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Original Contribution

ECOTOXICOLOGICAL EXAMINATION OF ACUTE TOXICITY OF SOME SPIROHYDANTOINS AND THEIR DERIVATIVES TOWARDS SEA LETTUCE (*ULVA LACTUCA*)

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Abstract: *This article presents an ecotoxicological study of acute toxicity deleterious effect of cyclopentanespiro-5-hydantoin, cyclohexanespiro-5-hydantoin, cyclopentanespiro-5-(2,4-dithiohydantoin) and 1-aminocyclopentanecarboxylic acid towards Sea lettuce (Ulva lactuca) conducted in accordance with international standards adopted by the Organisation for Economic Co-operation and Development (OECD). Dose-response modeling was carried out by R language for Statistical Computing, drc package.*

Key words: *spirohydantoins, Sea lettuce, Ulva lactuca, toxicity, drc, R language*

I. Introduction

It is well known that the hydantoins (imidazolidines) and their derivatives have application in the medicine and the clinical practice as aldose reductase inhibitors [1, 2]. They also possess antitumor [3], anticonvulsant, antiepileptic [4] and antiarrhythmic action [5].

Sea lettuce (*Ulva lactuca*) is bright green algae which is a common inhabitant of seas. The plant can be found attached to rocks and shells by a holdfast, but it is also commonly found free floating. Large volumes of sea lettuce often indicate high levels of nutrient pollution. It is often found in areas where sewage runoff is heavy. That's why sea lettuce is used as a standard indicator species to monitor pollution trends for indication of large amounts of nutrients [6].

During recent years there even are projects for using *Ulva lactuca* as human and animal food source which makes this plant potentially significant for agricultural economy.

As a common sea macroalga sea lettuce is a typical organism for ecotoxicological research of eventual deleterious action of

chemical substances according to the marine plants [7-9].

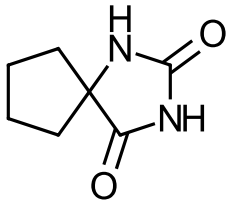
During this study we examine the presence or lack of acute toxic action of cyclopentane-spiro-5-hydantoin, cyclohexane-spiro-5-hydantoin, cyclopentane-spiro-5-(2,4-dithiohydantoin) and 1-aminocyclopentanecarboxylic acid towards sea lettuce which is one of the most common and widely spread alga for the region of the Bulgaria Black Sea Coast and Aquatoria.

II. Materials and methods

II.1. Synthetic compounds

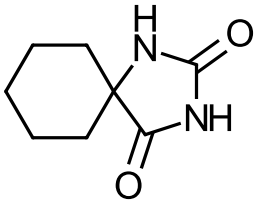
All chemicals used were purchased from Merck and Sigma-Aldrich. The cyclopentanespiro-5-hydantoin (CPSH, Fig. 1a) and cyclohexanespiro-5-hydantoin (CHSH, Fig. 1b) were synthesized *via* the Bucherer-Lieb method [10]. The cyclopentanespiro-5-(2,4-dithiohydantoin) (CPSDTH, Fig. 1c) was synthesized in accordance with Marinov et. al. [11]. The 1-aminocyclopentanecarboxylic acid (ACPCA, Fig. 1d) was obtained in accordance with Stoyanov and Marinov [12]. Melting points were determined with a Koffler apparatus and with a digital melting point apparatus SMP 10. 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.

a)



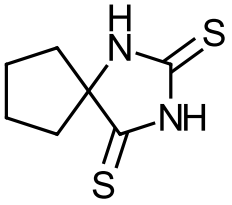
cyclopentanespiro-5-hydantoin
(CPSH)

b)



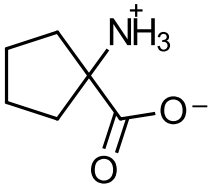
cyclohexanespiro-5-hydantoin
(CHSH)

c)



cyclopentanespiro-5-(2,4-dithiohydantoin)
(CPSDTH)

d)



1-aminocyclopentane-carboxylic acid
(ACPCA)

Fig. 1. *Structures of the compounds*

All 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 [11-13].

In addition, in this paper we present Raman and DRIFT spectral data for the compounds. The Raman spectra of the compounds (the stirred crystals placed in aluminium disc) were measured on RAM II (Bruker Optics) with a focused laser beam of 200 mW (for CPSH, CHSH and ACPCA) and 100 mW (for CPSDTH) power of Nd:YAG laser (1064 nm) from 4000 cm^{-1} to 51 cm^{-1} at resolution 2 cm^{-1} with 25 scans. Their Diffuse Reflectance FTIR (DRIFT) spectra were recorded with a VERTEX 70 FT-IR spectrometer (Bruker Optics). DRIFT accessory used is Praying Mantis™ (Harrick Scientific). Crystals of the compounds were stirred with KBr; the spectra are from 4000 cm^{-1} to 400 cm^{-1} at resolution 2 cm^{-1} with 49 scans. The spectral data obtained are presented in Table 1 below.

II.2. Ecotoxicological tests

Conduction of ecotoxicological tests was in accordance with OECD Standard № 201 – Freshwater Alga and Cyanobacteria, Growth Inhibition Test [14].

Natural occurring Ulva lactuca algae were collected from the Bulgarian Black Sea Coast, the Burgas beach region.

Ten concentrations using natural sea water of each compound were prepared for the purpose of the test. Saturated concentrations of the compounds in water were as follows:

- CPSH – 1 %;
- CHSH – 0.1 %;
- CPSDTH – 0.025 %;
- ACPCA – 0.1 %.

The test design included three replicates at each test concentration plus control variant.

The duration of test was 72 h (3 days). At the end of the test, tested plants were visually observed for phytotoxicological manifestations as whitening, discoloring, deforma-tions, necrosis and other signs. Plant biomass was measured before and after test. Based on this, a dose–response modeling was conducted for determination of NOAEC (LD_{05}), LOAEC (LD_{25}) and LD_{50} *via* R language for statistical computing (R Development Core Team (2011). R: A language and environment for statistical computing [15] and R packages drc [16].

Physical/chemical properties of tested compounds, required for such ecotoxicological studies, were estimated by using the US EPA EPI (Estimation Programs Interface) Suite™ – Windows®-based suite of physical/chemical property and environmental fate estimation programs developed by the EPA's Office of Pollution Prevention Toxics and Syracuse Research Corporation (SRC) [17].

Table 1. *Raman and DRIFT spectral data for the compounds*

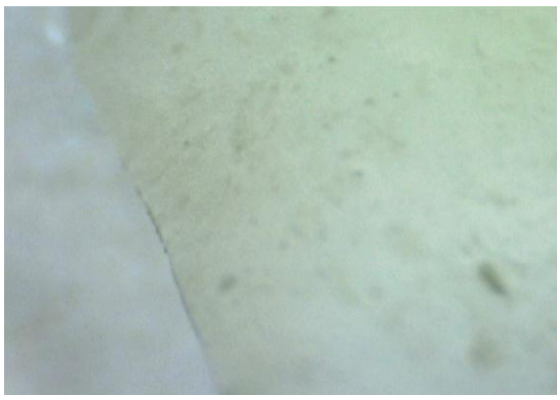
Compound	Raman spectral bands, cm^{-1}	DRIFT spectral bands, cm^{-1}
CPSH	3011, 2962, 2924, 2878, 2641, 1731, 1703, 1479, 1454, 1446, 1434, 1420, 1317, 1249, 1207, 1180, 1105, 1075, 1037, 1020, 1007, 948, 919, 890, 834, 776, 725, 642, 455, 350, 329, 204	3899, 3439, 3413, 3207, 2961, 2877, 2761, 2490, 2351, 2051, 1776, 1734, 1680, 1446, 1415, 1314, 1245, 1177, 1073, 1046, 1023, 1004, 949, 917, 888, 790, 750, 717, 645, 471, 445, 411
CHSH	3072, 2996, 2936, 2878, 2520, 1754, 1600, 1582, 1452, 1401, 1358, 1233, 1195, 1157, 1106, 1071, 1028, 1018, 1000, 912, 877, 788, 764, 752, 675, 640, 618, 536, 484, 350, 243	3955, 3869, 3722, 3656, 3477, 3205, 3067, 2990, 2937, 2811, 2706, 2514, 2471, 2421, 2375, 2320, 2116, 2033, 1983, 1952, 1883, 1769, 1720, 1599, 1539, 1496, 1444, 1400, 1367, 1317, 1291, 1231, 1191, 1161, 1106, 1072, 1018, 1000, 983, 961, 915, 876, 852, 786, 766, 752, 735, 696, 668, 640, 629, 537, 482, 453, 448, 411
CPSDTH	3147, 2939, 2925, 2845, 2677, 2098, 1529, 1437, 1397, 1357, 1300, 1268, 1232, 1214, 1162, 1130, 1111, 1091, 1042, 1021, 981, 950, 921, 852, 773, 665, 628, 585, 525, 458, 442, 351, 324, 271, 241, 220	3882, 3848, 3808, 3772, 3693, 3148, 3058, 2934, 2924, 2845, 2798, 2696, 2660, 2555, 2487, 2434, 2348, 2320, 2285, 2253, 2219, 2192, 2112, 2075, 1967, 1881, 1788, 1654, 1543, 1478, 1453, 1446, 1428, 1384, 1350, 1328, 1306, 1285, 1270, 1260, 1232, 1217, 1203, 1161, 1128, 1109, 1090, 1048, 1019, 981, 959, 921, 892, 851, 842, 803, 772, 733, 665, 652, 628, 584, 527, 496, 465, 443, 421, 406
ACPCA	2962, 2948, 2877, 2541, 2159, 1710, 1591, 1448, 1391, 1295, 1263, 1223, 1149, 1038, 1017, 954, 884, 788, 719, 557, 496, 435, 407, 353, 304	3855, 3745, 3467, 2961, 2541, 2079, 1740, 1673, 1577, 1527, 1475, 1447, 1403, 1331, 1229, 1196, 1035, 1013, 960, 882, 770, 579, 555, 511, 470, 438, 419, 413, 406

III. Results and discussions

All tested compounds, except CPSH, did not manifest any phytotoxic signs at their saturated concentrations in water. ANOVA analysis conducted with R language for statistical computing in respect to plant biomass did not show significant differences ($p > 0.05$ at 95 % confidence level) between tested variants and control.

However, the CPSH substance showed extremely high deleterious effect on plants at the saturated concentration in water – 1 %, at the form of completely discoloring and whitening of the plants – Fig . 2.

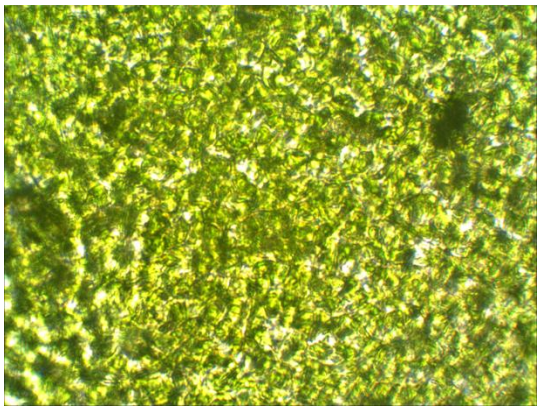
a)



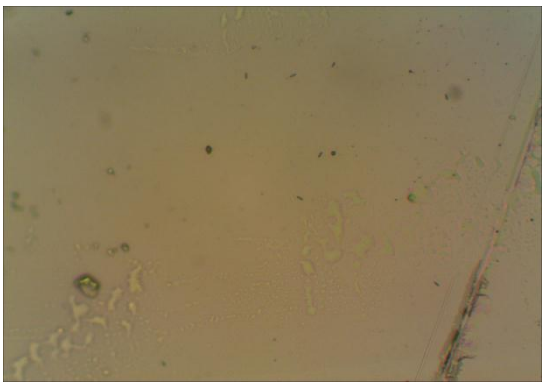
b)

Fig. 2. *Pictures taken with Chronos USB 2.0 digital microscope (150x):*
a) control variant;
b) CPSH (1 %) treated variant

Fig. 3. shows control variant and CPSH variant at 1 % concentration under light inverter microscope (400x) magnification:



a)



b)

Fig. 3. *Pictures taken with light inverter microscope (400x) – Boeco BIB-100:*
a) control variant;
b) CPSH (1 %) treatment variant

A dose-response curve describing the acute toxic action of CPSH compound is presented on Fig. 4.

Calculated values of NOAEC (LD_{05}), LOAEC (LD_{25}) and LD_{50} are as follows:

- **NOAEC (LD_{05}) = 0.165 %;**
- **LOAEC (LD_{25}) = 0.427 %;**
- LD_{50} = 0.750 %.

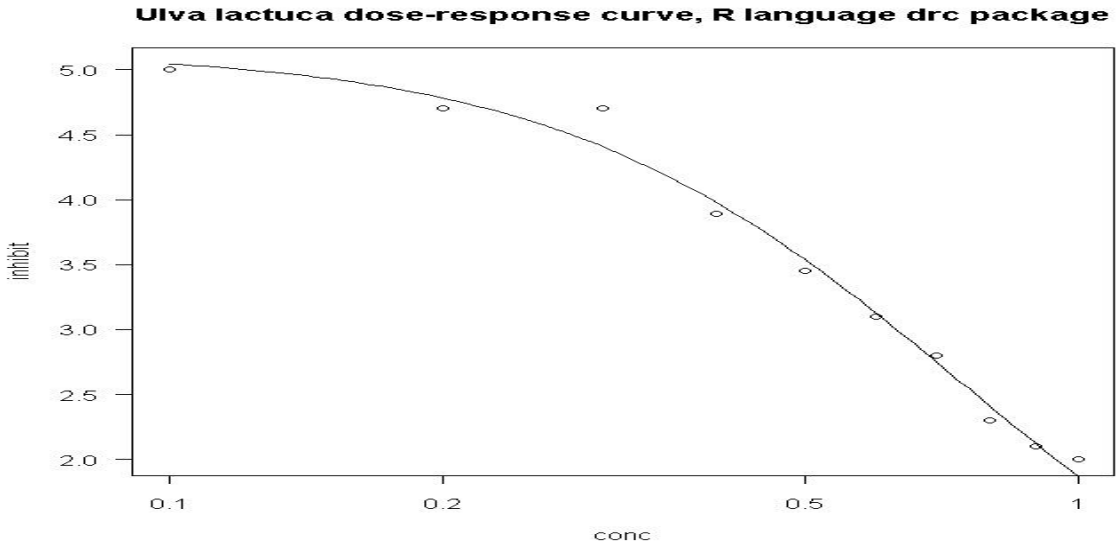


Fig. 4. *Dose-response curve described the acute toxic action of CPSH*

It is obvious from these results that relatively high levels of CPSH compound concentration will be able to produce acute deleterious effect on populations of Sea lettuce (LD_{50} = 0.750 % = 7500 ppm).

Physical/chemical properties of compounds tested as calculated by EPI Suite are presented in Table 2:
According to the Regulation (EC) № 1107/2009 of The European Parliament and the Council dated 21 October 2009, concerning the placing of plant protection products on the market and repealing Council Directives 79/117/EEC and 91/414/EEC, the persistence criterion for half-life (DT_{50}) in marine water is higher than 60 days – 1440 hours.

All compounds can be characterized as non-persistent based on predicted values as calculated by EPI Suite.

Future research of tested compounds is necessary in order to further reveal their behavior in the environment in terms of PECs (Predicted Environmental Concentrations) by FOCUS ecotoxicological software models as adopted by the European Parliament and the Council [18] based on field tests of selected synthetic compounds.

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Table 2. *Physical/chemical properties of tested compounds calculated by EPI Suite*

physical/chemical properties	CPSH	CHSH	CPSDTH	ACPCA
Log Kow	0.60	1.09	0.12	-1.67
Vapour Pressure P(mm Hg,25 deg C)	3.78e-007	1.59e-007	4.74e-007	9.49e-010
Water Solubility at 25 deg C (mg/L)	2.245e+004	7371	1987	6.982e+004
DT_{50} Water (hours)	900	900	900	360

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