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Original scientific paper

## Toxicity of five anilines to crustaceans, protozoa and bacteria\*

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**Abstract:** Aromatic amines (anilines and related derivatives) are an important class of environmental pollutants that can be released to the aquatic environment as industrial effluents or as breakdown products of pesticides and dyes. The toxicity of aniline, 2-chloroaniline, 3-chloroaniline, 4-chloroaniline and 3,5-dichloroaniline towards a multitrophic test battery comprised of bacteria *Aliivibrio fischeri* (formerly *Vibrio fischeri*), a ciliated protozoan *Tetrahymena thermophila* and two crustaceans (*Daphnia magna* and *Thamnocephalus platyurus*) were investigated. Under the applied test conditions, the toxicity of the anilines notably varied among the test species. The bacteria and protozoa were much less sensitive towards the anilines than the crustaceans:  $EC_{50}$  values 13–403 mg L<sup>-1</sup> versus 0.13–15.2 mg L<sup>-1</sup>. No general tendency between toxicity and the chemical structure of the anilines (the degree of chloro-substitution and the position of the chloro-substituents) was found in the case of all the tested aquatic species. The replacement of the artificial test medium (ATM) by the river water remarkably decreased the toxicity of anilines to crustaceans but not to protozoa. This research is part of the EU 6<sup>th</sup> Framework Integrated Project OSIRIS, in which ecotoxicogenomic studies of anilines (e.g., for *Daphnia magna*) will also be performed that may help to clarify the mechanisms of toxicity of different anilines.

**Keywords:** ecotoxicity; anilines; test battery; river water; ECOSAR.

### INTRODUCTION

Aromatic amines (anilines and related derivatives) are widely used industrial chemicals and are therefore an important class of environmental pollutants. Aniline is the parent molecule of a vast family of aromatic amines. Since its discovery in 1826, it has become one of the hundred most important building blocks in

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chemistry. Aniline and its derivatives containing chloro-substituents are used as intermediates in many different fields of applications, such as the production of isocyanates, rubber processing chemicals, dyes and pigments, agricultural chemicals and pharmaceuticals.<sup>1</sup> These compounds can be released into the surface water as industrial effluents or as break-down products of pesticides and dyes.

According to Registration, Evaluation, Authorization and Restriction of Chemicals (REACH) regulation,<sup>2</sup> all substances on the European Market, which are manufactured or imported in a quantity of 1 tonne or more per year will have to be registered by June 1, 2018. The latest evaluation made by Rovida and Hartung<sup>3</sup> in 2009 suggests that around 68,000 to 101,000 chemicals will have to be registered in the EU under the REACH regulation. This is a huge task and responsibility for industry, regulators and scientists to manage the risks that chemicals may pose to health and the environment.

This article focuses on the ecotoxicity of aniline and four of its derivatives: aniline, 2-chloroaniline (2-CA), 3-chloroaniline (3-CA), 4-chloroaniline (4-CA) and 3,5-dichloroaniline (3,5-DCA). According to European Chemical Substances Information System (ESIS) and data on chemical production from 1990–1994, aniline and 2-CA are high production volume (HPV) chemicals (placed on the EU market in volumes exceeding 1000 tonnes per year per producer or importer) and 3-CA, 4-CA and 3,5-DCA are LPV (low production volume) chemicals, *i.e.*, volumes of 10–1000 tonnes per year.<sup>4</sup> Aniline and 4-CA are classified as hazardous substances in Annex I of Directive 67/548/EEC,<sup>5</sup> whereas 2-CA, 3-CA and 3,5-DCA were not evaluated at the EU-level under previous legislation, suggesting the need to collect information on their environmental and health properties and to classify them under REACH-legislation. The (eco)toxicity data available for aniline and its derivatives show that 2-CA, 3-CA and 3,5-DCA could also be dangerous to humans and the environment. For example, according to International Agency for Research on Cancer (IARC), 4-CA is classified as possibly carcinogenic to humans. Chen *et al.*<sup>6</sup> showed that 2-CA is also potentially carcinogenic to humans. Aniline and 4-CA are also classified as dangerous for the environment according to European Chemical Substances Information System (ESIS).

The main aim of REACH is not only to provide a high level of protection of human health and the environment, but also to reduce animal testing to a minimum, to promote the use of alternative methods and to combine all sources of data<sup>7</sup> (available existing data, *in silico*, *in vitro* and *in vivo* approaches) for the assessment of the hazardous properties of substances. Thus, expectations towards *in vitro* studies and QSARs (quantitative structure-activity relationship) are very high.

In the field of aquatic toxicology, QSARs have been developed as alternative tools for predicting the toxicity of chemicals, when little or even no empirical data are available. Elaboration of SARs (structure-activity relationships) or some other computational toxicity prediction models is primarily based on experiment-

ally measured toxic effects of chemicals. Therefore, there is a direct relationship between the amount and quality of available information on toxicity of different chemicals towards different test species and adequacy of the models.

The fate and biological effects of chemicals in aquatic ecosystems depend, above all, on the chemical composition of natural water.<sup>8</sup> However, the majority of toxicity data for chemicals available for standard freshwater test organisms has been generated using standard test media, and, as a result, the available information concerning toxicity of chemicals, including anilines, in natural waters is limited. Environmentally irrelevant conditions in standard toxicity tests reduce their predictive power for environmental risk assessment.

The objectives of this study were: 1) to establish the relationship between chemical structure and the toxicity of five anilines (aniline, 2-CA, 3-CA, 4-CA and 3,5-DCA) toward different aquatic test species belonging to different trophic levels and 2) to evaluate the effect of replacement of the artificial test medium by the natural water on the toxicity test results.

## EXPERIMENTAL

### Chemicals

Aniline, 2-chloroaniline, 3-chloroaniline and 4-chloroaniline were purchased from Sigma-Aldrich and 3,5-dichloroaniline from Acros-Organics. Stock solutions (aniline – 8000 mg L<sup>-1</sup>, 2-CA – 500 mg L<sup>-1</sup>, 3-CA – 1100 mg L<sup>-1</sup>, 4-CA – 550 mg L<sup>-1</sup> and 2,3-DCA – 200 mg L<sup>-1</sup>) were prepared in MilliQ water, taking into account their solubility (Table I), and stored in the dark.

TABLE I. Selected characteristics of the five tested anilines

Chemical	CAS No.	Purity %	Measured water solubility mg L <sup>-1</sup>	Estimated water solubility <sup>a</sup> mg L <sup>-1</sup>	Measured log K <sub>ow</sub> <sup>b</sup>	Estimated log K <sub>ow</sub> <sup>c</sup>
Aniline	62-53-3	≥ 99.5	36000 (25 °C) <sup>b</sup> 34000 <sup>d</sup>	20820	0.90	1.08
2-Chloroaniline (2-CA)	95-51-2	≥ 99.5	8160 (25 °C) <sup>b</sup>	2241	1.90	1.72
3-Chloroaniline (3-CA)	108-42-9	99	5400 (20 °C) <sup>b</sup>	2331	1.88	1.72
4-Chloroaniline (4-CA)	106-47-8	98	3900 (25 °C) <sup>b</sup> 2000 <sup>e</sup>	2572	1.83	1.72
3,5-Dichloroaniline (3,5-DCA)	626-43-7	98	784 (25 °C) <sup>b</sup> 600 (26 °C) <sup>f</sup>	223	2.90	2.37

<sup>a</sup>EPI Suite™ program WSKOWWIN, v. 1.41; <sup>b</sup>U.S. EPA ECOSAR; <sup>c</sup>EPI Suite™ program KOWWIN™, v. 1.67; <sup>d</sup>Ref. 10; <sup>e</sup>Provider's Material Safety Data Sheet (MSDS) (Sigma-Aldrich); <sup>f</sup>Provider's MSDS (Acros-Organics)

### Bioassays

The toxicity of five anilines was studied toward four aquatic organisms: bacteria, protozoa and two crustaceans, using the following bioassays:

The kinetic luminescent bacteria test (modified Flash assay) with *Aliivibrio fischeri* (formerly *Vibrio fischeri*) is based on the inhibition of the light output of naturally bioluminescent bacteria by toxic compounds. The acute test (exposure time 15 min) was performed at room temperature ( $\approx 20$  °C) in 96-well microplates using a modified Flash test protocol described in Mortimer *et al.*<sup>11</sup> Reconstituted *Aliivibrio fischeri* Reagent (Aboatox, Turku, Finland) was used as the test bacteria suspension and all chemicals and their dilutions were tested in 2 % NaCl. Inhibition of bacterial bioluminescence by the tested compounds was calculated as a percentage of the unaffected control (2 % NaCl).

Daphtoxkit F<sup>TM</sup>, Thamnotoxkit F<sup>TM</sup> and Protoxkit F<sup>TM</sup> were purchased from MicroBio-Tests, Inc. (Mariakerke-Gent, Belgium) and tests were performed according to the procedures described in the instruction supplied with the corresponding Toxkits.

The 48-h acute immobilization test with the crustacean *Daphnia magna* (Daphtoxkit F<sup>TM</sup>) adhered to OECD 202 guideline. The tests with neonates less than 24 h old, obtained by the hatching of ephippia, were performed at 20 °C.

The 24-h mortality test with the crustacean *Thamnocephalus platyurus* (Thamnotoxkit F<sup>TM</sup>) was performed at 25 °C with larvae of shrimp *T. platyurus* (< 24 h old) obtained by the hatching of cysts.

The growth inhibition test (24-h) with the ciliated protozoan *Tetrahymena thermophila* (Protoxkit F<sup>TM</sup>) is based on the measurement of the population density of protozoa. Briefly, the investigated chemical and *T. thermophila* culture (strain BIII) were added to the food substrate suspension in MilliQ water. While normal proliferating protozoan culture clears the substrate suspension in the test vessels during exposure, inhibition of the growth of protozoa is reflected by the residual turbidity of the food substrate, measured as the optical density (OD) of the test samples at 440 nm. The incubation was performed at 30 °C.

The acute inhibition test (24-h) of the viability of *Tetrahymena thermophila* was conducted essentially as described in Mortimer *et al.*<sup>12</sup> Briefly, *T. thermophila* (strain BIII, the growth inhibition test) was grown axenically in nutrient medium. During the exponential growth phase ( $5 \times 10^5$  cells mL<sup>-1</sup>), the cells were harvested by centrifugation and washed with Osterhout's medium, which was also used as the test medium. The test plates with protozoa were incubated for 24 h at 25 °C without shaking. Cell viability was tested using the fluorescent dye propidium iodide (PI, Fluka) and by measuring the ATP content of the cellular suspensions using the luciferin–luciferase method.

To prevent potential photolytic breakdown of anilines the exposure of protozoan and crustacean tests were conducted in the dark.<sup>13</sup>

The EC<sub>50</sub> values were determined using Regtox software for Microsoft Excel.<sup>14</sup> The average EC<sub>50</sub> values and standard deviations (SD) were calculated from 3–5 independent experiments, each in several replicates (four for *D. magna*, three for *T. platyurus*, and two for *T. thermophila* and *A. fischeri*).

#### Test media

The artificial test medium – ATM (test medium used in the standard test procedure) in the crustacean assays had the following composition (mg L<sup>-1</sup>): for *D. magna* - CaCl<sub>2</sub>·2H<sub>2</sub>O, 294; MgSO<sub>4</sub>·7H<sub>2</sub>O, 123.25; NaHCO<sub>3</sub>, 64.75; KCl, 5.75; pH 7.8 ± 0.2 and for *T. platyurus* - CaSO<sub>4</sub>·2H<sub>2</sub>O, 60; MgSO<sub>4</sub>·7H<sub>2</sub>O, 123; NaHCO<sub>3</sub>, 96; KCl, 4; pH 7.8±0.2, dissolved in MilliQ water. MilliQ water or Osterhout's medium (NaCl, 104; MgCl<sub>2</sub>, 8.5; MgSO<sub>4</sub>, 4; KCl, 2.3; CaCl<sub>2</sub>, 1 mg L<sup>-1</sup>; pH 6.6, dissolved in MilliQ water) were used as the standard test medium for *T. thermophila*, and a 2 % solution of NaCl for *A. fischeri*. Thus, the ATM used in the assays did not contain any organic compounds.

Natural waters were sampled from a well (subsurface water) in a small village in northern Estonia and from the River Jägala (Estonia). Chemical analyses of the natural water samples (Table II) were performed using standard analytical methods in an accredited laboratory.

TABLE II. Characterization of the natural waters used as test media

Parameter	Unit	Water from the well	Water from the River Jägala
pH	–	7.5	8
Conductivity	µS	156	282
DOC <sup>a</sup>	mg C L <sup>-1</sup>	9.6	16.1
BOD <sub>7</sub> <sup>b</sup>	mg O <sub>2</sub> L <sup>-1</sup>	1.4	1.6
Nitrate	mg N L <sup>-1</sup>	0.27	2.3
Phosphate	mg P L <sup>-1</sup>	0.195	0.018
Ca <sup>2+</sup>	mg L <sup>-1</sup>	33	68
HCO <sub>3</sub> <sup>-</sup>	mg L <sup>-1</sup>	96.4	192.2
SO <sub>4</sub> <sup>2-</sup>	mg L <sup>-1</sup>	4	25
Fe <sub>tot</sub>	mg L <sup>-1</sup>	0.21	0.76

Before the biotesting, suspended solids and plankton were separated from the water samples by filtration through a 0.45 µm pore size standard filter (Millipore).

#### *Use of ECOSAR for predicting the aquatic toxicity of anilines*

The toxicity of the anilines ( $EC_{50}$ ) to *D. magna* were calculated using the ECOSAR model – a computerized predictive system used by the United States Environmental Protection Agency (US EPA) to estimate the aquatic toxicity of industrial chemicals. The ECOSAR model uses Structure Activity Relationships (SARs) for the prediction of the aquatic toxicity of untested chemicals based on their structural similarity to chemicals for which aquatic toxicity data are available. The SARs in the ECOSAR model express correlations between the physico-chemical properties and aquatic toxicity of a compound within specific chemical classes. ECOSAR version 1.00a (February 2009), downloadable from the US EPA website,<sup>9</sup> was used in the current study.

## RESULTS AND DISCUSSION

The results of the toxicity testing of the five anilines using the above-listed bioassays in ATM (the respective artificial test medium) are presented in Table III. The experimental data on the toxicity of the investigated anilines are comparable with the data published by other authors (Table IV).

It should be mentioned that the 48-h  $EC_{50}$  values for *D. magna* available in the literature vary considerably. However, when averaged (Table IV), these data are in agreement with the present results (Table III). Unfortunately, no information on the toxicity of the anilines to *T. platyurus* and *T. thermophila* could be found, but the toxicity of investigated anilines to close protozoan species *T. pyriformis* (Table IV) were comparable to the present data (Table III). In the current study, much higher  $EC_{50}$  values were obtained in the acute inhibition test (exposure of protozoa during 24 h with no food added, see Experimental) than in the growth inhibition test with *T. thermophila* (Table III). Exposure of *T. thermophila* to aniline in the acute inhibition test yielded the following  $EC_{50}$  values:

2007 mg L<sup>-1</sup>, measured with propidium iodide, and 2140 mg L<sup>-1</sup>, according to the measurement of the ATP level. Considering that the EC<sub>50</sub> value from the acute inhibition test is over 5 times higher than the EC<sub>50</sub> value of the growth inhibition test (2007 vs. 358 mg L<sup>-1</sup>), it can be assumed that in case of toxicity testing of aniline, the growth inhibition test of *T. thermophila* is more relevant than the acute inhibition test. The difference in the EC<sub>50</sub> values of the two test formats could be attributed to the mode of action of aniline, which has been classified as a polar narcotic which exerts non-covalent bioreactivity by disturbing the structure and functioning of biomembranes.<sup>19</sup> As a result of the slow narcotic mechanism of action, aniline probably inhibits the normal functioning of the cell, including cell proliferation, but does not kill the cells during that time, rendering the mortality endpoint (propidium iodide assay) less sensitive. However, this supposition has to be verified.

TABLE III. Toxicity of anilines (EC<sub>50</sub>, mg L<sup>-1</sup>, mean±SD) towards four aquatic species tested in ATM

Compound	Exposure time			
	24 h	15 min	48 h	24 h
	Protozoa <i>Tetrahymena thermophila</i> <sup>a</sup>	Bacteria <i>Aliivibrio fischeri</i>	Crustacean <i>Daphnia magna</i>	Crustacean <i>Thamnocephalus platyurus</i>
Aniline	358±180	403±101	0.13±0.04	2.8±0.6
2-Chloroaniline	252±16	43±19	1.2±0.4	15.2±4.5
3-Chloroaniline	135±9.0	59±14	0.24±0.07	2.0±0.6
4-Chloroaniline	36±3.5	13±0.5	0.19±0.04	4.4±1.1
3,5-Dichloroaniline	29±2.4	36±3.8	0.48±0.24	3.9±0.8

<sup>a</sup>Growth inhibition test (Protoxkit F™)

TABLE IV. EC<sub>50</sub> values (mg L<sup>-1</sup>) for the five anilines published by other authors

Chemical	<i>Tetrahymena pyriformis</i>	<i>Aliivibrio fischeri</i> ( <i>V. fischeri</i> , <i>P. phosphoreum</i> ) <sup>a</sup>	<i>Daphnia magna</i> <sup>b</sup>
Aniline	158.1 <sup>c</sup> 190 <sup>d</sup>	69 (15 °C) 488 (15 °C)	0.39±0.23
2-Chloroaniline	188.7 <sup>c</sup> 200 <sup>d</sup>	15 (15 °C) 36.5 (20 °C)	0.94±0.68
3-Chloroaniline	76.9 <sup>c</sup> 100 <sup>d</sup>	13.4 (15 °C) 39.5 (20 °C)	0.23±0.13
4-Chloroaniline	113.7 <sup>c</sup> 10 <sup>d</sup>	3.77 (15 °C) 21 (20 °C)	0.24±0.13
3,5-Dichloroaniline	31.6 <sup>c</sup>	10.7 (15 °C)	1.16±0.06

<sup>a</sup>Ref.15, exposure time 15 min; testing temperature indicated in the brackets; <sup>b</sup>mean±STD from U.S. EPA ECOSAR and Ref.16, exposure time 48 h; <sup>c</sup>Ref.17, exposure time 40 h; <sup>d</sup>Ref.18, exposure time 24 h

The toxicity of investigated anilines varied notably among the test species (Table III). All tested compounds were remarkably more toxic (10–100 times) to



crustaceans than to bacteria and protozoa (both unicellular organisms). *D. magna* was the most sensitive species. Other authors<sup>20,21</sup> also showed that *D. magna* was more sensitive than other aquatic species, *i.e.*, algae and fish, to anilines. Although it was previously demonstrated that *T. platyurus* can be more sensitive than *D. magna*, *e.g.*, to pyrene<sup>22</sup> and insecticides,<sup>23</sup> in case of anilines, *D. magna* was about an order of magnitude more sensitive than *T. platyurus*. It should be emphasized, however, that the acute assays with the two crustacean test species used different exposure times (24-h for *T. platyurus* vs. 48-h for *D. magna*) which could explain the different results obtained. The high sensitivity of *D. magna* to aromatic amines, compared to other crustaceans, was also shown by Ramos *et al.*<sup>24</sup>

Thus, the present study confirms that extrapolation of toxicity data from one species to another (even if the species are taxonomically similar) could lead to incorrect deductions.

#### *Relationship between toxicity and the chemical structure of the anilines*

There was no common relationship between the toxicity and chemical structure of the anilines (the degree of chlorosubstitution and the position of chloro-substituents) for all the tested aquatic species (Table III). In case of protozoa, the toxicity of anilines depended on the position of chloro-substituents and increased in accordance with the degree of chlorosubstitution, with aniline ( $EC_{50} = 358 \text{ mg L}^{-1}$ ) being about 12-fold less toxic than 3,5-DCA ( $EC_{50} = 29 \text{ mg L}^{-1}$ ). Aniline was also approximately 10-fold less toxic than the substituted anilines to the bacteria *A. fischeri* ( $403 \text{ mg L}^{-1}$  vs.  $13\text{--}59 \text{ mg L}^{-1}$ ; Table III). As mentioned above, both crustaceans and especially *Daphnia magna* were remarkably (up to 3 orders of magnitude) more sensitive towards anilines than protozoa and bacteria. For both crustaceans, it was difficult to recognize a clear relationship between toxicity and the chemical structure of the tested compounds. Interestingly, for both crustaceans, 2-CA was noticeably more toxic than the other four tested anilines. This indicates that, regardless of the different sensitivity of two species, the mechanism of action of anilines is probably the same for both crustaceans.

A comparison of the present results with the predicted toxicity values for *D. magna* obtained with the ECOSAR model (experimentally obtained octanol-water partitioning coefficient,  $K_{ow}$ , values were used for the calculations, Table I) shows that the predictive power of the ECOSAR model, at least in case of anilines, is limited. Moreover, the ECOSAR model under predicted the toxicity of four anilines by almost one order of magnitude (Fig. 1).

As a rule, there is a correlation between the toxicity of an organic chemical and its  $K_{ow}$  value: the higher the  $\log K_{ow}$ , the lower the  $L(E)C_{50}$  value, *i.e.*, the higher the toxicity. For example, in previous studies on MEIC chemicals, a good correlation was shown between the toxicity of 24 MEIC chemicals to photobacteria and their  $K_{ow}$  value; the correlation coefficient of the linear regression

(log–log) was  $-0.84$ ).<sup>25</sup> Lee *et al.*<sup>26</sup> showed that the toxicity of 16 phenols toward *Selenastrum capricornutum* and *D. magna* was closely related to the log  $K_{ow}$  values. In the current study, this trend was observed for 5 tested anilines in the case of protozoa and bacteria. However, the most toxic compound to crustaceans was aniline, which is the least hydrophobic of the five tested compounds (Fig. 2).

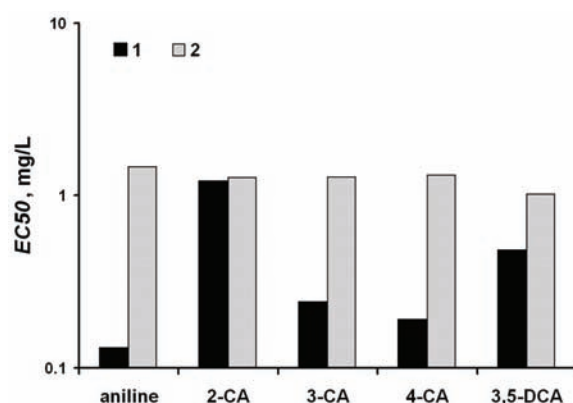


Fig. 1. Toxicity of anilines to crustacean *Daphnia magna*: measured (1) and predicted by ECOSAR (2). Note the logarithmic y-scale.

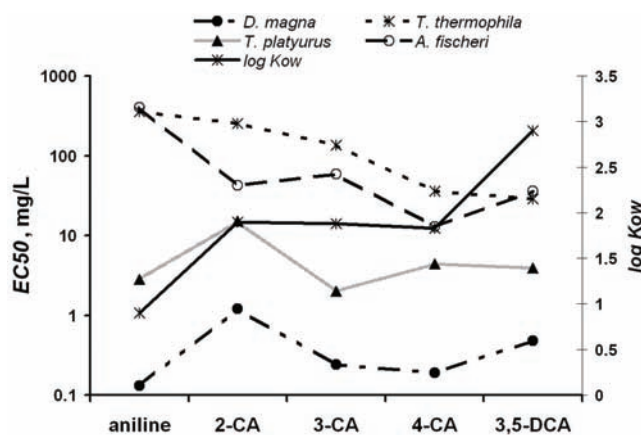


Fig. 2. Toxicity of anilines to the four test species ( $EC_{50}$  values obtained in the current study) vs. log  $K_{ow}$ .

As was shown above, the existing tools for the prediction of the toxicity of aniline (ECOSAR) to aquatic species yields inaccurate toxicity data. There are many reasons why the predictive power of QSAR models is not reliable. Firstly and most importantly, experimentally determined physico-chemical properties should be used to develop QSARs. Secondly, the descriptors should be selected very carefully and the toxicity of chemicals should be predicted by more than one descriptor. Certainly, QSAR models are rapid and cost-effective methods, which can



be used as important alternative screening tools for prioritising and predicting the toxicity of untested chemicals, but it must be born in mind that the calculated values may differ considerably from the experimental ones.

#### *Modulation of the toxicity of anilines in natural water*

There are an increasing number of studies showing the modulating effect of the composition of natural water on the toxicity of different chemicals, mostly heavy metals but also metal oxide nanoparticles.<sup>27</sup> The presence of humic compounds in natural water may also modulate the toxicity of organic chemicals. For example, it was shown that dissolved humic materials (DHM) significantly reduced the toxicity of 4-CA to *D. magna*, but the effect of DHM on the toxicity of 4-CA to zebrafish (*Brachydanio rerio*) was not observed.<sup>28</sup> In the present study, the effect of natural water on toxicity of anilines to bacteria, protozoa and crustaceans was evaluated.

It is known that photolysis and microbial degradation are the most important degradative processes affecting anilines in aquatic environments.<sup>29</sup> To prevent breakdown of the chemical structures by photolysis, the exposure of protozoa and crustaceans to the anilines was realised in the dark (see Experimental).<sup>13</sup> In addition, it was previously shown<sup>29,30</sup> that during short incubation periods (up to 3 days in the dark), there was no measurable microbial degradation of aniline and the chloroanilines in natural water. Therefore, it could be presumed that in the short-term tests performed in the current study, the tested compounds remained stable and that the differences between the results obtained with ATM and natural water indicate the impact of water composition on the bioavailability of anilines to different aquatic species.

The mitigation effect of natural water on the toxicity of anilines to four test species is presented in Table V. The tests organisms were exposed to the anilines at concentrations that were close to the  $EC_{50}$  values obtained in the respective standard test media (Table II). The results are presented as a ratio of the toxic effect (%) in natural waters and in ATM (Table V). Thus, values lower than one indicate a decrease of toxicity in natural water and values exceeding one, accordingly, indicate an increase in toxicity. For example, when the immobilization of *D. magna* exposed to 2-CA at a concentration  $0.2 \text{ mg L}^{-1}$  in ATM was 80 % and in natural water only 40 %, the toxicity in natural water decreased 2 times ( $40/80 = 0.5$ ).

In general, the effect of natural water on the toxicity of anilines was minimal. However, some tendencies were observed: *i*) different to particle-feeding organisms (protozoa and crustaceans), the toxicity of anilines to bacteria was practically the same in natural water and ATM and *ii*) toxicity of anilines for protozoa *T. thermophila* and crustacean *T. platyurus* seemed to be slightly increased when exposed in natural water, and for *D. magna*, natural water slightly decreased the toxic effect of chloroanilines (but not of aniline). These data are in accordance with the data of Lee *et al.*<sup>28</sup> (see above). However, the data on the other crus-

tacean *T. platyurus* did not confirm this tendency (Table V). This discrepancy may be explained by the different sensitivity of the test species to background pollution. It seems that in case of anilines, the mitigation effect of natural water on toxicity to crustaceans depended mainly on the integrated effect of the water composition (including background pollution) and tested chemical, but not on the dissolved organic matter (DOC) content. Thus, the current data on anilines are different from the data of a previous study on the effect of natural waters on the toxicity of CuO nanoparticles to *D. magna* and *T. platyurus*, in which it was shown that natural waters remarkably (up to 100-fold) decreased the toxicity of nano-CuO to both crustaceans and this effect depended mainly on the DOC concentration.<sup>27</sup>

TABLE V. The ratio between the toxicity of anilines in natural water (NW) and artificial test medium (ATM) tested at the same concentrations (effect in NW / effect in ATM)

Compound	<i>Tetrahymena thermophila</i> <sup>a</sup>		<i>Aliivibrio fischeri</i>		<i>Daphnia magna</i>		<i>Thamnocephalus platyurus</i>	
	Well	River	Well	River	Well	River	Well	River
Aniline	2.4	2.2	1.1	1	2.1	1.9	1.8	1.5
2-Chloroaniline	1.3	1.5	1.2	1.1	0.6	0.5	0.8	1
3-Chloroaniline	0.95	1.2	0.9	0.85	0.7	0.5	2.2	1.9
4-Chloroaniline	1.2	1.6	0.8	0.9	0.7	0.7	0.8	0.9
3,5-Dichloroaniline	1.8	2.4	1.1	1.1	0.3	0.5	1.1	1.3

<sup>a</sup>Growth inhibition test (Protoxkit F<sup>TM</sup>)

## CONCLUSIONS

It may be concluded that the opinion stated 15 years ago: "...at present no prediction about the behaviour of a previously untested chemical can be made, which is based on the physico-chemical or structural properties of the organic chemical."<sup>28</sup> – is still valid, at least in the case of anilines.

QSARs can be used as an initial evaluation of the toxicity of a chemical, however, tests with bioassays must be performed for confirmation.

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## ИЗВОД

### ТОКСИЧНОСТ ПЕТ АНИЛИНСКИХ ЈЕДИЊЕЊА ПРЕМА ЉУСКАРИМА, ПРОТОЗОАМА И БАКТЕРИЈАМА

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Ароматични амини (анилини и деривати) су важна класа загађујућих супстанци које могу бити испуштене у животну средину као индустријски ефлуенти или као производи раз-

градње пестицида и боја. Испитали смо токсичност анилина, 2-хлоранилина, 3-хлоранилина, 4-хлоранилина, и 3,5-дихлоранилина за мултитрофичну тест батерију која се састоји од бактерија *Aliivibrio fischeri* (раније *Vibrio fischeri*), протозоа бичара *Tetrahymena thermophila* и два љускара (*Daphnia magna* и *Thamnocephalus platyurus* уурус). У примењеним условима токсичност анилина је приметно варирала међу тестираним врстама. Бактерије и протозое су биле много мање осетљиве према анилинима него љускари: вредности  $EC_{50}$  су биле 13–403  $mg L^{-1}$  према 0,13–15,2  $mg L^{-1}$ . Није откривен никакав општи тренд између токсичности и хемијске структуре анилина (степен супституције хлора и позиција хлорних супституената) ни у једном случају тестираних водених врста. Замена вештачког тест медијума (АТМ) речном водом уочљиво је смањила токсичност анилина за љускаре, али не и за протозое. Ово истраживање је део интегрисаног пројекта OSIRIS у оквиру европског FP6 програма, у коме ће се спровести и екотоксикогеномске студије анилина (нпр. за *D. magna*), које могу помоћи у разјашњавању механизма токсичности различитих анилина.

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