Acute toxicity of some spirohydantoins and their derivatives towards *Planorbis planorbis* (Ram's Horn Snail)

Marin Marinov, Donyo Ganchev, Petja Marinova, Stefan Krustev, Plamen Penchev, Milena Zlateva, Nadezhda Atanasova, Neyko Stoyanov

Acute toxicity of some spirohydantoins and their derivatives towards Planorbis planorbis (Ram's Horn Snail): *The article represent an investigation with freshwater snail (Planorbis planorbis) for revealing the eventual acute toxic effect of cyclopentanespiro-5-hydantoin, cyclohexanespiro-5-hydantoin, cyclopentanespiro-5-(2,4-dithiohydantoin) and 1-aminocyclopentanecarboxylic acid. Planorbis species are very common air-breathing freshwater snails in Europe. They are commonly used as species for ecotoxicological test in order to be determined the eventual deleterious action of chemicals on freshwater invertebrates.*

Key words: *Planorbis planorbis, Spirohydantoins.*

INTRODUCTION

Planorbis planorbis (Ram's Horn Snail) is a species of air-breathing freshwater snail, an aquatic gastropod mollusk in the family *Planorbidae*. It is one of the most common freshwater snails in Europe, occurs in numerous water body types with a preference for standing water such as ponds, swamps or lakes with slow moving or stagnant waters. As typical freshwater invertebrate it is commonly used as test species for ecotoxicological investigations of various chemicals including pesticides [1, 2].

The aim of this study is to be reveal the acute toxicity of cyclopentanespiro-5-hydantoin (CPSH), cyclohexanespiro-5-hydantoin (CHSH), cyclopentanespiro-5-(2,4-dithiohydantoin) (CPSDTH) and 1-aminocyclopentanecarboxylic acid (ACPCA) towards *Planorbis planorbis*.

RESULTS AND DISCUTIONS

1. Test animals

Naturally occurring freshwater *Planorbis planorbis* individuals were collected from lake Srebarna, Bulgaria. Snails with similar size 5-6 mm shell diameter were placed in aquarium filled with natural lake water under continuously aerated conditions, temperature of 25°C and natural light.

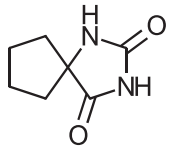
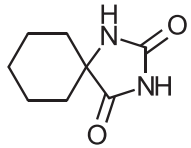
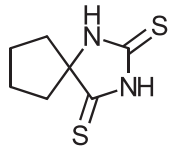
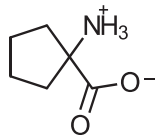
2. Synthetic compounds

All used chemicals were purchased from Merck and Sigma-Aldrich. The initial cyclopentanespiro-5-hydantoin (CPSH, Table 1) and cyclohexanespiro-5-hydantoin (CHSH, Table 1) were synthesized *via* the Bucherer-Lieb method [3]. The cyclopentanespiro-5-(2,4-dithiohydantoin) (CPSDTH, Table 1) was synthesized in accordance to Marinov et. al. [4]. The 1-aminocyclopentanecarboxylic acid (ACPCA, Table 1) was obtained according to Stoyanov and Marinov [5]. 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 [4-6].

The Attenuated Total Reflection FTIR (ATR) spectra were recorded with a VERTEX 70 FT-IR spectrometer (Bruker Optics). The ATR accessory is MIRacle with a one-reflection ZnSe element (Pike) and the stirred crystals of CPSH, CHSH, CPSDTH and ACPCA were pressed by an anvil to the reflection element; the spectra are from 4500 cm^{-1} to 600 cm^{-1} at resolution 2 cm^{-1} with 16 scans (see Table 1).

Table 1. Structures and ATR spectral data of the compounds

Compound	Structure	ATR spectral bands, cm^{-1}
cyclopentanespiro-5-hydantoin (CPSH)		3193, 3070, 2960, 2877, 2761, 1729, 1679, 1456, 1446, 1412, 1384, 1323, 1312, 1298, 1276, 1244, 1178, 1105, 1072, 1045, 1023, 1004, 949, 917, 887, 789, 748, 713, 643
cyclohexanespiro-5-hydantoin (CHSH)		3278, 3199, 3065, 2991, 2704, 2365, 1766, 1728, 1711, 1599, 1536, 1495, 1450, 1444, 1432, 1398, 1366, 1337, 1317, 1291, 1226, 1190, 1160, 1106, 1080, 1071, 1017, 1000, 983, 960, 915, 875, 851, 783, 764, 751, 732, 693, 669, 648, 639, 627, 616
cyclopentanespiro-5-(2,4-dithiohydantoin) (CPSDTH)		3138, 3058, 2933, 2924, 2845, 1602, 1539, 1453, 1446, 1424, 1383, 1349, 1327, 1306, 1283, 1270, 1260, 1230, 1216, 1202, 1156, 1125, 1108, 1089, 1046, 1018, 981, 958, 921, 892, 850, 841, 803, 772, 726, 664, 650, 628
1-aminocyclopentane-carboxylic acid (ACPCA)		3462, 3341, 3216, 2947, 2764, 2540, 2361, 2066, 1669, 1637, 1619, 1597, 1561, 1522, 1472, 1447, 1395, 1331, 1295, 1246, 1228, 1196, 1161, 1073, 1035, 1012, 965, 909, 882, 768

Ten concentrations of the tested compounds were prepared using the natural lake water from aquariums. The saturated concentrations of the compounds in the water were as follows: CPSH – 1 %, CHSH – 0.1 %, CPSDTH – 0.025 %, ACPCA – 0.1 %. Snails were placed in separated mini aquariums filled with test solution with volume 10 ml and snail density – 10 snails / aquarium at 12 hours photoperiod daily. 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. Snails mortality

After finishing the test, the immobilization the tested animals were visually observed with digital microscope [8]. The percents of mortality (response to current compound) were calculated using Abbot's formula.

4. Statistical analisys

On base of snail mortality (immobilization) LD_{05} (NOEL), LD_{25} (LOEL) and LD_{50}, were calculated using R language for statistical computing [9] and R packages drc [10] with logistic model at 95 % confidence level.

For CPSH compound model fitting the three-parameter logistic function with lower limit 0 was used by R language drc package function drm() for general model fitting function for concentration/dose/time-response models [10].

The values of LD_x (LD_{05}, LD_{25}, LD_{50}) were determined by ED() function, R language drc package [10] and are as follows:

- LD_{05} = 0.130752 %
- LD_{25}= 0.230346 %
- LD_{50}= 0.322668 %

The graphical presentation of dose-response modelling as dose-response curve (Figure 1) was created by R language function plot() with function drm() as main argument [10].

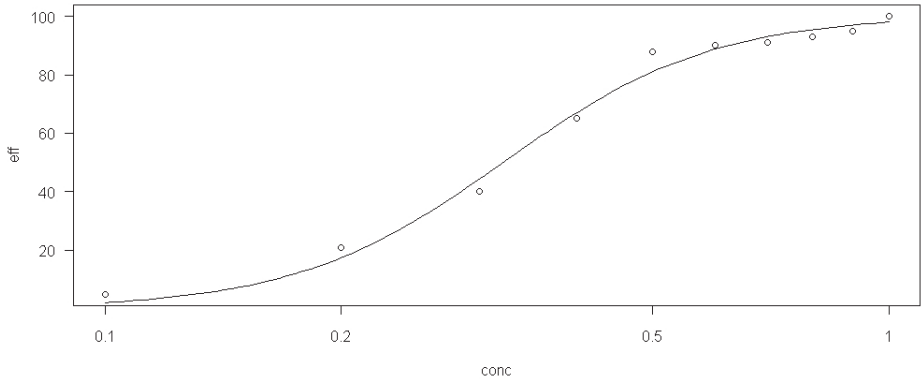


Figure 1. Dose-response curve of CPSH

For CPSDTH compound the same dose-response modeling by R language drc package was conducted. Figure 2 represent dose-response curve for this compound.

Received values of LD_x (LD_{05}, LD_{25}, LD_{50}) are as follows:

- LD_{05}= 7.8203e-07
- LD_{25}= 2.8945e-05
- LD_{50}= 2.4834e-04

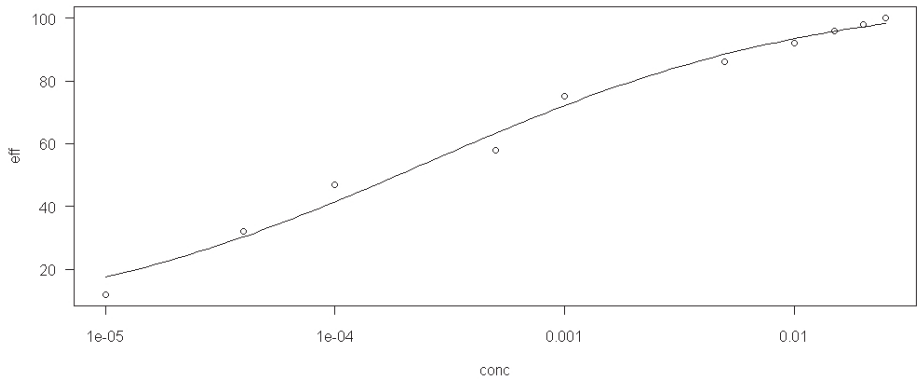


Figure 2. Dose-response curve of CPSDTH

CONCLUSIONS

All tested substances except CPSH and CPSDTH have not cause any toxic manifestation on snails at the saturated concentration of the compounds in water. However CPSH manifest acute toxic effect at relatively high concentration – LD_{50}= 0.322668 (3226.68 ppm). CPSDTH on the other side show that can be potentially

dangerous for the freshwater invertebrates with in LD_{50}=2.4834e-04 (24.834 ppm) concentration.

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About the autors:

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

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

Plamen Penchev, Assoc. Prof., PhD, Faculty of Chemistry, University of Plovdiv, e-mail: plamen@uni-plovdiv.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.

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.