Effect of terbutryn on aquatic organisms

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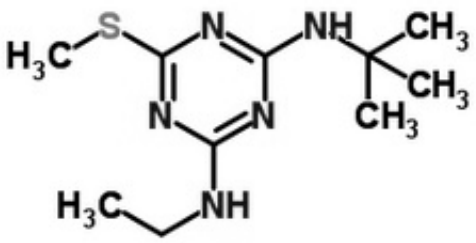
Abstract: The aim of the study was to determine the growth inhibition effect of terbutryn on freshwater algae *Desmodesmus communis*, *Chlorella kessleri* and cyanobacteria *Anabaena* sp. The experimental inhibition concentrations ranged from 0.004 to 1.250 mg/L. IC50 values analysed by the non-linear regression were as follows: 0.012 mg/L for *Desmodesmus communis*, 0.188 mg/L for *Chlorella kessleri* and 0.666 mg/L for *Anabaena* sp.

Key Words: algae, cyanobacteria, inhibition, microtiter plate, toxicology

INTRODUCTION

Terbutryn (2-(terc-butylamino)-4-(ethylamino)-6-(methylthio)-s-triazine) belongs to a group of substituted symmetric triazines (s-triazines). There are two groups of triazine herbicides: asymmetric triazines or triazinones on one hand and symmetric triazines on the other hand (Plhalová et al. 2010). Symmetric triazines are substances similar to herbicides used in agriculture for inhibiting a broad-leaved weed and grasses (Arufe et al. 2004). The main commercial symmetric triazines are ametryn, prometryn and terbutryn (Breckenridge et al. 2010). Terbutryn was used worldwide to weed control in crops such as cereals, legumes, potatoes, corn, sugar cane and under fruit trees. Terbutryn is selective and systemic herbicide inhibiting photosynthesis. It is used also against submerged and floating macrophytes, algae and cyanobacteria in watercourses, lakes and ponds (Moretti et al. 2002, Daho 2006). Terbutryn does not affect soil microorganisms and has low toxicity for birds. On the other hand, terbutryn is highly toxic for algae (even in low concentrations, Rioboo et al. 2007), toxic for fish and moderately toxic for cladocerans (Daho 2006). The substance is moderately soluble in water (22 mg/L) and is potentially bioaccumulative in aquatic organisms. It is a lipophilic substance biologically available for monocellular algae and hence could be a part of the food chain (Rioboo et al. 2007). Although the terbutryn application is forbidden in many countries, it can still be found in the water systems (Daho 2006, Rioboo et al. 2007). Preparations containing terbutryn have not been registered in the Czech Republic since 2005 (Plhalová et al. 2010).

Figure 1 The structural formula of terbutryn (Royal Society of Chemistry 2015).



The application of chemical substances to the environment caused by human activities can be of high risk for both the nature and human's health. Legislation of Europeans and other industrial countries needs appropriate data to evaluate the risks of the registration of new chemical preparations

such as pesticides, biocides and medicines. This data contains information about toxicity on various trophic levels (Scholz et al. 2008). Acute toxicity assays together with the growth inhibition assay serves as a basic tool for the evaluation of the potential toxic effect of the particular substance on live organisms (Kočí 2006). Growth inhibition assays are the basic ecotoxicological practise for a risk assessment of industrial chemical substances and pesticides (Brust et al. 2001). Algae are common test organisms sensitive to many toxic preparations and therefore are widely used in toxicity assays (Zhang et al. 2012). Algae, as primary producers, are key functional organisms in aquatic food chains (Machado and Soares 2012). Planktonic algae and cyanobacteria such as *Desmodesmus communis, Chlorella kessleri*, *Pseudokirchneriella subcapitata*, and *Anabaena* sp. can be used in growth inhibition assays (ÚNMZ 2012). The first part of the organism which is in contact with the chemical substance is the cell membrane. The cell membrane integrity is crucial for the functioning and viability of the cell itself. Cells with the damaged membrane are usually classified as dead cells. The cell membrane integrity evaluation is based on fluorescence methods (Machado and Soares 2012). Fluorescence shows a photosynthetic pigment of algae and cyanobacteria in living cells (Gregor and Maršálek 2006).

Even though terbutryn has been banned in many countries including the Czech Republic, the substance is still detected in the water. Terbutryn can leach from soil to the aquatic ecosystems. Its metabolites can be found in the drinking water long time after its last application. In this study, we aimed at the toxic effect of terbutryn determined by the comparable standardize method on various aquatic organisms, namely algae *D. communis*, *C. kessleri* and cyanobacteria *Anabaena* sp.

MATERIAL AND METHODS

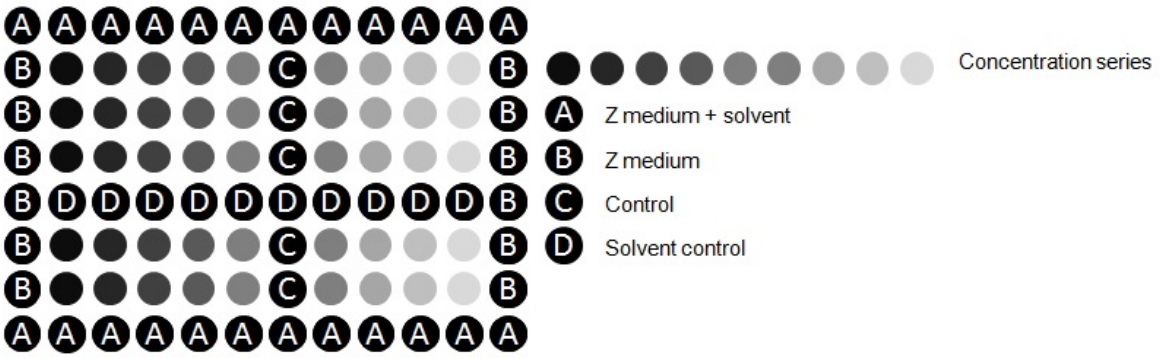
Inoculum preparation

The assay was performed on a green algae *D. communis, C. kessleri* and cyanobacteria *Anabaena* sp. The algae inoculum was taken during the exponential growth of the inoculum culture and put to the assay two days before the cultivation. The inoculum was added to the nutrient solution (Z medium) in a density of 10^4 cells.ml/L to allow growth throughout the assay without the risk of nutrient depletion. Erlenmeyer flasks of 150 ml were used for the pre cultivation which took place in a constant temperature of 26±1°C and a constant light intensity of 7000 lx.

Preparation of concentration series

Terbutryn was dissolved in distilled water. Two concentration series with the dilution factor of two were prepared: 0.004883; 0.009766; 0.019531; 0.039063; 0.078125; 0.15625; 0.3125; 0.625; 1.25 mg/L and 0.003906; 0.007813; 0.015625; 0.03125; 0.0625; 0.125; 0.25; 0.5; 1 mg/L. Altogether 18 different stock solutions were prepared.

Figure 2 Microtiter plate – pipetting scheme.



Legend: See the text for the details.

The inoculum samples were pipetted to a microtiter plates (Figure 2). The microtiter plate has nine tested concentrations in five replicates and the control group. Tested series were prepared by mixing

each stock solution with the inoculum (190 µl of the inoculum + 10 µl of the stock solution). The control group with Z medium (190 µl of the inoculum + 10 µl of Z medium) and the control with solvent only (190 µl of the inoculum + 10 µl of distilled water).

Incubation

Microtiter plates were closed by a cap during the incubation to prevent air contamination and to reduce evaporation. Incubation was carried out in the same conditions as the inoculum preparation (temperature: 26±1°C; light intensity: 7000 lx).

Fluorescence measurement

Measurement of fluorescence was made every 24 hours using the spectrophotometer (TECAN Infinite M1000 PRO) with the excitation wave length of 590 nm and the emission of 680 nm. The content of microtiter plates was re-mixed before each measurement. The assay lasted for 72 hours.

The IC50 estimation

The results were evaluated in software MS Excel (Microsoft) to figure the percentage of inhibition. Then, a dose-response curve based on a nonlinear regression was constructed in GraphPad Prism 7.04 (GraphPad Software, La Jolla California USA, www.graphpad.com). The inhibition concentration in which 50% of tested organisms died (IC50) was estimated as the fitted midpoint of the curve.

RESULTS AND DISCUSSION

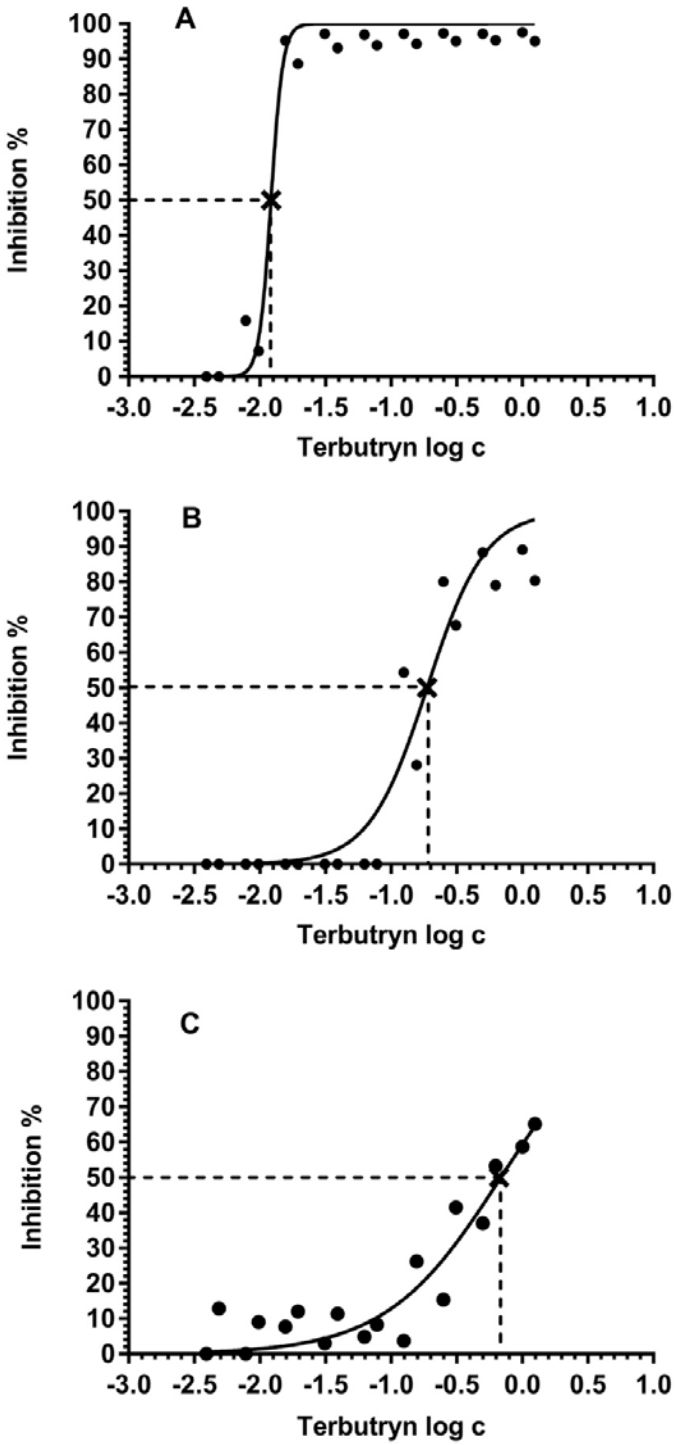
The inhibition concentration of terbutryn in 72 hours (72hIC50) for *D. communis* was **0.012 mg/L** (log concentration 1.915, R^2 = 0.977), for *C. kessleri* **0.188 mg/L** (log concentration 0.7255, R^2 = 0.976) and for cyanobacteria *Anabaena* sp. **0.666 mg/L** (log concentration = 0.164 (R^2 = 0.887). The most sensitive taxon was *D. communis*, the most resistant cyanobacteria *Anabaena* sp. (Figure 3).

There are plenty of published data about the content of terbutryn in surface waters. Concentration of 0.02 µg/L of terbutryn was found in samples from river Elbe (Saxony, Germany). Concentrations in surface waters in Bavaria ranged from 0.6 to 1.2 µg/L (Brust et al. 2001). The highest concentration found in surface waters of the Czech Republic is 0.02 µg/L (Velíšek et al. 2009).

Arufe et al. (2004) presented the 72hLC50 of 1.41 mg/L for *Sparus aurata* yolk sac fry exposed to the commercial herbicide with terbutryn (59.4%). The same author presented results of toxicity assays with various time exposition: 24hLC50 – 3.66 mg/L, 48hLC50 – 2.18 mg/L. As a conclusion, the toxicity increases with increasing exposition time. The results show different sensitivity between *S. aurata* and *Vibrio fisheri* to commercial herbicide containing terbutryn (59.4%). Herbicide toxicity was more than one order lower in *V. fisheri* (15 min EC50 – 15.94 mg/L) than for *S. aurata* yolk sac fry (72hLC50 – 1.41 mg/L) (Arufe et al. 2004). Terbutryn is moderately toxic for fish. Bathe et al. (1973) presented LC50 being 4 mg/L for *Cyprinus carpio*, Arufe et al. (2004), Kidd and James (1991) presented 96hLC50 for *Oncorhynchus mykiss* 3 mg/L. Arufe et al. (2004) and Daho (2006) mentioned the results of acute toxicity assays and growth inhibition assays of other aquatic organisms, e.g. *Skeletonema costatum* 9dEC50 – 0.91 µg/L, *Dolichospermum flos-aquae* 7dEC50 – 3.4 µg/L, *Daphnia magna* 48hEC50 – 7.1 mg/L and *Lepomis macrochirus* 96hLC50 – 4 mg/L. Those results showed high toxicity of terbutryn for *S. costatum* and *D. flos-aquae*.

Plhalová et al. (2010) compared the acute toxicity of terbutryn using *Danio rerio* and *Poecilia reticulata.* The average value of terbutryn toxicity for embryonic stage of *D. rerio* was 8.04 mg/L, for juvenile *D. rerio* was the average value 96hLC50 5.71 mg/L. The results shows higher sensitivity of juvenile stages compared to the embryonic stage of *D. rerio*. The average value of terbutryn 96hLC50 for juvenile stage of *P. reticulata* was 2.85 mg/L. Comparison of the average LC50 of terbutryn for *D. rerio* and *P. reticulata* showed significantly higher sensitivity of *P. reticulata*.

Figure 3 Dose-Response curve of tested organisms.



Legend: A – Desmodesmus communis, B – Chlorella kessleri, C – Anabaena sp. Crosses shows IC50.

CONCLUSION

In conclusion, the terbutryn is highly toxic to green algae and cyanobacteria according to the calculated inhibitory concentrations (72hIC50). The most sensitive species was the green algae *D. communis* (72hIC50 was 0.012 mg/L) followed by *C. kessleri* (72hIC50 was 0.188 mg/L), the most

resistant cyanobacteria *Anabaena* sp (72hIC50 was 0.666 mg/L). The results are confirmed by other authors who show that the acute toxicity of terbutryn is also high to other aquatic organisms. Based on our results, the natural concentrations causing the acute toxicity of the most sensitive green algae should be 600 times higher than the highest concentration found in surface waters in the Czech Republic (0.02 µg/L). The potential environmental risk is bioaccumulation which can be important even in relatively low natural concentration of terbutryn.

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REFERENCES

Arufe, M.I. et al. 2004. Toxicity of a commercial herbicide containing terbutryn and triasulfuron the seabream (*Sparus aurata* L.) larvae: comparsion with the Microtox test. Ecotoxicology and Environmental Safety, 59(2): 209–216.

Bathe, R. et al. 1973. Determination of pesticide toxicity to fish. Schriftenr. Ver. Wasses-Boden-Lufthxg. Berlin-Dahlem 37, 241–256.

Breckenridge, Ch.B. et al. 2010. Symmetrical Triazine Herbicides: A Review of Regulatory Toxicity Endpoints. In Hayes´ Handbook of Pesticide Toxicology. University of California: Robert Krieger, pp. 1711–1723.

Brust, K. et al. 2001. Effects of terbutryn on aufwuchs and *Lumbriculus variegates* in artificial indoor streams. Environmental Toxicology and Chemistry, 20(9): 2000–2007.

Daho, M.B. 2006. Ecotoxicological evaluation of the herbicide terbutryn. Master´s thesis, University of Uppsala.

Gregor, J., Maršálek, B. 2006. Fluorescenční metody kvantifikaci planktonních cyanobakterií. In Cyanobakteria 2006. Brno, Czech Republic, 24–25 May. Botanický ústav AV ČR Průhonice, pp. 91–95.

Kidd, H., James, D.R. 1991. The Agrochemicals Handbook third ed. Royal Society of Chemistry Information Services, Cambridge, UK.

Kočí, V. 2006. Význam testů toxicity pro hodnocení vlivu látek na životní prostředí. Chemické listy, 100(10): 882–888.

Machado, M.D., Soares, E.V. 2012. Development of short-term assay based on the evaluation of the plasma membrane integrity of the alga *Pseudokirchneriella subcapitata*. Applied Microbiology and Biotechnology, 95(4):1035–1042.

Moretti, M. et al. 2002. In vitro testing for genotoxicity of the herbicide terbutryn: cytogenetic and primary DNA damage. Toxicology in Vitro, 16(1): 81–88.

Plhalová, L. et al. 2010. Comparison of Terbutryn Acute Toxicity to *Danio rerio* and *Poecilia reticulata*. Acta Veterinaria Brno, 79(4): 593–598.

Rioboo, R. et al. 2007. Population growth study of the rotifer *Brachionus* sp. fed with triazine-exposed microalgae. Aquatic Toxicology, 83(4): 247–253.

Royal Society of Chemistry. 2015. ChemSpider Search and share chemistry. [Online]. Available at: http://www.chemspider.com/Chemical-Structure.12874.html. [2018-07-2].

Scholz, S. et al. 2008. The zebrafish embryo model in environmental risk assessment-applications beyond acute toxicity testing. Environmental Science and Pollution Research, 15(5): 394–404.

ÚNMZ. 2012. Kvalita vod – Zkouška inhibice růstu sladkovodních zelených řas. ČSN EN ISO 8692 (75 7740). Praha: Úřad pro technickou normalizaci, metrologii a státní zkušebnictví.

Velíšek, J. et al. 2009. Effects of sup-chronic exposure to terbutryn in common carp (*Cyprinus carpio* L.). Ecotoxicology and Environmental Safety, 73(3): 384–390.

Zhang, L.J. et al. 2012. Development and application of whole sediment toxicity est using immobilized freshwater microalgae *Pseudokirchneriella subcapitata*. Environmental Toxicology and Chemistry, 13(2): 377–386.