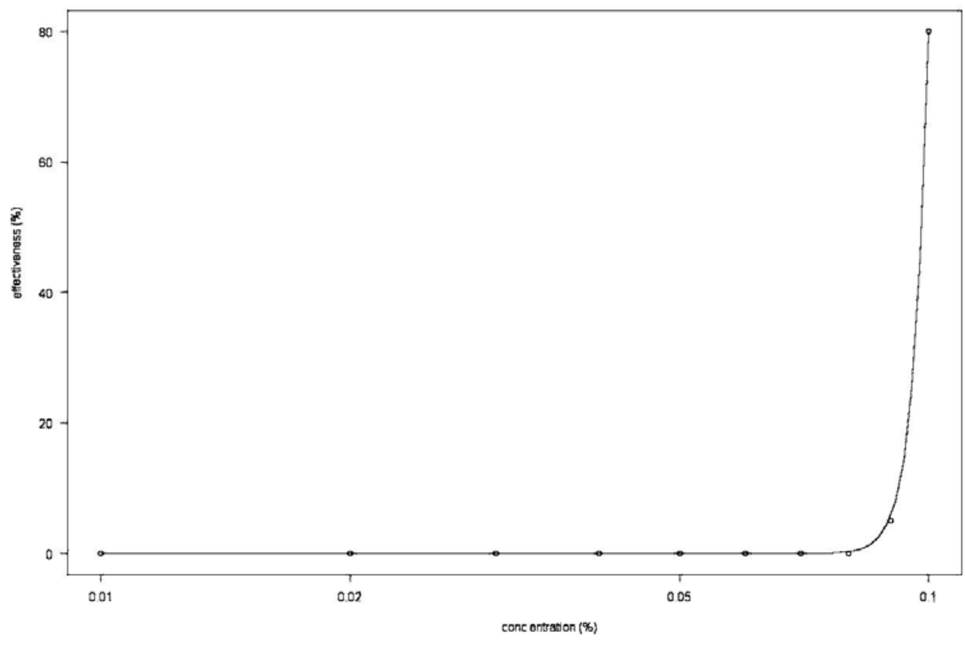
III. Results and discussion

The conducted test reveal that as per mortality rates recorded, the cyclopentanespiro-5-(2,4-dithiohydantoin) has absolutely no adverse effect at the saturated concentration in water – 0.025 %. Dose-response curves and toxicological data of the other two compounds are presented on the figures below:



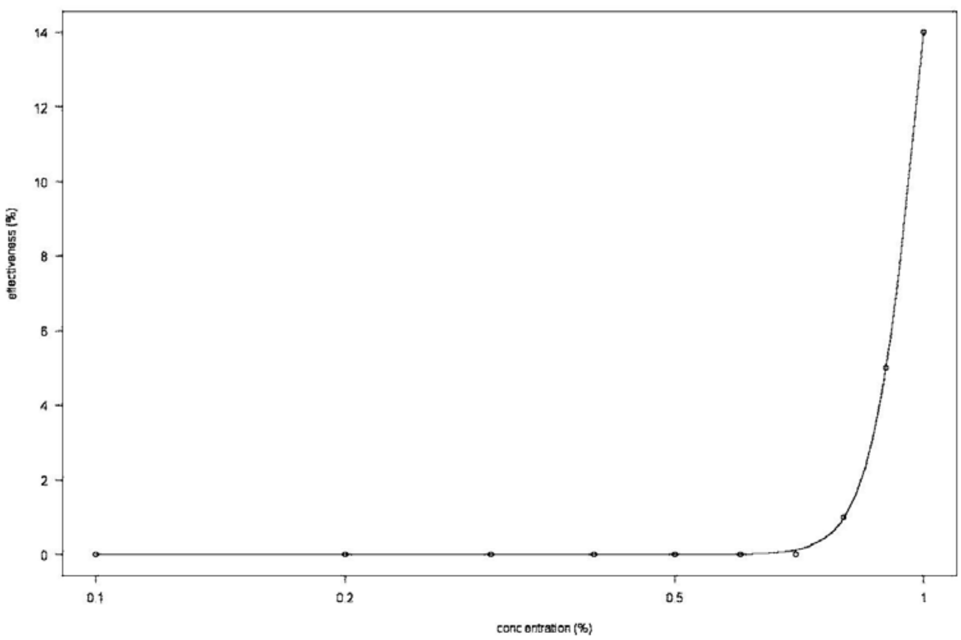
NOAEC = 0.62 %; LOAEC = 0.72 %; LC_{50} = 0.8 %.

Figure 4. *Dose-response curve of cyclopentanespiro-5-hydantoin*



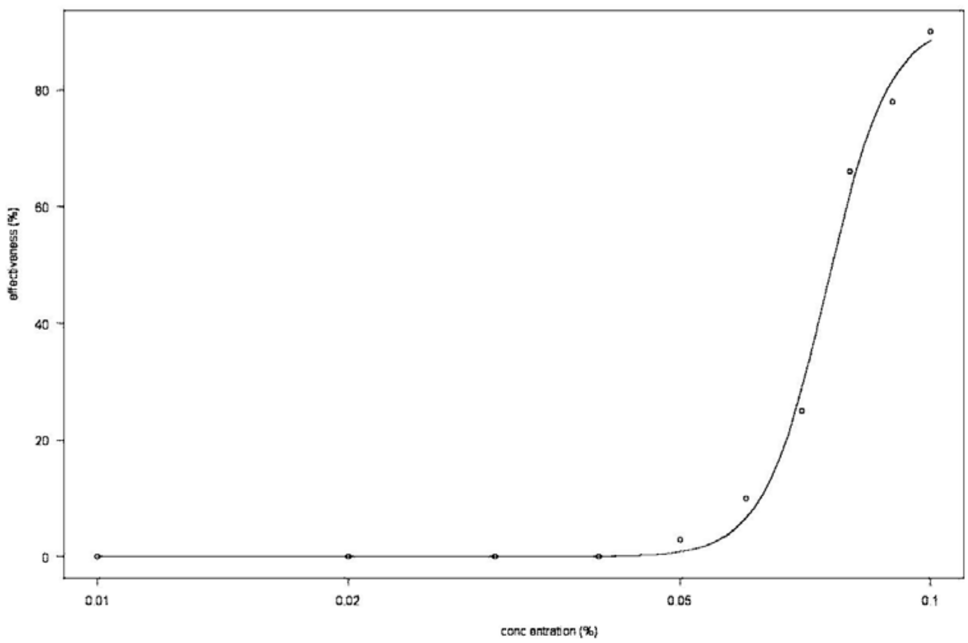
NOAEC = 0.07 %; LOAEC = 0.08 %; LC_{50} = 0.1 %.

Figure 5. *Dose-response curve of 1-aminocyclopentane-1-carboxylic acid*



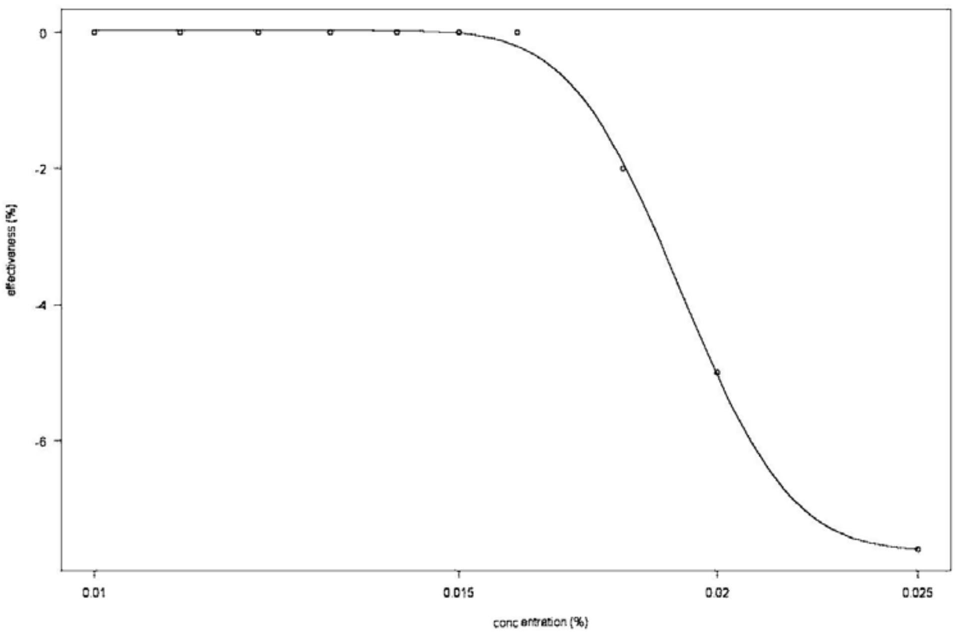
NOAEC = 0.8 %; LOAEC = 0.9 %; LC_{50} = 0.98 %.

Figure 6. *Dose-response curve of cyclopentanespiro-5-hydantoin (weigh)*



NOAEC = 0.05 %; LOAEC = 0.06 %; LC_{50} = 0.07 %.

Figure 7. *Dose-response curve of 1-aminocyclopentane-1-carboxylic acid (weigh)*



NOAEC = 0.016 %; LOAEC = 0.018 %; SC_{50} = 0.02 %.

Figure 8. *Dose-response curve of cyclopentanespiro-5-(2,4-dithiohydantoin) (weigh)*

It is obvious from the results obtained that cyclopentanespiro-5-hydantoin is much safer for earthworms than 1-aminocyclopentane-1-carboxylic acid. However, both compounds can be dangerous to tested animals at very low concentrations – LC_{50} between 0.1 and 0.8 % as per the weight loss ratio. As figure 8 shows, cyclopentanespiro-5-(2,4-dithiohydantoin) even has a stimulation effect on earthworm growth, where SC_{50} is a stimulation concentration 50 %. Data received from the weight loss ratio of the other compounds confirm a more severe effect of 1-aminocyclopentane-1-carboxylic acid as compared to cyclopentanespiro-5-hydantoin.

IV. Conclusion

The conducted trials reveal completely different effects of the spirohydantoin derivatives used on one test organism. While 1-aminocyclopentane-1-carboxylic acid and cyclopentanespiro-5-hydantoin have an inhibitory effect in various degrees, cyclopentanespiro-5-(2,4-dithiohydantoin) reveals a growth stimulation effect. This proves the necessity to perform ecotoxicological characterization of such chemical substances as opposed to the non-reliability of the Structure-Activity-Relation analysis.

V. Acknowledgements

Financial support by the Agricultural University – Plovdiv, Bulgaria (Contract 03-14) is gratefully acknowledged.

VI. References

[1]. Ganchev, D., M. Marinov, P. Marinova, S. Krustev, M. Zlateva, N. Atanasova, A. Nikolov, N. Stoyanov, 2012. Ecotoxicological examination of some spirohydantoins and their derivatives towards Black Sea Mussel (*Mytilus galloprowincialis*). University of Ruse "Angel Kanchev" Proc., 51 (9.2), 14-17.

[2]. Marinov, M., D. Ganchev, P. Marinova, S. Krustev, P. Penchev, M. Zlateva, N. Atanasova, N. Stoyanov, 2012. Acute toxicity of some spirohydantoins and their derivatives towards *Planorbis planorbis* (Ram's Horn Snail). University of Ruse "Angel Kanchev" Proc., 51 (9.2), 18-21.

[3]. Marinov, M. N., D. H. Ganchev, P. E. Marinova, P. N. Penchev, S. V. Krustev, M. R. Zlateva, N. I. Atanasova, N. M. Stoyanov, 2012. Ecotoxicological examination of acute toxicity of some spirohydantoins and their derivatives towards Sea lettuce (*Ulva lactuca*). J. Sci. Appl. Res., 2, 74-81.

[4]. Ganchev, D. H., M. N. Marinov, P. E. Marinova, S. V. Krustev, M. R. Zlateva, N. I. Atanasova, N. M. Stoyanov, 2012. *In vivo* phytotoxicological study of cyclopentanespiro-5-hydantoin and its derivatives towards some cultural and non-cultural plants. J. Sci. Appl. Res., 2, 82-89.

[5]. Ganchev, D., M. Marinov, A. Nikolov, P. Marinova, S. Krustev, M. Zlateva, N. Atanasova, N. Stoyanov, 2013. Phytotoxicity study of cyclopentanespiro-5-hydantoin and its derivatives towards wheat. Agric. Sci., 12, 103-107.

[6]. Ganchev, D. H., M. N. Marinov, S. V. Krustev, M. R. Zlateva, N. I. Atanasova, N. M. Stoyanov, 2013. Phytotoxicological study of some spirohydantoins and their derivatives towards *Pseudocrossidium revolutum*. J. Sci. Appl. Res., 3, 20-25.

[7]. Ganchev, D., M. Marinov, M. Zlateva, R. Prodanova, A. Nikolov, S. Krustev, N. Stoyanov, 2013. *In vivo* insecticidal activity of cyclopentanespiro-5-hydantoin and its two derivatives towards Mealy plum aphid (*Hyalopterus pruni*) and effect on *Prunus cerasifera*. University of Ruse "Angel Kanchev" Proc., 52 (10.2), 16-20.

[8]. Ganchev, D., M. Marinov, R. Prodanova, S. Krustev, M. Zlateva, N. Stoyanov, 2013. *In vitro* screening for fungicidal activity of cyclopentanespiro-5-hydantoin and its two derivatives towards *Alternaria solani* and effect on Tomato plant varieties. University of Ruse "Angel Kanchev" Proc., 52 (10.2), 57-60.

[9]. Marinov, M. N., D. H. Ganchev, P. E. Marinova, A. S. Nikolov, R. Y. Prodanova, S. V. Krustev, M. R. Zlateva, N. M. Stoyanov, 2013. *In vivo* insecticidal activity of cyclopentanespiro-5-hydantoin and its two

derivatives towards Oleander aphid (*Aphis nerii*) and effect on *Buddleja davidii*. J. Sci. Appl. Res., 4, 171-177.

[10]. Marinov, M. N., D. H. Ganchev, A. S. Nikolov, S. V. Krustev, N. M. Stoyanov, 2013. Ecotoxicological study of cyclopentanespiro-5-hydantoin and its derivatives towards *Spirogyra* sp. Scientific Researches of the Union of Scientists in Bulgaria – Plovdiv, XVI, 103-107.

[11]. Ganchev, D., M. Marinov, S. Krustev, P. Marinova, M. Zlateva, N. Atanasova, N. Stoyanov, 2014. Cyclopentanespiro-5-(2,4-dithiohydantoin): Synthetic pathways and phytotoxic examination towards oil yielding rose (*Rosa damascena Mill*). Bulg. J. Agric. Sci., 20, 293-296.

[12]. Marinov, M., D. Ganchev, P. Marinova, S. Krustev, N. Atanasova, M. Zlateva, N. Stoyanov, 2012. Synthesis of cyclopentanespiro-5-(2,4-dithiohydantoin) and *in vitro* insecticidal activity against *Cladius pectinicornis*. Bulg. J. Agric. Sci., 18 (6), 929-933.

[13]. Marinov, M., D. Ganchev, A. Nikolov, P. Marinova, S. Krustev, V. Madzharova, N. Stoyanov, 2013. *In vitro* fungicidal activity of cyclopentanespiro-5-hydantoin and its derivatives towards *Blumeria graminis* f. sp. *tritici*. Agric. Sci., 12, 97-101.

[14]. Bucherer, H. T., V. A. Lieb, 1934. Über die bildung substituierter hydantoine aus aldehyden und ketonen. Synthese von hydantoinen. J. Prakt. Chem., 141, 5-43.

[15]. Curphey, T. J., 2002. Thionation with the reagent combination of phosphorus pentasulfide and hexamethyldisiloxane. J. Org. Chem., 67, 6461-6473.

[16]. Stoyanov, N., M. Marinov, 2012. Two methods for spirothiohydantoin synthesis. Acta Chim. Slov., 59, 680-685.

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

[18]. Naydenova, E., N. Pencheva, J. Popova, N. Stoyanov, M. Lazarova, B. Aleksiev, 2002. Aminoderivatives of cycloalkanespirohydantoins: Synthesis and biological activity. Il Farmaco, 57 (3), 189-194.

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

[20]. OECD Guideline for testing of chemicals, 1984. № 207 “Earthworm, Acute Toxicity Tests”. http://www.oecd.org/chemicalsafety/risk-assessment/1948293.pdf.

[21]. Abbott, M. S., 1925. A method of computing the effectiveness of an insecticide. J. Econ. Entomol., 18, 265-267.

[22]. 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, http://www.R-project.org/.
[23]. Ritz, C., J. C. Streibig, 2005. Bioassay analysis using R. J. Statist. Software, Vol. 12, Issue 5.