QSAR acute aquatic ecotoxicological evaluation of cyclopentanespiro-5-(2,4-dithiohydantooin) towards species of phylum *Mollusca*

Donyo Ganchev, Agricultural University – Plovdiv, Bulgaria, Email: donyo@abv.bg  
Marin Marinov, Agricultural University – Plovdiv, Bulgaria, Email: m_n_marinov@abv.bg

**Abstract**- QSAR aquatic ecotoxicological evaluation of cyclopentanespiro-5-(2,4-dithiohydantooin) was conducted in order to be revealed the toxicity of tested compound towards animal species from phylum Mollusca with QSAR Toolbox software developed by Laboratory of Mathematical Chemistry (LMC), Bourgas, Bulgaria. The present paper also makes a comparison between a real trial with Planorbis planorbis (Ram’s Horn Snail) and QSAR modelling for determination of the accuracy of the method and modeling algorithms used. This approach – comparison between real tests and QSAR modeling can provide a valuable information about accuracy of the QSAR method and future perfection of it, especially for such non-popular for research studies objects like species from *Mollusca* phylum.

**Keywords**: QSAR, ecotoxicology, spirohydantoins, cyclopentanespiro-5-(2,4-dithiohydantooin, Planorbis planorbis, Mollusca.

**I. INTRODUCTION**

QSAR modeling and evaluation is the most novel and cutting-edge method for evaluation of the chemicals toxicological properties and respectively – chemical registration [1]. The recent REACH Policy of the European Union has force scientists and regulators to focus their attention on establishing general validation principles for QSAR models in the context of chemical regulation (previously known as the Setubal, nowadays, the OECD principles) [2]. The QSAR method allow cheap, rapid and humane way for determination of the toxicity of chemicals and their safety for the environment, just using computer models and software programs [3].

The aquatic ecotoxicity is one of the most important evaluations due to the role of the aquatic organisms for normal functioning of ecosystems and the fact that in most cases, chemicals (especially pesticides) can be very toxic to the aquatic flora and fauna which can have extremely serious impact onto all ecosystems [4].

The conduction of full scale in vivo trials in this case is often completely impossible due to the necessity or the risk of contamination of the tested chemical/chemicals of all water bodies and underground waters [5]. Even when there is a possibility for such kind test, they are very limited in their scope and require a lot of time, money and staff to be conducted. Using QSAR analysis can safe do all of this [6].

*Mollusks* compose the large phylum of invertebrate animals. They are the largest marine phylum comprising about 23% of all the named marine organisms which also live in freshwater and terrestrial habitats, playing important role in all ecosystems [7]. For centuries they have been used for food by humans, as well as source of medicals, jewelry goods and dyes. Some of them are extremely dangerous agricultural pests especially in the cold and humid areas [8]. They, together with other organisms can be considered as indicators of chemical pollution and impact onto ecosystems [9, 10].
In 2012, a scientific paper for evaluation of acute toxicity of some spirohydantoins and their derivatives towards *Planorbis planorbis* (Ram's Horn Snail), revealed that this chemical substance - cyclopentanespiro-5- (2,4-dithiohydantoin) - CPSDTH, (Fig. 1) can cause acute toxicity towards Ram's Horn Snail (*Planorbis planorbis*) [11]:

- \( LC_{50} = 7.8203 \times 10^{-7} \% \) (v/v)
- \( LC_{25} = 2.8945 \times 10^{-5} \% \) (v/v)
- \( LC_{50} = 2.4834 \times 10^{-4} \% \) (v/v)

![Figure 1. Cyclopentanespiro-5-(2,4-dithiohydantoin) – CPSDTH, structural formula](image)

Figure 1. Cyclopentanespiro-5-(2,4-dithiohydantoin) – CPSDTH, structural formula

/Systematic name: 1,3-diazaspiro[4.4]nonane-2,4-dithione/

The tested compound cyclopentanespiro-5-(2,4-dithiohydantoin) was synthesized in accordance with Reference [12]. The melting point was determined with a digital melting point apparatus SMP 10. The elemental analysis data were obtained with an automatic analyzer Carlo Erba 1106. The IR spectrum was taken on spectrometer Bruker-113 in KBr disc. The NMR spectra were taken on a Bruker DRX-250 spectrometer, operating at 250.13 and 62.90 MHz for 1H and 13C, respectively, using the standard Bruker software. The chemical shifts were referenced to tetramethylsilane (TMS). The measurements were carried out at ambient temperature.

*Planorbis planorbis* is a species of air-breathing freshwater snail, an aquatic gastropod mollusk in the family *Planorbidae*. The habitat of this species is shallow standing and slowly running freshwaters on a mud substrate, also ponds and temporarily drying flood waters, up to 1 meter depth [13]. *Planorbis planorbis* does not tolerate intensive water movements but is tolerant to eutrophic conditions [14].

The purpose of the present study is to use QSAR analyses in order to reveal the toxicity of cyclopentanespiro-5-(2,4-dithiohydantoin) towards other species from phylum *Mollusca* in order to be uncover the potential of the tested chemical compound, to be toxic for animals from phylum *Mollusca* and to be compared the accuracy of the QSAR analysis with real *in vivo* conducted test with species from phylum *Mollusca* - *Planorbis planorbis* (Ram's Horn Snail). This approach - comparison between real tests and QSAR modeling can provide a valuable information about accuracy of the QSAR method and future perfection of it especially for such non-popular for research studies objects like species from *Mollusca* phylum [15].
II. MATERIALS AND METHODS

The QSAR Toolbox v. 3.4 software [16] was used for performing the current QSAR analysis. In order to uncover the aquatic toxicity of the tested compound, profiling with two general mechanic profilers was carried out: protein binding by OASIS v1.4 - developed by Laboratory of Mathematical Chemistry (LMC), Bourgas, Bulgaria and Protein binding by OECD - developed by School of Pharmacy and Chemistry, Liverpool John Moores University, UK; together with three endpoint specific profilers: Acute aquatic toxicity classification by Verhaar (Modified) - developed by Henk J.M. Verhaar, Cees J. van Leeuwen & Joop L.M. Hermens, Acute aquatic toxicity MOA by OASIS - developed by Laboratory of Mathematical Chemistry (LMC), Bourgas, Bulgaria and Aquatic toxicity classification by ECOSAR - developed by U.S. Environmental Protection Agency was conducted [17, 18].

From one side - this is a standard approach according to establishing the aquatic toxicity via QSAR analysis, however - from the other - the using of given profilers is in accordance with chemical structure and mode of action of tested chemical compound.

III. RESULTS

Fig. 2 shows the information about tested chemical gained from ChemSpider [19]:
After inserting the SMILES string into the QSAR Toolbox, the software shows the following information about the chemical tested (Fig. 3):

![Initial information about the tested chemical substance gained from the QSAR Toolbox](image1)

Figure 3. The initial information about the tested chemical substance gained from the QSAR Toolbox

It can be clearly seen, that the structural and molecular formulas are completely the same as to what is presented in ChemSpider. This proves the right recognition of the given chemical from the software based on the SMILES string.

After application of the selected profilers, the results are:

**General mechanic profilers:**
- Protein binding by OASIS v1.4 - no alerts found - The query chemicals do not match the structural criteria specified in the boundaries of the profiler
- Protein binding by OECD – Fig. 4

![Profiling results](image2)

Figure 4. The result from application of protein binding by OECD, general mechanic profiler
Endpoint specific profilers:

- Acute aquatic toxicity classification by Verhaar (Modified) - Class 5 (not possible to classify according to these rules)
- Acute aquatic toxicity MOA by OASIS - reactive unspecified
- Aquatic toxicity classification by ECOSAR – Fig. 5

The running of the module "Endpoints" shows that "There are no experimental data available for the chemical of interest".

In the module "Category Definition", the most appropriate way of category definition was by using of general mechanistic profiler "Protein binding by OECD". In this case the QSAR Toolbox found 13823 similar compounds.

The QSAR Toolbox was able to provide information about 96 hours acute test for mortality about species of phylum Mollusk grouped in two classes Bivalvia and Gastropoda class.

For the species of the Bivalvia class by the QSAR Toolbox was able to conduct modeling according to Corbicula manilensis - freshwater bivalve mollusk in the family Cyrenidae. The mollusk occurs in freshwater environments of Eastern Asia, including Russia, Thailand, the Philippines, China, Taiwan, Korea, and Japan [20].

In order analysis to be more correct, additional subcategorization with endpoint specific profiler acute aquatic toxicity classification by Verhaar (Modified) was conducted. The predicted LC50 value of tested chemical was 7.05 e+05 mg/l (Fig. 6):

![Figure 5. The result from application of aquatic toxicity classification by ECOSAR, endpoint specific profiler](image)

![Figure 6. Trend analysis prediction of tested compound according to Corbicula manilensis](image)
For the species of the *Gastropoda* class by the QSAR Toolbox was able to conduct modeling according to:

- *Lymnaea acuminata* - freshwater snail in the family *Lymnaeidae* native to South Asia, where it occurs in Bangladesh, Burma, India, Nepal, and Pakistan. It is a widespread and common species there. The species lives in water bodies like lakes, streams, and wetlands with thick vegetation. It easily can survive in polluted waters [21].

- *Indoplanorbis exustus* - an aquatic pulmonate gastropod mollusk in the family *Planorbidae* very similar species to *Planorbis planorbis*. The species is widely distributed on the Earth [22].

- *Aplexa hypnorum* - small air-breathing freshwater snail, an aquatic pulmonate gastropod mollusk in the family *Physidae*. The species inhabits very shallow ponds and ditches, usually ones that dry out periodically in Europe [23].

- *Lymnaea peregra ssp. ovata* - air-breathing freshwater snail found in Europe, Newfoundland and north Asia. The species is common in slow-moving or still water [24].

- *Viviparus bengalensis* - river, freshwater snail found in Middle East and East Africa [25].

The received results as predicted LC$_{50}$ (96 h acute mortality test) for species from *Gastropoda* class according to cyclopentanespiro-5-(2,4-dithiohydantoin) were:

- *Lymnaea acuminata* - 3.22 mg/l.

- *Indoplanorbis exustus* - 4.11 mg/l (Fig. 7).

![Figure 7. Trend analysis of prediction of LC$_{50}$ for Indoplanorbis exustus](image)

- *Aplexa hypnorum* - 1.19 mg/l

- *Lymnaea peregra ssp. ovata* - 9.64 e+05 mg/l

- *Viviparus bengalensis* - 1.23 e+08 mg/l
IV. CONCLUSIONS

From the modeling conducted via the QSAR Toolbox, a clear toxic action of cyclopentanespiro-5-(2,4-dithiohydantoin) towards species from Mollusca phylum can be seen. The LC50 of in vivo conducted acute toxicity trials with Planorbarius barbatus is 2.4834e-04 % (v/v) [11], the predicted LC50 value from the current QSAR analyses of the closest to this species, snail - Indoplanorbarius exustus is 4.11 mg/l which is equal to 4.11 e-04 % (v/v). This proves the accuracy of the present QSAR analysis due to the very close values received from real trials and the QSAR prediction.

For the other species of the Gastropoda class, LC50 for Lymnaea acuminata and Aplexa hypnorum are also close to the value of LC50 of Planorbarius barbatus received from real in vivo conducted test. However, for other two species: Lymnaea peregra ssp. ovata and Viviparus bengalensis the LC50 is too large: 9.64 e+05 and 1.23 e+08 mg/l as well as, the predicted LC50 for Corbicula manilensis from Bivalvia class - 7.05 e+05 mg/l.

From Fig 6 and Fig. 7 can be seen that, a very low number of values is used for establishing the QSAR predicted LC50 values via trend analyses due to the lack of real trials and the QSAR modeling of the tested chemical for Mollusca phylum. The species from this phylum are not among the standard test organisms according to the OECD standards for testing chemicals. They are relatively rarely used in scientific ecotoxicological researches. The negligence of species from Mollusca phylum in the area of ecotoxicology should be corrected due to their big ecological significance, widely distribution around the world, easy to be grown in laboratory conditions, small size and relatively high breeding potential. Such kind approach - comparison between real tests and QSAR modeling can provide a valuable information about accuracy of the QSAR method and future perfection of it, especially for such non-popular for research studies objects like species from Mollusca phylum.

V. REFERENCES