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The toxic effect of oxytetracycline and trimethoprim in the aquatic environment

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ABSTRACT

The objective of our study was the investigation of the toxic properties of two antimicrobial drugs: oxytetracycline (OTC) and trimethoprim (TMP) in the aquatic environment. The toxic effects were tested according to the OECD guidelines for the testing of chemicals, on the cyanobacteria *Anabaena flos-aque*, on the alga *Pseudokirchneriella subcapitata*, on the daphnid *Daphnia magna* as well as on the activated sludge. We discussed the short term and long term results of tests on cyanobacteria and microalgae. Both experiments were concluded in 72 h allowing direct comparison of sensitivity of the two tested species. The results of our study showed toxic effect in the same range for both groups. In the test on the toxicity of OTC to *P. subcapitata* we obtained the 72 h ErC_{50} of 1.04 mg L^{-1} (72 h ErC_{10} 0.47 mg L^{-1}) which are lower in comparison to the results on the toxicity to *A. flos-aque* of ErC_{50} of 2.7 mg L^{-1} (72 h ErC_{10} 1.5 mg L^{-1}). TMP is less toxic to both photosynthetic plankton species. Similar to the test results on OTC, the *P. subcapitata* is more sensitive to TMP (ErC_{50} 129 mg L^{-1}; ErC_{10} 65 mg L^{-1}) than *A. flos-aque* (72 h ErC_{50} 253 mg L^{-1}; 72 h ErC_{10} 26 mg L^{-1}). OTC is toxic to the activated sludge (3 h EC_{50} 17.9 mg L^{-1}), while the calculated 3 h EC_{50} value for TMP exceeded solubility for the compound. In comparison to other species, both tested antimicrobials showed low toxicity to daphnids.

1. Introduction

Antimicrobial agents are extensively used in human and veterinary medicine and in aquaculture. Worldwide estimation of antimicrobial agents consumption lies between 100,000 and 200,000 ton per year (Wise, 2002). According to the European Federation of Animal Health report, in the year 1999, 65% of antimicrobials were used in human medicine (Kümmerer, 2009). In the survey by the European medical agency (EMA), the sales of veterinary antimicrobial agents was compared among 10 European countries, resulting from 18 to 188 mg kg^{-1} of antimicrobials per kilogram of biomass of food producing animals (Grave et al., 2010). The EMA report On Sales of veterinary antimicrobial agents in 2011 reveals a total EU sell of 82 tons. Data on the usage of human antimicrobials in the EU are not available, however, due to their substantial use, scattered distribution and direct exposure, the effect of biologically active molecules to the environmental compartments should not be overlooked.

Since VICH (International Cooperation on Harmonization of Technical Requirements for Registration of Veterinary Products) has released The Guidance for environmental impact assessment for veterinary medicinal products (VICH 6, 2000) (VICH 38, 2005) and the EMA (European Medical Agency) has released the Guideline On The Environmental Risk Assessment Of Medicinal Products For Human Use (CHMP, 2006), the increased demand for new ecotoxicological data on active substances is created. The European Union legislation set the obligation to take environmental risk into account at the registration of veterinary medicinal products (Directive, 2004/28/EC). The applicant is required to provide data on environmental fate and behaviour and ecotoxicological properties that enable an assessment of the potential risks posed by the medicinal product to the environment. When the potential risk is identified, regulators should reduce it to the acceptable level by mitigation measures or even by preventing the registration of the product (Montforts et al., 2004).

The objective of our study was to investigate the environmental toxicity properties of two antimicrobial substances, oxytetracycline (OTC) and trimethoprim (TMP), in the aquatic environment. Both of the substances are commonly used in human medicine and for the treatment of animals reared in terrestrial environment and in aquaculture. OTC belongs to the broad-spectrum tetracycline group, widely used in human and veterinarian medicine. As a drug, it undergoes minimal metabolism and is mainly excreted

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via urine as an unchanged drug (CVMP, 1995). Urinary recovery of OTC within 72 h post-treatment ranges between 42–62% of the administered dose in pigs and between 62–88% in cows (Mevius et al., 1986). TMP belongs to the class of chemotherapeutic agents acting on dihydrofolate reductase, inhibiting the synthesis of tetrahydrofolic acid. In veterinary medicine the most often-used combination is TMP with sulphonamide. In humans, pigs and poultry, 46% of the applied dose is excreted through urine and feces, 22% of which as unchanged TMP (CVMP, 1997).

In line with the Guidance for environmental impact assessment for veterinary medicinal products (VICH Expert Working Group, 2005), published data can only be used to substitute studies, if the publication contains a sufficient amount of data and sufficient details on the design and conduct of the study to allow a full and independent assessment (EMA, 2012).

In support to the assessment of the effect of OTC and TMP on the aquatic environment we selected four toxicity tests and performed experiments following the OECD guidelines for the testing of chemicals. In addition to the acute toxicity test on daphnids and the multigeneration test on eukaryotic single cell green algae, we chose the multigeneration test on prokaryotic cyanobacteria as recommended in the VICH guidance, Phase II (VICH 38, 2005). Additionally, we conducted the test on the inhibition of respiration on activated sludge according to the Guideline On The Environmental Risk Assessment Of Medicinal Products For Human Use (CHMP, 2006). In the tests, we met the validity criteria which enable the comparison of the results from our study with the published data.

2. Materials and methods

In the study, antimicrobial substances were tested according to the OECD guidelines for the testing of chemicals on the toxicity to the cyanobacteria *Anabaena flos-aque*, to the alga *Pseudokirchneriella subcapitata*, to the daphnid *Daphnia magna* and to the microbial community of active sludge.

Test compounds of pharmacopeia purity were donated by the pharmaceutical company Krka d.d., the chemicals used for the growth medium were all of analytical grade and purchased from Merck. The physicochemical properties of OTC and TMP are included in Table 1.

2.1. Chemical measurements

In order to provide information on exposure concentrations to antimicrobials during the tests, the concentrations of OTC and TMP in toxicity tests to cyanobacteria, algae, daphnids and the microbial community of active sludge were determined by the LC–MS–MS analysis.

An Agilent 1200 HPLC system coupled with an AB Sciex API 4000 tandem mass spectrometer with electrospray ionization (ESI) interface operating in positive Multiple Reaction Monitoring (MRM) mode was used for both analytes. For OTC, the transition m/z 461 → 426 and m/z 461 → 443 were chosen. For TMP the transition m/z 291 → 230 and m/z 291 → 123 were chosen. The column used for separation was Luna C18(2) 100 × 2 mm with 3 μm particles. The mobile phase A consisted of 0.1% formic acid and acetonitrile (95/5) and the mobile phase B consisted of 0.1% formic acid in acetonitrile. The gradient elution conditions were initially A–B (95–5) programming to 40% B over 8 min. The flow rate was 0.3 ml min^{-1} with oven temperature 40 °C and the injection volume of 20 μl.

The limit of detection (LOD) was 0.01 mg L^{-1} and the limit of quantification (LOQ) was 0.025 mg L^{-1} for both analytes. The average recovery for OTC was 95% and for TMP 96% with the corresponding RSD of 9.8% for OTC and 15.7% for TMP.

The WTW Multiline P4 multimeters with WTW Cell Oxi 325 and the pH probe WTW Sen Tix ORP for oxygen and pH measurements were used during tests.

Table 1
Physicochemical properties of two tested antimicrobial agents.

Substance	CAS	WS at 25 °C (mg L^{-1})	pK_a	Log K_{ow}
Oxytetracycline	79-57-2	313	9.5	−0.90
Trimethoprim	738-70-5	400	7.12	0.91

WS = water solubility.
Data were obtained from the TOXNET, Hazardous Substances Data Bank http://toxnet.nlm.nih.gov/cgi-bin/sis/htmlgen?HSDB.

2.2. Toxicity to the freshwater alga P. subcapitata and to the cyanobacteria A. flos-aque

A 72 h toxicity test was performed on two photosynthetic plankton species: green alga *P. subcapitata* (formerly known as *Selenastrum capricornutum*) and the cyanobacteria *A. flos-aque* to determine the effects of the given substance on the growth of freshwater photosynthetic plankton organisms. The test was performed according to the OECD TG 201 "Freshwater Alga and Cyanobacteria, Growth Inhibition Test" (OECD, 2011). Both test species are recommended in these guidelines.

2.2.1. Preparation of test solutions

Green algae and cyanobacteria were cultivated and tested in the OECD TG 201 medium and in the growth medium BG 11(Stanier et al., 1971) respectively.

The reference substance 3,5-dichlorophenol was tested as a means of verifying the test procedure.

2.2.2. Tested strains

The algal species *P. subcapitata*, strain CCAP 278/4 and the cyanobacteria *A. flos-aque* strain CCAP 1403/13A were obtained from the supplier SAMS Research Service Ltd. Scottish Marine Institute, Dunbeg, Argyll, PA 37 1QA, UK. The strains were cultivated in in the appropriate growth medium for at least 10 d prior to the test.

2.2.3. The test

Exponentially growing algae and cyanobacteria were exposed to the test substance in batch cultures over a period of 72 h. The test endpoint was inhibition of growth, expressed as the ErC_{50} and ErC_{10}. Five concentrations arranged in a geometric series were prepared for each test. In the test with cyanobacteria the geometric series for OTC and TMP were 2 and 1.3 respectively. For algae exposed to OTC and TMP the geometric series were 3 and 2 respectively. The tests on algae were conducted in 250 mL Erlenmeyer test flasks in 3 replicates, incubated for 72 h at 20 ± 1 °C in the growth chamber under continuous illumination and the light intensity of 7000 lux. The cyanobacteria were incubated at 24 ± 2 °C under continuous light at 3500–4000 lux. Each vessel was filled with 100 mL of the test medium. Algae were constantly shaken at approximately 120 rpm, while cyanobacteria were stirred just before sampling. Algae and cyanobacteria showed typical form without any inclusions seen under 500-fold magnifications.

The cell concentration in each flask was determined 24, 48 and 72 h after the start of the test by using fluorometric measurements (440–680 nm), Perkin Elmer Victor 3, 1420 Multilabel Counter (Perkin Elmer, Singapore, Republic of Singapore). Fluorometry as a surrogate measurement was validated by the measurement of cyanobacteria dry biomass and by counting the cell number of algae .The pH was measured at the beginning of the test and after 72 h of exposure.

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2.2.4. Statistical analyses

The average specific growth rate and the percent inhibition of growth rate for each replicate treatment were calculated using TOXCALC – Toxicity Data Analysis Software, Version 5.0.32. The percentage reduction in average growth rate at each test substance concentration compared to the control value was plotted against the logarithm of the concentration. The value for ErC_x was read from the resulting graph.

2.3. The toxicity to the aquatic invertebrate, D. magna

The purpose of this test is to determine the effects of OTC and TMP on the mobility of daphnids. The test was performed according to the OECD TG 202 "*Daphnia* sp., Acute Immobilisation Test" (OECD, 2004).

2.3.1. Preparation of test solutions

Reconstituted test water was prepared according to the OECD TG 202 (OECD, 2004).

2.3.2. Daphnids

A strain of *D. magna* was obtained from MicroBioTests Inc., Belgium. Several generations were cultivated in tap water and fed with dried algae fish food. At the start of the test, the animals were 8–24 h old and, to reduce variability, they were not first brood progeny.

2.3.3. The test

Young daphnids, aged less than 24 h at the start of the test, were exposed to the test substance at a range of concentrations for a period of 48 h. Five concentrations per test substance in a geometric series with a separation factor 2 including the control were placed into the specially designed plates with 5×6 vessels. Five juveniles were added to each vessel. The mobility of the test organisms was checked after 24 and 48 h and compared with control values. The results were analyzed in order to calculate the EC_{50} and EC_{10} at 48 h. $K_2Cr_2O_7$ was used as a reference substance.

2.3.4. Statistical analyses

Data were analysed by appropriate statistical methods (EPA Probit Analysis Program, Version 1.5) to calculate the slopes of the curves and the EC_{50} with 95% confidence limits ($p = 0.95$).

2.4. Inhibition of respiration of activated sludge

The test was performed according to the OECD TG 209 "Activated Sludge, Respiration Inhibition Test" (OECD, 2010). The purpose of the test is to assess the effect of a test substance on microorganisms of activated sludge by measuring the respiration rate (oxygen consumption) under defined conditions in the presence of different concentrations of the test substance.

2.4.1. Preparation of test solutions

Isotonic stock solution and synthetic sewage feed was prepared according to the OECD TG 209.

2.4.2. Inoculum

Inoculum was obtained from the exit of the aeration tank, from a well-operated wastewater treatment plant receiving predominantly domestic sewage in the municipal sewage treatment plant in the village Rače in north-east Slovenia. Initial dry matter concentration in the inoculum was 4.2 mg L^{-1}. The oxygen uptake rate was 23.4 mg oxygen per gram of activated sludge (dry weight of suspended solids) per hour.

2.4.3. The test

In the test, total oxygen consumption of an activated sludge fed with a standard amount of synthetic sewage feed was measured after a contact time of 3 h. Five concentrations arranged in a geometric series with a separation factor 2 were prepared. The inhibitory effect was calculated as 3 h EC_{50} at the concentration of the test substance at which the respiration rate is 50% of the mean respiration rates of two controls. The test was conducted at 20 ± 2 °C in 300 mL vessels for O_2 measurement. The pH was controlled. Prior to the test, the test solution was aerated using a magnetic stirrer. 3,5-Dichlorophenol was used as a reference substance.

2.4.4. Statistical analyses

Data were analyzed by appropriate statistical methods (EPA Probit Analysis Program, Version 1.5) to calculate the slopes of the curves and the EC_{50} with 95% confidence limits ($p = 0.95$).

3. Results

Results of ecotoxicological testing of OTC and TMP on cyanobacteria, green algae, daphnids and activated sludge are presented in Table 2. Toxicity endpoints calculation are based on measured initial concentrated as presented in Table 3.

3.1. The toxicity to the cyanobacteria A. flos-aque and the freshwater alga P. subcapitata

72 h of exposure of *A. flos-aque* to OTC resulted in the growth inhibition ErC_{50} 2.7 mg L^{-1}. The estimated ErC_{10} of 1.57 mg L^{-1} is lower than the lowest tested concentration. Toxicity endpoints for TMP are an order of magnitude higher in comparison to OTC, with the resulting ErC_{50} of 253 mg L^{-1}. The estimated ErC_{10} of 26 mg L^{-1} for TMP is lower than the lowest tested concentration.

Alga *P. subcapitata* is more sensitive to OTC than *A. flos-aque*, however, the 72 h ErC_{50} of 1.04 mg L^{-1} and the ErC_{10} of 0.47 mg L^{-1} are in the same order of magnitude. Similar to the toxic effect on cyanobacteria, in comparison to OTC, TMP is less toxic to alga *P. subcapitata*. After 72 h of exposure to TMP we calculated the ErC_{50} of 169 mg L^{-1} and the ErC_{10} of 65 mg L^{-1}.

Test conditions for cyanobacteria and green algae differ as they were adapted to follow the ecological strategy of these two taxonomic groups. Tests on cyanobacteria were conducted under a reduced light intensity during the test, no shaking was applied and the temperature was higher in comparison to the test on algae. The illuminance in the test with cyanobacteria was lower (3500–4000 lux) compared to the illuminance in the test on algae (7000 lux).

During the test, surrogate fluorometric measurements were validated at three points. That was done by counting the cell number of algae and by measurement of cyanobacteria dry biomass. The initial number of cells of *P. subcapitata* was 5.6×10^3 cells ml^{-1} (surrogate measurement r^2 0.98). The initial biomass of *A. flos-aque* was 0.008 g L^{-1} (surrogate measurement r^2 0.99).

OTC was proved to be unstable under illumination with the artificial light. At the end of the test under the reduced light regime on *A. flos-aque*, 40% of the initial concentration of OTC could be detected in the test chambers. Following the guidance OECD TG 201, the geometrical mean of concentration on the day 0, 2 and 3 was used to calculate the effect concentrations. Under intensive illumination during the incubation of algae, only 2% of the initial concentration of OTC was detected, failing to meet the criteria of mass balance of the parent compound. As proposed in the OECD TG 201 guideline, the calculated OTC ErC_x for *P. subcapitata* was based on initial measured concentrations as the decrease in the

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Table 2
Overview of ecotoxicological test results on OTC and TMP.

Test species	Endpoint	OTC		TMP	
		Toxicity (mg L^{-1})	95% CI (mg L^{-1})	Toxicity (mg L^{-1})	95% CI (mg L^{-1})
A. flos-aque	72 h ErC_{50}	2.7	0.01–10.4	253	187–319
A. flos-aque	72 h ErC_{10}	1.5	0–3.5	26	7–44
P. subcapitata	72 h ErC_{50}	1.04	0.98–1.1	129	123–136
P. subcapitata	72 h ErC_{10}	0.47	0.35–0.56	65	0.01–103
D. magna	48 h EC_{50}	669[a]	n.a.	100	89–112
D. magna	48 h EC_{10}	197	126–492[a]	66	53–76
Activated sludge	3 h EC_{50}	17.9	9.9–64.9	671[a]	n.a.
Activated sludge	3 h EC_{10}	0.2	0.03–0.51	9.8	1.9–19.9

Toxicity endpoints were calculated based on measured concentrations. CI = confidence interval; n.a. = not applicable.
[a] The value exceeded the water solubility.

Table 3
Concentrations of OTC and TMP used for the calculation of toxicity endpoints.

Test species	OTC (mg L^{-1})	TMP (mg L^{-1})
A. flos-aque	25.3[a]	300
P. subcapitata	10.1	353
D. magna	234	320
Activated sludge	12.7	270

[a] Average concentration between the measured concentrations at the beginning and the end of test.

concentration of the test substance in the course of the test was not accompanied by a decrease in growth inhibition.

The toxicity of TMP to green algae and cyanobacteria is based on the initial measured test concentration, as the measured concentration at the end of the tests did not deviate for more than 20% to the one at the beginning.

The biomass specific growth rate of *A. flos-aque* in the control was 0.92 d^{-1}, the mean coefficient of variation (CV) of daily specific growth rate in the replicate control cultures was 35.9% and CV% of average specific growth rates for control during the whole test was 11.7%. pH values for OTC and for TMP during the test were between 7.1–7.6 and 7.1–7.4 respectively.

The biomass specific growth rate of *P. subcapitata* in the control was 1.25 d^{-1}, the mean CV of daily specific growth rate in replicate control cultures was 21% and CV% of average specific growth rates for control during the whole test was 4.48%. pH values for OTC and for TMP during the test were 7.4 to 7.5 and 7.6 to 8.3 respectively.

3.2. The toxicity to the aquatic invertebrate, D. magna

The results of the testing on *D. magna* are based on the initial measured concentration. The calculated 48 h EC_{50} value for OTC exceeds water solubility (313 mg L^{-1} at 25 °C) for this compound. The pH varied during the test from 6.4 to 7.1. The oxygen concentrations in the tested solutions dropped from 6.4 to 5.6 mg L^{-1}.

The TMP 48 h EC_{50} to daphnids is 100 mg L^{-1}. During the test the pH of solutions varied from 8.1 before and 7.6 after incubation. The oxygen concentration in the test solution was in the range of 6 mg L^{-1} for the entire duration of the test.

The EC_{50} of reference substance $K_2Cr_2O_7$ was 1.1 mg L^{-1}; 95% confidence limits: 0.1–1.4 mg L^{-1}.

3.3. Inhibition of respiration of activated sludge

In the 3 h test on inhibition of respiration of activated sludge, we calculated the EC_{50} of 17.9 mg L^{-1} and the EC_{10} of 0.2 mg L^{-1} for OTC. the acute toxic endpoint EC_{50} for TMP exceeds water solubility (400 mg L^{-1} at 25 °C), while the 3 h EC_{10} was 9.8 mg L^{-1}. The calculations of test endpoints was based on the initial measured concentration. pH values during the test were monitored, but no correction was needed as they were stable at 7.2 (±0.2). The concentration of suspended solids in the solution was 4.8 mg L^{-1} for OTC and 4.5 mg L^{-1} for TMP respectively.

The 3 h EC_{50} of the reference substance 3,5-dichlorophenol was 11.4 mg L^{-1}.

4. Discussion

We studied the toxic effect of oxytetracycline (OTC) and trimethoprim (TMP) in the aquatic environment. Tests were performed according to the OECD guidelines for the testing of chemicals on the cyanobacteria *A. flos-aque*, on the alga *P. subcapitata*, on the daphnid *Daphnia magna* and on the microbial community of activated sludge. The tests on cyanobacteria and microalgae were both concluded in 72 h allowing a direct comparison of the sensitivity of both species. Although cyanobacteria are expected to be more sensitive to antimicrobials, the results of our study showed toxic effect in the same range for both tested groups.

4.1. Toxicity to the freshwater alga P. subcapitata and to the cyanobacteria A. flos-aque

In multigeneration studies on algae and cyanobacteria, it is generally accepted that a 72 h EC_{50} value may be considered as equivalent to a short-term result and that the 72 h EC_{10} or NOEC value can be considered as a long-term result, where the endpoint EC_{10} is used preferably (European Chemicals Agency, 2008). In our study the ErC_x was calculated from the percentage of reduction in average growth rate at each test substance concentration compared to the control value. Two species of green algae (*P. subcapitata* and *Desmodesmus subspicatus*) and two species of cyanobacteria (*A. flos-aquae* and *Synechococcus leopoliensis*) are suitable to be used according to the Annex II to the guideline OECD 201(OECD, 2011). In the study we performed tests on *A. flos-aque* and *P. subcapitata*.

The results on the toxicity to *P. subcapitata* (OTC 72 h ErC_{50} of 1.04 mg L^{-1}, TMP 72 h ErC_{50} of 129 mg L^{-1}) obtained in our study are consistent with the data from literature. Authors that followed comparable standardized test methods reported similar results. Holten Lützhøft found the OTC 72 h ErC_{50} of 4.5 mg L^{-1} and TMP 72 h ErC_{50} of 130 mg L^{-1} (Holten Lutzhøft et al., 1999), while Eguchi reports values for OTC 72 h ErC_{50} of 0.34 mg L^{-1} and for TMP 72 h ErC_{50} of 80.3 mg L^{-1} which is the lowest reported concentration on the acute toxicity of TMP to algae (Eguchi et al., 2004). The lowest short term toxicity of OTC for algae (72 h EC_{50} 0.17 mg L^{-1}) was reported by Isidori (Isidori et al., 2005), but the test was conducted at higher temperature (25 °C). Few data on long term toxicity of the studied antimicrobials on green algae are published in open literature. The 72 h NOEC for OTC of 0.183 mg L^{-1} and the 72 h NOEC for TMP of 25.5 mg L^{-1} (Eguchi et al., 2004) are lower, but in the same order of magnitude as values obtained in our study

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(72 h ErC_{10} of 0.47 mg L^{-1} for OTC mg L^{-1} and 72 h ErC_{10} of 65 mg L^{-1} for TMP).

Since early studies on the effect of antimicrobials on the aquatic compartment, the cyanobacteria were regarded as the most sensitive group (Harrass et al., 1985; Halling-Sørensen et al., 2000; Ando et al., 2007). However, public literature mostly cites data for only one cyanobacteria species (*Mycrocistis aeuroginosa*) which were obtained according to the standardized test method (Holten Lutzhøft et al., 1999). A 7 d well documented test based on nominal concentrations resulted in OTC ErC_{50} of 0.207 mg L^{-1} and TMP ErC_{50} of 112 mg L^{-1}. *M. aeuroginosa* proved to be an order of magnitude more sensitive to OTC and twice more sensitive to TMP than *P. subcapitata*.

We adjusted our test design in order to obtain a growth rate of 0.92 as required in the OECD TG 201 and to finalize the test in 72 h. Beside better control of the mass balance of tested substances during the experiment, the shortening of the test from 5 or 7 d to only 3 d allows a direct comparison of the results on toxicity between green algae and cyanobacteria. Based on the measurement of growth inhibition after 72 h of exposure to antimicrobials, we calculated the short and long term toxicity results on *A. flos-aque* of ErC_{50} 2.7 mg L^{-1} (ErC_{10} 1.5 mg L^{-1}) for OTC and of ErC_{50} 253 mg L^{-1} (ErC_{10} 26 mg L^{-1}) for TMP.

The impact of exposure duration was discussed in the study on the fluorescence measurement of the yield of photosystem II. Photosynthetic efficiency was measured as the acute endpoint after 24 h of exposure to a group of antimicrobials (Grinten et al., 2010). Cyanobacteria *M. aeuroginosa* proved to be less sensitive to OTC than the green alga *P. subcapitata*. The study proved that cyanobacteria are not ultimately more sensitive to antimicrobials than green algae. The important parameter for the comparison of the two groups was the test duration. Shortening the time of exposure for cyanobacteria leads to higher endpoint values. In the toxicity test on cyanobacteria, an exposure time of 5 instead of 7 d results in an EC_{50} value of one order of magnitude higher than after 7 d of exposure (Robinson et al., 2005).

The comparison of the short and long term results on toxicity for both photosynthetic taxonomic groups used in our study showed that the fresh water green alga *P. subcapitata* is two times more sensitive to OTC and to TMP than the cyanobacteria *A. flos-aque*. Our conclusions are in line with the results of Ando (Ando et al., 2007). In the study on the toxicity of OTC and TMP, the endpoints of a 6 d test on different cyanobacteria species can be compared with their effect on green algae. The 144 h EC_{50} for OTC was in the same concentration range for *M. aeuroginosa*, *A. flos-aque* and the green algae *P. subcapitata* (0.23 to 0.39 mg L^{-1}). OTC was less toxic for cyanobacteria *Nostoc sp.* (144 h EC_{50} 7.0 mg L^{-1}) and two species of *Synechococcus* (144 h EC_{50} 0.63 mg L^{-1}).The most sensitive to TMP was *Nostoc sp.* (144 h EC_{50} 7.0 mg L^{-1}) followed by *P. subcapitata* (144 h EC_{50} 80.3 mg L^{-1}). In that study, the long term toxicity endpoints are based on the area under the growth curve. The comparison of sensitivity of both tested photosynthetic plankton species with the published data is difficult due to differences in test duration and statistical analysis and because of the lack of demonstrated validity criteria of the test. Very few studies discuss the disappearance of the tested compound and document the concentrations used for calculations of the endpoint.

4.2. The toxicity to the aquatic invertebrate, D. magna

In our study, OTC was not toxic to *D. magna* as the calculated 48 h EC_{50} exceeded the solubility of this antimicrobial in water. The reported acute toxicity concentrations for OTC are generally substantially higher than water solubility concentrations (Wollenberger et al., 2000). According to several authors, TMP is moderately toxic to *D. magna*. The 48 h EC_{50} of 100 mg L^{-1} obtained in the presented study was not significantly lower in comparison with the 48 h EC_{50} for *D. magna* of 123 mg L^{-1} (Halling-Sorensen et al., 1998), 149 mg L^{-1} (Liguoro et al., 2009) and 167.4 mg L^{-1} (Park and Choi, 2008).

4.3. Inhibition of respiration of activated sludge

The information on the toxicity of active substances to activated sludge is needed to design the aerobic biodegradability experiments. Nevertheless, the effect on aerobic microbial community can be discussed from the view of self-purification in the assessment of surface water ecotoxicity (Straub, 2013). The TMP showed low effect on the respiration of activated sludge. The calculated EC_{50} exceeds water solubility for this substance, while the EC_{10} is in the range of 10 mg L^{-1} which indicates that the inoculum used in our study is less sensitive compared to the reported EC_{50} of 17.8 mg L^{-1} (Halling-Sørensen et al., 2000). OTC effected the respiration of activated sludge at lower concentrations compared to TMP. The OTC EC_{50} is 20 mg L^{-1} and the EC_{10} 0.2 mg L^{-1}.

5. Conclusion

In the presented study the antimicrobials oxytetracycline (OTC) and trimethoprim (TMP) were tested according to the OECD guidelines for the testing of chemicals on toxicity to cyanobacteria, to green algae, to daphnids and to activated sludge. Compared to other tested species, OTC was moderately toxic to organisms of activated sludge (EC_{50}17.9 mg L^{-1}) and not toxic to *D.magna,* while TMP showed effects to daphnids (EC_{50} 100 mg L^{-1}), but not to the microbial community of activated sludge. In the study of toxic effect on cyanobacteria we used the species *A. flos-aque* which proved to be less sensitive to the tested antimicrobials than *Microcystis aeruginosa*, which is a frequently cited cyanobacteria test species in public literature. The experiments on cyanobacteria *A. flos-aque* and on green algae *P. subcapitata* were carried out in 72 h allowing a direct comparison between the toxicity results for the tested species. The *P. subcapitata* 72 h ErC_{50} of 1.04 mg L^{-1} is an order of magnitude more sensitive to OTC than to TMP (72 h ErC_{50} 129 mg L^{-1}). The response of cyanobacteria to the exposure to both antimicrobials was similar. *A. flos-aque* showed an order of magnitude higher sensitivity to OTC (72 h ErC_{50} 2.7 mg L^{-1}) compared to TMP (72 h ErC_{50} 253 mg L^{-1}). In the study the eukaryote photosynthetic plankton green alga was more sensitive to both tested antimicrobials than prokaryote cyanobacteria; however, the difference in sensitivity was less than an order of magnitude.

A review of the literature reveals numerous results on aquatic toxicity of OTC and TMP. Algae and cyanobacteria are the most sensitive groups for antimicrobials. The comparison of study results in order to find the most sensitive species is hindered by differences in the applied test methodology, the statistical analysis in the calculation toxicity endpoints and the duration of tests.

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