University of South Bohemia in České Budějovice
Faculty of Science

Degradation of atrazine by homogeneous photocatalysis using Fe(III)/UV/air system and evaluation of potential toxicity of atrazine and its metabolites

Master thesis

Lucie Keltnerová

Supervisor: Doc. RNDr. Šárka Klementová, CSc.

České Budějovice 2016

Keltnerová, L., 2016: Degradation of atrazine by homogeneous photocatalysis using Fe(III)/UV/air system and evaluation of potential toxicity of atrazine and its metabolites. Mgr. Thesis, in English. – 33 pp., Faculty of Science, University of South Bohemia, České Budějovice, Czech Republic.

Annotation

Atrazine photochemical degradation in homogeneous phase using Fe(III)/UV/air system was studied. Two toxicity assessments, a *Lemna minor* growth inhibition test and a *Daphnia magna* acute immobilisation test, were employed to test potential toxicity of atrazine and its degradation products. The occurrence of atrazine in rivers from the Vltava River basin was evaluated from the analyses performed by Povodí Vltavy, State Enterprise.

Prohlášení

Prohlašuji, že svoji diplomovou práci jsem vypracovala samostatně pouze s použitím pramenů a literatury uvedených v seznamu citované literatury.

Prohlašuji, že v souladu s § 47b zákona č. 111/1998 Sb. v platném znění souhlasím se zveřejněním své diplomové práce, a to v nezkrácené podobě elektronickou cestou ve veřejně přístupné části databáze STAG provozované Jihočeskou univerzitou v Českých Budějovicích na jejích internetových stránkách, a to se zachováním mého autorského práva k odevzdanému textu této kvalifikační práce. Souhlasím dále s tím, aby toutéž elektronickou cestou byly v souladu s uvedeným ustanovením zákona č. 111/1998 Sb. zveřejněny posudky školitele a oponentů práce i záznam o průběhu a výsledku obhajoby kvalifikační práce. Rovněž souhlasím s porovnáním textu mé kvalifikační práce s databází kvalifikačních prací Theses.cz provozovanou Národním registrem vysokoškolských kvalifikačních prací a systémem na odhalování plagiátů.

....

V Českých Budějovicích dne Podpis

Poděkování

Ráda bych touto cestou poděkovala mé školitelce Doc. RNDr. Šárce Klementové, CSc. za vedení práce, cenné rady při provádění experimentů a zejména za její přátelský přístup a ochotu s čímkoliv pomoci.

Dále chci poděkovat RNDr. Michalu Šorfovi, Ph.D. za pomoc a velkou vstřícnost při provádění testů toxicity.

Děkuji také Ing. Davidu Kahounovi za provedení LC-MS analýz.

Rovněž děkuji Povodí Vltavy s. p. za poskytnutí dat měřených koncentrací atrazinu a jeho produktů v řekách povodí Vltavy.

Děkuji Davidovi Klementovi za vyhotovení obrázku Fig. 1.

Na závěr děkuji Ondřeji Hornychovi za pomoc s korekturou textu této práce.

Table of contents

1 Introduction

1.1 Basic facts about atrazine

For several last decades a considerable amount of pesticide has been used in agricultural production and other applications. Among the most frequently used pesticides are s-triazine herbicides and atrazine plays a major role in this group (Scribner et al., 2005).

Atrazine (6-chloro-N-ethyl-N´-(1-methylethyl)-1,3,5,-triazin-2,4-diamin) is a white, crystalline compound used for control of weed growth on agricultural soils, along roadways as well as on private lawns (Olsberg, 2007). Since its introduction by the Syngenta Corporation in 1959 on the US market, atrazine has become one of the most used herbicides in the USA and in many other countries. It is estimated that as many as 76.5 million pounds of atrazine are used in the USA every year (Land Stewardship project & Pesticide action network, 2010), with 85% used on corn cultivation (Saas & Colangelo, 2006).

Atrazine has been demonstrated to be a persistent chemical compound resistant to chemical and biological degradation. Specifically, approximate half-life of atrazine is 146 days in aerobic soils and as many as 742 days in freshwater (Saas & Colangelo, 2006). Slow degradation of atrazine alongside with washing and leaching processes results in its inflow into surface and ground waters. Indeed, the presence of atrazine has been confirmed in 75% of streams and 40% of ground-water reservoirs in the USA (Scribner et al., 2005; Saas & Colangelo, 2006) and, also, atrazine was detected at least in trace amounts in 68% of sampled European rivers (Loos et al., 2009).

1.2 Potential harmful effects of atrazine

Because of the presence of atrazine in natural waters including sources of drinking water, scientists have begun to investigate potential harmful effects of atrazine on animal and human health.

Several studies reported higher incidence of breast carcinoma at Sprague-Dawley rats after being exposed to atrazine (Mayhew, 1986; Morseth, 1998) and the influence of atrazine on the length of their estrous cycle (Wetzel et al, 1994; Morseth, 1998).

O´Connor et al. (1987) studied the effects of ingestion of atrazine on dogs. Results suggested that higher doses of atrazine (about 1000 ppm) could cause severe heart disorders at dogs, such as cardiomyopathy.

Sanderson et al. (2000) found that atrazine can alter natural function of CYP19 aromatase, which is responsible for the conversion of androgens to estrogens. Atrazine

is able to increase the rate of this conversion, which can result in the disruption of the endocrine system, higher incidence of some tumours or male feminization. Male feminization induced by atrazine exposure has been observed by Hayes et al. (2002) in several species of frogs.

In addition to studies conducted on animals, there have been various studies and surveys investigating potential harmful effects of atrazine on human health. Van Leeuwen et al. (1999) reported a close connection between atrazine in drinking water (in concentration 50 – 649 ng/l) and gastric cancer. However, there are several studies which did not confirm the positive connection between exposition of humans to atrazine and various types of cancer, e.g. breast, colon, bone, lung or prostate cancer (Van Leeuwen et al., 1999; Hopenhayn-Rich et al., 2002; Muir et al., 2004; Rusiecki et al., 2004; Thorpe & Shirmohammadi, 2005; Mills & Yang, 2006). Thus, carcinogenic effects of atrazine remain unclear.

1.3 The restriction of atrazine application

Reported negative influence of atrazine on animal and human health triggered numerous discussions on whether atrazine should be added among prohibited substances. The result was that the European Union announced a ban on atrazine usage in 2005, while the US Environmental Protection Agency approved its continued use due to lack of appropriate data (Saas & Colangelo, 2006). According to EEC Directive from 2004, the concentration of atrazine in drinking water should not exceed 0.1 μg/l and the limit of atrazine in the mixture with other pesticides is 0.5 μg/l (European Commission Directive, 2004).

1.4 Photochemical degradation of atrazine

Due to the continuous use of atrazine in the USA and especially in large amounts in developing countries as well as emerging economies, there have been plentiful efforts to examine degradation mechanisms of atrazine in natural waters or to develop efficient methods of atrazine removal from polluted and waste waters.

Among the most efficient methods of pesticide removal are Advanced Oxidation Processes which include photocatalytic oxidation reactions (Malato & Agüera, 2004). Photocatalytic oxidation comprises two types: homogeneous and heterogeneous.

Heterogeneous photocatalysis is based on the use of a semiconductor as a catalyst present in the solid state. After being irradiated by light of suitable wavelength, the semiconductor is activated, which means that electrons from the valence band are excited into the conduction band. Thus a pair of an electron and a hole is created. These electrons and holes then generate (in reaction with dissolved oxygen and water) reactive oxygen species, e.g. $OH^{\bullet}$, $O_2^{\bullet -}$, $HO_2^{\bullet}$, responsible for substrate oxidation. The most frequently used semiconductors are TiO_2 and ZnO (Canle et al., 2005).

Fenoll et al. (2012) studied photocatalytic degradation of atrazine by TiO_2 and ZnO. Results showed that after 4 hours of irradiation by UV light, 85% and 30% of initial concentration of atrazine was removed using ZnO and TiO_2, respectively.

Homogeneous photocatalysis differs from the heterogeneous type in the phase state of the catalyst. The catalysts are metal ions dissolved in the reaction solution; they are transformed to the catalytically active state when irradiated by light of suitable wavelength. The example can be the photochemical reduction of ferric ions to ferrous ions in photoinitiated degradation of triazines and phenyl-urea pesticides (Klementová, 2011). The most often used system is the so called Fenton agent, which is a mixture of ferrous ions and hydrogen peroxide. A photochemical modification of the Fenton agent is a mixture of ferric ions and hydrogen peroxide called photo-Fenton agent (Canle et al., 2005; Farré et al., 2005); in this system ferrous ions are formed by photochemical reduction of ferric ions *in situ*. Copper or manganese ions were also investigated but they seem to be less efficient in comparison with ferric ions (Klementová & Hamsová, 2000).

There are several photo-Fenton-like systems which can be used for the degradation of pesticides, namely Fe(III)/H_2O_2/UV-VIS, Fe(II)/H_2O_2/UV-VIS, Fe(III)/UV-VIS and H_2O_2/UV-VIS (De Laat et al., 1999; Derbalah et al., 2004; Du et al., 2009; Kassinos et al., 2009). All of these systems are based on the production of $OH^{\bullet}$ which oxidize the substrate; but differ in the mechanism of $OH^{\bullet}$ generation.

In Fe(III)/H_2O_2/UV-VIS system $OH^{\bullet}$ are generated by several photochemical reactions (Derbalah et al., 2004).

First, ferric ions undergo photoreduction (Eq. (1)).

$$Fe^{3+} + H_2O + h\upsilon \rightarrow Fe^{2+} + OH^- + OH^{\bullet} \quad (1)$$

Then, ferrous ions react with hydrogen peroxide to form other $OH^{\bullet}$ radicals (Eq. (2)). This reaction is called photo-Fenton reaction.

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH^- + OH^\bullet \quad (2)$$

Finally, hydrogen peroxide undergoes photolysis which leads to $OH^\bullet$ generation (Eq. (3)).

$$H_2O_2 + h\upsilon \rightarrow 2\ OH^\bullet \quad (3)$$

Moreover, ferric ions often form complexes with carboxylic acids, e.g. oxalate, and these complexes undergo photodecomposition which results in the production of ferrous ions (Eq. (4)). Ferrous ions then react in photo-Fenton reaction, thus, this reaction indirectly enhance the rate of $OH^\bullet$ generation.

$$Fe^{3+}\text{-}(RCO_2^-) + h\upsilon \rightarrow Fe^{2+} + \bullet RCO_2^- \quad (4)$$

The mechanism of Fe(II)/H_2O_2/UV-VIS system is similar to the previous one, however, the first reaction (Eq. (1)) is skipped. In Fe(III)/UV-VIS system $OH^\bullet$ radicals are produced merely by photodecomposition of Fe^{3+} and in H_2O_2/UV-VIS system $OH^\bullet$ radicals are generated by photolysis of hydrogen peroxide (Derbalah et al., 2004).

The very important aspect of homogeneous photocatalysis is the reoxidation of ferrous ions to ferric ions which is responsible for the disappearance of the catalytically active form. Du et al. (2009) studied the mechanism of reoxidation of ferrous ions in the presence of air. Reoxidation appeared to be a complex of several reactions in which two reactive species, namely $HO_2^\bullet$ and H_2O_2, are responsible for oxidation of ferrous ions to ferric ions (Appendix I).

1.5 Kinetics of homogeneous photocatalysis of atrazine and degradation products

Du et al. (2009) investigated the kinetics of homogeneous photocatalysis of atrazine in the presence of Fe and the results showed that it followed pseudo-first-order kinetics with respect to the concentration of atrazine. Pseudo-first-order kinetics describe a system in which one of the reactants, in this case OH• radicals, in the chemical reaction is overabundant and its concentration change during the reaction can be neglected. Therefore, the reaction rate is only affected by the organic substrate and first-order kinetics behaviour is observed. After 15 minutes of irradiation using Fe(III)/UV/air system,

64.4% of initial atrazine concentration was degraded; this corresponds with the rate constant equal to 6.5×10^{-2} min^{-1}.

Huston & Pignatello (1999) studied the removal of atrazine by Fe(III)/H_2O_2/UV system. The amount of degraded atrazine was 98.8% after 30 minutes of irradiation and the amount of removed TOC (total organic carbon) was 46.5% after 120 minutes of irradiation. The rate constant of the reaction was equal to 399.4×10^{6} min^{-1}.

The study on degradation of atrazine by Fe(II)/H_2O_2/UV system by Kassinos et al. (2009) showed that atrazine was completely eliminated in the first 5 minutes of the oxidation process. However, the remaining concentration of TOC was 43% after 2 hours of atrazine photodecomposition, which suggests that atrazine is not mineralised to CO_2, but transformed into compounds retaining the triazine ring which is resistant to oxidation. Results of all these studies suggest that the Fe(III)/UV/air system is least effective while systems with H_2O_2 are quite similar; differences in the rate of atrazine degradation can be caused by different reaction conditions.

As mentioned above, atrazine is transformed into various compounds during photocatalytic oxidation. Degradation of atrazine consists of several processes, such as side-chain oxidation, dealkylation, dehalogenation and deamination (Minero et al., 1992; Scribner et al., 2005). The whole degradation pathway for atrazine (as proposed by Scribner et al., 2005) is illustrated in Fig. 1.

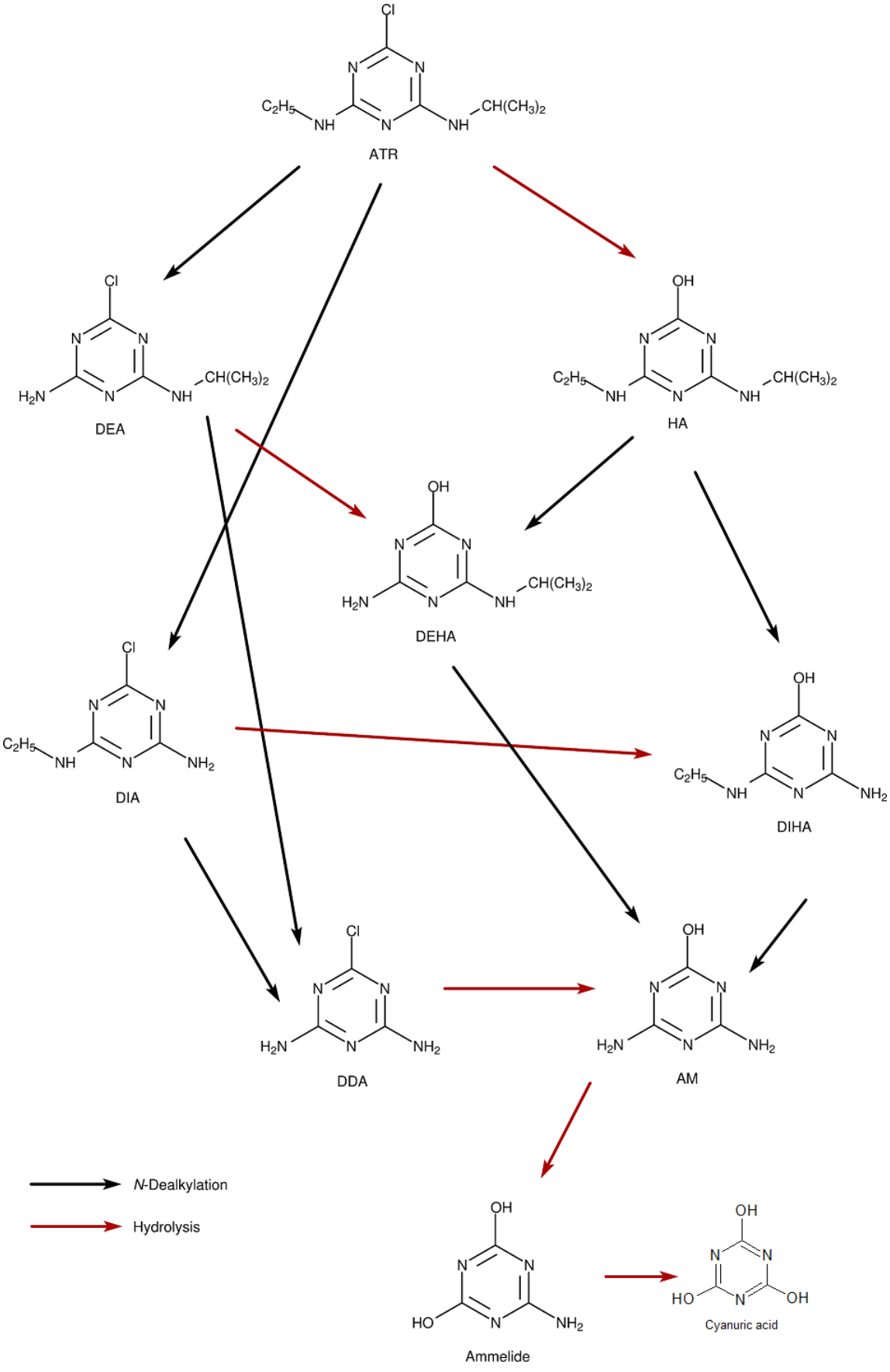

Fig. 1. Degradation pathways for atrazine.

The first-step dealkylation and formation of hydroxyatrazine are quite rapid processes. In contrast, further dehalogenation and especially deamination, leading to the formation of cyanuric acid, are very slow processes demanding long-term irradiation (Konstantinou & Albanis, 2003). Cyanuric acid has been reported as the final product in photochemical degradation of triazines because of its chemical stability (Minero et al., 1992).

Evgenidou & Fytianos (2002) investigated atrazine metabolites (i.e. degradation products) emerging during homogeneous photocatalysis of atrazine. Two dealkylated photoproducts, desethyl-atrazine and desisopropyl-atrazine, and one dechlorinated photoproduct, hydroxyatrazine have been found in the reaction solution.

1.5 Toxicity assessment of atrazine

In order to evaluate the efficiency of degradation methods for pollutants, it is worth coupling them with toxicity assessments because some degradation products may be more toxic than the parent compound (Tixier et al., 2002).

Widely used toxicity assessments are those based on microorganisms, mainly because of the short exposition time, relative ease of manipulation with the organism or result reproducibility in laboratories (Lapertot et al., 2008). One of the most frequently employed toxicity assessments is Microtox© which uses a bacterium *Vibrio fischeri*. In this test, the inhibition of bioluminescence is measured (Bulich, 1979).

Kross et al. (1992) studied the luminescent inhibition of *Vibrio fischeri* caused by atrazine and its metabolites, desethyl-atrazine and desisopropyl-atrazine. Measured EC_{50} (50% effective concentration = 50% inhibition of *V. fischeri* luminescence) values were 75, 675 and 360 mg/l for atrazine, desethyl-atrazine and desisopropyl-atrazine, resp. The results show that both atrazine metabolites are less toxic than atrazine itself. A similar EC_{50} for atrazine, namely 89 mg/l, has been reported in a study by Lapertot et al. (2008). Tchounwou et al. (2000) also investigated the toxicity of atrazine and its metabolites using *Vibrio fischeri*; the EC_{50} values for atrazine, desethyl-atrazine, desisopropyl-atrazine and desethyl-desisopropyl-atrazine were 39.87; 81.86; 82.68 and 12.74 mg/l, resp. Though the measured values are lower than that from Kross et al. (1992) or Lapertot et al. (2008), both studies show that desethyl-atrazine and desisopropyl-atrazine are less toxic than atrazine. In contrast, the didealkylated metabolite, desethyl-desisopropyl-atrazine, seems to be more toxic for *Vibrio fischeri* than atrazine itself.

Other organisms can be used for assessing the toxicity of atrazine. For instance, growth inhibition of a bacterium *Escherichia coli* (Baek & An, 2011), immobilization of a crustacean *Daphnia magna* (Palma et al., 2008), FDA-esterase activity of a ciliate *Tetrahymena pyriformis* (Bogaerts et al., 2001) or mortality of a crustacean *Thamnocephalus platyurus* (Palma et al., 2008) have been used for toxicity assessment. In a study from 2008, Palma et al. compared the acute toxicity of atrazine for *Vibrio fischeri*, *Daphnia magna* and *Thamnocephalus platyurus*. The results showed comparable sensitivity to atrazine in *Daphnia magna* (EC_{50} = 35.5 mg/l) and *Thamnocephalus platyurus* (EC_{50} = 36.7 mg/l), while *Vibrio fischeri* was less sensitive (EC_{50} = 69.4 mg/l).

Apart from microorganisms and animals, algae and water macrophytes are often used for testing toxicity of pollutants. Commonly tested are the green alga *Raphidocelis subcapitata* (Ma et al., 2006) and the water vascular plant *Lemna* sp. (Fairchild et al., 1997; Fairchild et al. 1998), inhibition of their growth is measured.

Sensitivity of these two organisms to atrazine is compared by Fairchild et al. (1997). The values of EC_{50} were 153 and 235 μg/l for *Lemna minor* and *Raphidocelis subcapitata*, resp. In another study by Fairchild et al. (1998), the toxicity of atrazine for 6 algae species and 6 vascular plant species was tested. Of all the studied species, *Raphidocelis subcapitata* and *Lemna minor* have comparatively lower sensitivity to atrazine, while the alga *Microcystis* and the vascular plant *Elodea* are the most sensitive.

These distinct results of studies mentioned above indicate that a suite of species should be tested to reliably assess the effects of atrazine and its metabolites on the environment.

1.6 The aims of this thesis

In the first part, degradation of atrazine by homogeneous photocatalysis is described. Additionally, toxicity of atrazine and its metabolites is assessed using *Lemna minor* and *Daphnia magna* assays.

In the second part, the occurrence of atrazine in several rivers of Vltava River basin is studied to evaluate the impact of past atrazine use on the environment.

2 Materials and methods

Atrazine, desethyl-atrazine (DEA), desisopropyl-atrazine (DIPA), desethyl-desisopropyl-atrazine (DEDIPA) and hydroxyderivatives of these compounds (all of HPLC quality) were purchased from Dr. Ehrenstorfer GmbH, Germany.

A saturated solution of atrazine (33 mg/l) was prepared by dissolving the substance in ultrapure water (Ultrapur 10, Watrex). The solution was then filtered through a 0.20 μm nylon membrane filter (Millipore). A solution of 0.16 M $FeCl_3$ was prepared by dissolving the substance in ultrapure water and 5 μl was added to 25 ml of filtered atrazine solution, so the final concentration of $FeCl_3$ in atrazine solution was 3.2×10^{-5} mol/l. Samples containing 3 ml of atrazine plus $FeCl_3$ solution were transferred into 1 cm glass cuvettes and were irradiated for 10; 20; 30; 55 and 90 minutes; one sample was left unirradiated. After irradiation, each sample was transferred into a small glass vial with a screw cap. Irradiation of samples was conducted in a Rayonet reactor with RPR 3000Å lamps emitting light 254-350 nm; light below 300 nm was filtered out to imitate short-wavelength sun radiation. Radiant flux was measured by a Lutron UV A light meter and a total power of all electromagnetic radiation emitted per unit time was calculated; the value was 4.5 W.

Degradation kinetics was monitored by the HPLC method; parameters of the method are summarized in Tab. 1.

Tab. 1. Parameters of HPLC analysis.

HPLC conditions		
HPLC	pump specification	ConstaMetric 3200
	column specification	Phenomenex Luna (250 x 4.6 mm, 5 μm)
	UV detector specification	Delta Chroma UVD 200, Watrex
	software	ClarityLite
	MF composition	Water / Methanol (45/55 v/v)
	detection wavelength	230 nm
	gradient / isocratic	Isocratic
	flow rate	1 ml/min
	temperature	25°C
	pump pressure	18 MPa

Products of atrazine degradation were determined by the LC-MS method; parameters of the method are presented in Tab. 2.

Tab. 2. Parameters of LC-MS analysis.

LC/MS conditions		
LC	column specification	Phenomenex Luna (250 x 4.6 mm, 5 µm)
	MF composition	Water / Methanol (45/55 v/v)
	gradient / isocratic	Isocratic
	flow rate	1 ml/min
	column oven temperature	25°C
	sample volume	5 µl
PDA	wavelengths (channels)	230 nm
MS	IS-type	HESI Heated Electrospray Ionization
	IS-heater temperature	350°C
	IS-spray voltage	4000 V
	IO-capillary temperature	350°C
	IT-records	Full MS (100 - 250 m/z)

A *Lemna* sp. growth inhibition test:

A *Lemna* sp. growth inhibition test was performed according to the OECD Guideline 221 (OECD, 2002) using a vascular plant *Lemna minor* which was obtained from a natural water reservoir in Sumrakov in southern Bohemia. Prior to the test, plants were cultivated in Swedish Standard *Lemna* growth medium (SIS) for 3 weeks; the composition of the SIS medium is given in Appendix II. To test the effect of atrazine on *Lemna* growth, a concentration series of atrazine (0; 0.053; 0.212; 0.53 and 1.06 mg/l) was prepared by diluting a saturated solution of atrazine (filtered through a 0.20 µm nylon membrane filter) in the SIS medium. Then, colonies consisting of 9 to 12 fronds were exposed to the concentration series of atrazine for seven days. Each concentration treatment was tested in a total of 4 replicates.

To test the effect of degradation products of atrazine on *Lemna* growth, a mixture of photodegradation products (irradiated sample, irradiation time 40 minutes) was diluted in the same way as the saturated solution of atrazine, the procedure of the test was otherwise the same as in the case of atrazine. Each concentration treatment was tested in 2 replicates.

Additionally, the effects of atrazine metabolites (DEA, DIPA, DEDIPA and their hydroxyderivatives) on Lemna growth were tested. *Lemna* fronds were exposed for seven days to solutions of the individual compounds. Each solution of a metabolite

was prepared from its saturated solution (filtered through a 0.20 μm nylon membrane filter) by diluting it 30 times in the SIS medium. Each metabolite was tested in 2 replicates.

All *Lemna* sp. growth inhibition tests were performed in opened glass crystallising dishes (ø 9 cm), under natural daylight and at 23°C temperature for seven days. Total frond area was documented at the beginning of the test, the third and the fifth day of the experiment and at the seventh day. Total frond area was calculated using the Easy Leaf Area software (Easy Leaf Area, Department of Plant Sciences, University of California, USA). The average specific growth rate and the percent inhibition of growth were calculated and EC_{50} was estimated using an Excel add-in ED50plus v1.0 (Vargas, 2000). To determine the difference between treatments, the statistical test ANOVA was performed in Statistica 13 (StatSoft Inc., Tulsa, OK, USA).

A *Daphnia* sp. acute immobilisation test:

A *Daphnia* sp. acute immobilisation test was carried out according to the OECD Guideline 202 (OECD, 2004) using *Daphnia magna*. All female daphnids, aged 24 hours at the start of the test, were derived from a healthy stock. To test the effect of atrazine to *Daphnia* mortality, corresponding amounts of saturated solution of atrazine (filtered through a 0.20 μm nylon membrane filter) were added to bubbled tap water to form a geometric concentration series (0 and from 1.0×10^{-2} to 6.1 mg/l with the multiplying factor 2.5). Tests were conducted in opened glass beakers (70 ml) in which one female daphnid per beaker were exposed to atrazine solution of certain concentration with an addition of *Scenedesmus subspicatus* suspension (1 mg C/l). Mortality of daphnids was examined after 24 hours and 48 hours, resp. Each atrazine concentration was tested twice in 4 replicates and once in 7 replicates.

All tests with daphnids were performed in an incubator (Q-cell Compact series thermostatic chambers, POL-LAB, Poland), with a temperature of 20°C and light/dark period of 16/8 hours. The difference in mortality rates among treatments was analysed in Statistica 13 (StatSoft Inc., Tulsa, OK, USA) using Kruskal-Wallis test.

Evaluation of atrazine occurrence in the river basin of the Vltava River:

The occurrence of atrazine in several rivers of the Vltava river basin was evaluated using data provided by Povodí Vltavy s. p. (Povodí Vltavy s. p., Praha, Czech Republic). Concentrations of atrazine and its derivatives before and after 2005 were compared.

3 Results

3.1 Degradation and toxicity assessments of atrazine

Degradation of atrazine in a homogeneous solution saturated with air under irradiation by UV light is quite a rapid process if sufficient amount of Fe(III) is present in the reaction system (Fig. 2). After 30 minutes of irradiation, more than 95% of initial amount of atrazine was eliminated from the reaction solution. The process can be described by first order kinetics with a rate constant of 0,120 min^{-1}.

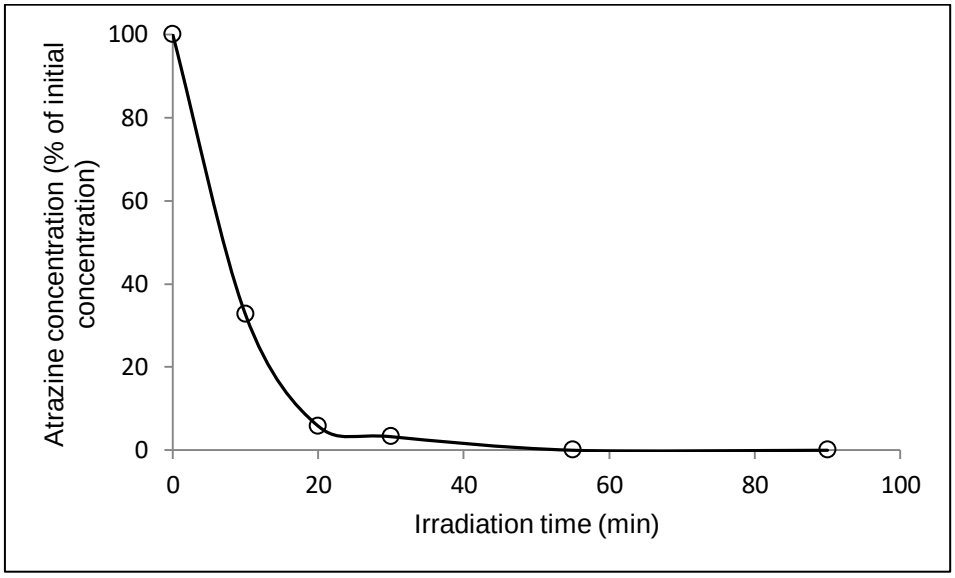

Fig. 2. Photodegradation of atrazine in Fe(III)/UV/air system. Initial atrazine concentration = 1.5 x 10^{-4} mol/l; concentration of added Fe(III) = 3.2 x 10^{-5} mol/l.

The irradiation procedure leads to the formation of several intermediates and final products. An LC-MS analysis (Fig. 3) showed detectable amounts of the following products of atrazine degradation: desethyl-atrazine (DEA), desisopropyl-atrazine (DIPA), desethyl-desisopropyl-atrazine (DEDIPA), 2-hydroxy-desethyl-atrazine (DE-2OH), 2-hydroxy-desisopropyl-atrazine (DIPA-2OH), 2-hydroxy-desethyl-desisopropyl-atrazine (DEDIPA-2OH) and cyanuric acid (CA). The results demonstrate that DEA and DIPA are formed in the largest extent.

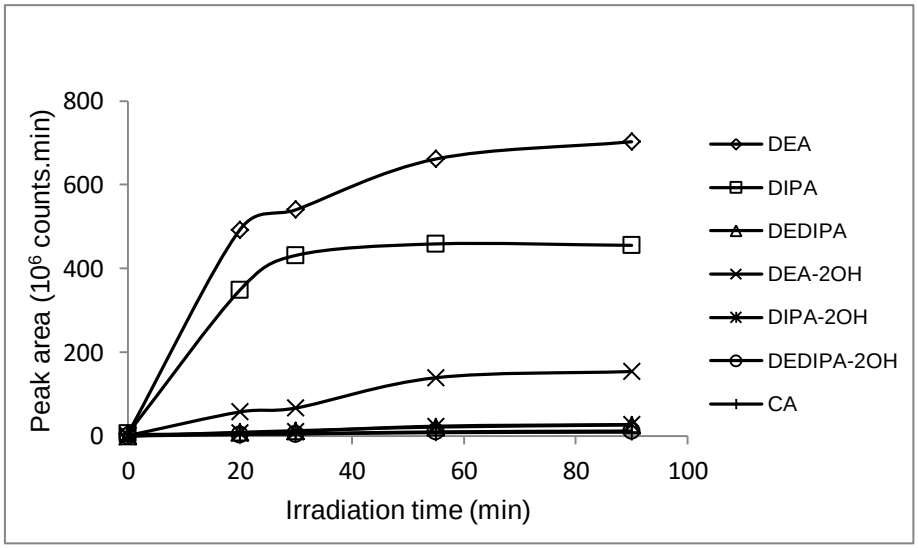

Fig. 3. The formation of atrazine degradation products. The abbreviations stand for: DEA=desethyl-atrazine, DIPA=desisopropyl-atrazine, DEDIPA=desethyl-desisopropyl-atrazine, DEA-2OH=2-hydroxy-desethyl-atrazine, DIPA-2OH=2-hydroxy-desisopropyl-atrazine, DEDIPA-2OH=2-hydroxy-desethyl-desisopropyl-atrazine, CA=cyanuric acid.

Retention times and m/z of atrazine and its degradation products as gained in LC-MS analysis are summarized in Tab. 3. Besides identified products of photodegradation there are several other products formed in the photochemical process.

Tab. 3. Retention times and m/z (where applicable) of atrazine and its photoproducts obtained from LC-MS analysis. The abbreviations stand for: DEA=desethyl-atrazine, DIPA=desisopropyl-atrazine, DEDIPA=desethyl-desisopropyl-atrazine, DEA-2OH=2-hydroxy-desethyl-atrazine, DIPA-2OH=2-hydroxy-desisopropyl-atrazine, DEDIPA-2OH=2-hydroxy-desethyl-desisopropyl-atrazine, Atr-2OH=2-hydroxy-atrazine, CA=cyanuric acid.

Chemical compound		
Retention time (min)	**Chemical abbreviation**	**m/z**
2.21	DEDIPA-2OH	128
2.37	DIPA-2OH	156
2.65	DEA-2OH	170
2.17	CA	128
3.6	DEDIPA	146
3.44	coelution of 3 compounds	×
3.74	coelution of 2 compounds	×
4.51	DIPA	174
6.24	DEA	188
6.4	ATR-2OH	198
9.32	elution of 1 compound	×
10.54	coelution of 3 compounds	×
17.3	atrazine	×

Data from *Lemna mino*r growth inhibition test showed significant (ANOVA: $F=35.71$; $df=4, 15$; $p<10^{-6}$) positive dose-response relationship of atrazine (Fig. 4). The resulting value of EC_{50} for atrazine was 128.4 μg/l. The change in leaf area decreases with atrazine concentration.

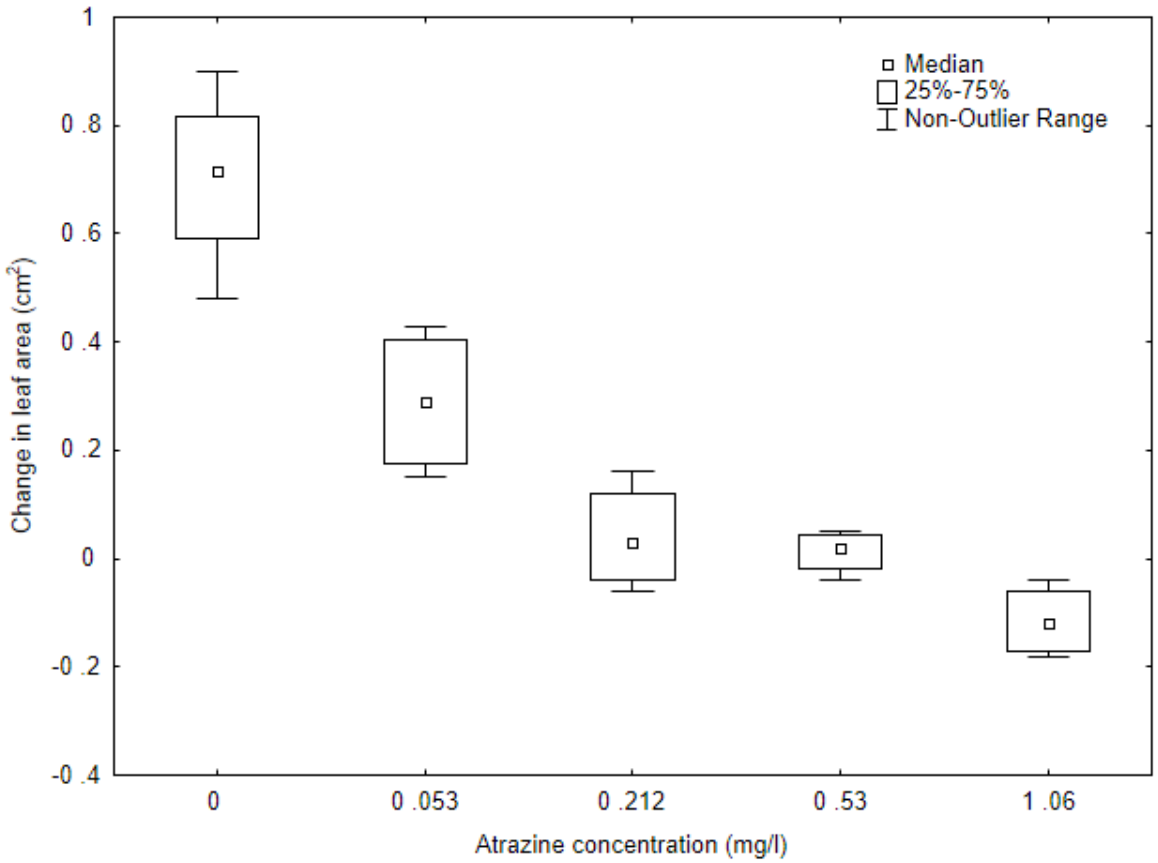

Fig. 4. *Lemna minor* growth inhibition test: change in leaf area after 7 days of atrazine exposure. The change in leaf area decreases with atrazine concentration.

Data from *Lemna minor* growth inhibition test of the mixture of atrazine photoproducts showed non-significant (ANOVA: F=3.23; df=4, 5; p=0.1153) dose-response relationship (Fig. 5). The relation of the change in leaf area and atrazine photoproducts concentration is erratic and no clear trend can be seen.

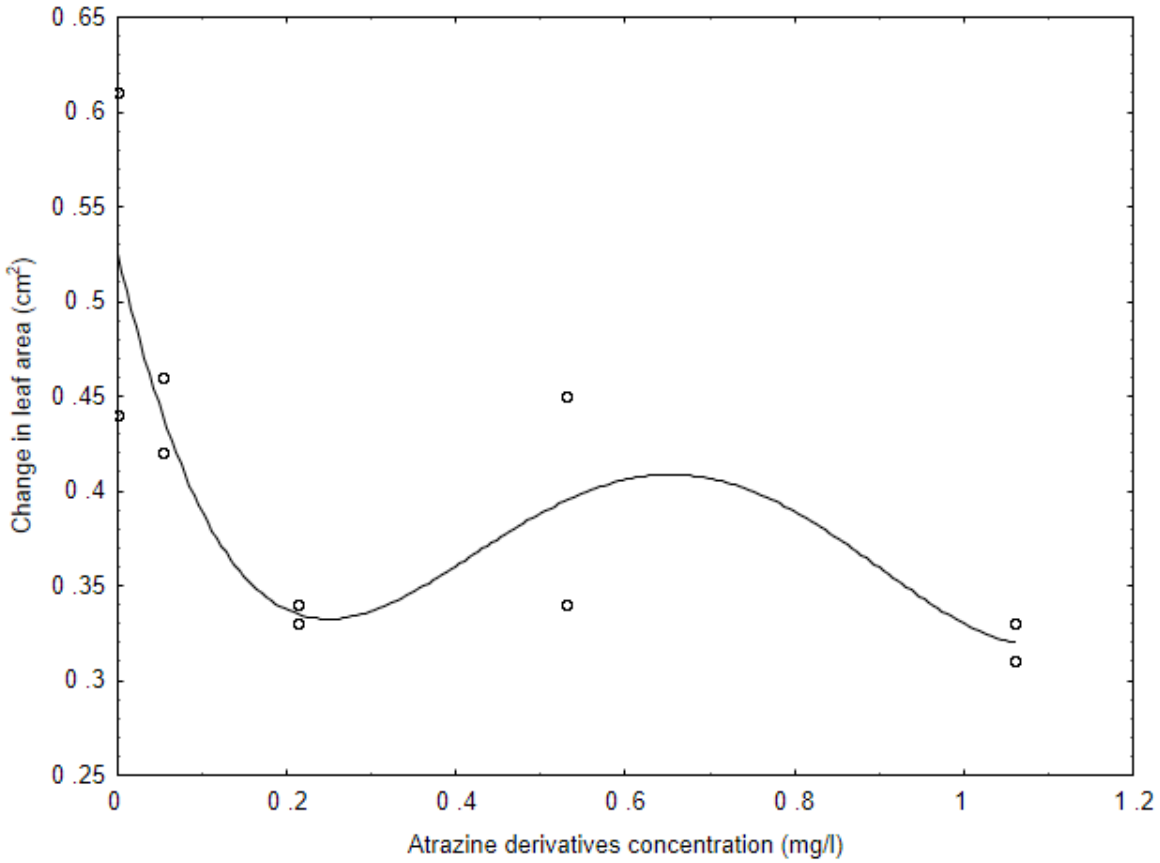

Fig. 5. *Lemna minor* growth inhibition test: change in leaf area after 7 days of atrazine photoproducts exposure. The relation of the change in leaf area and atrazine derivatives concentration is erratic and no clear trend can be seen.

Data from *Lemna minor* growth inhibition test of several atrazine derivatives are summarized in Fig. 6. From atrazine derivatives, desethyl-atrazine (DEA) exhibits the most inhibiting effect on the *Lemna minor* growth. Two other derivatives, desisopropyl-atrazine (DIPA) and desethyl-desisopropyl-atrazine (DEDIPA), seem to have a pronounced negative effect on the *Lemna* growth. On the other hand, hydroxyderivatives of atrazine seem to be significantly less toxic in comparison with DEA but also when compared with DIPA and DEDIPA.

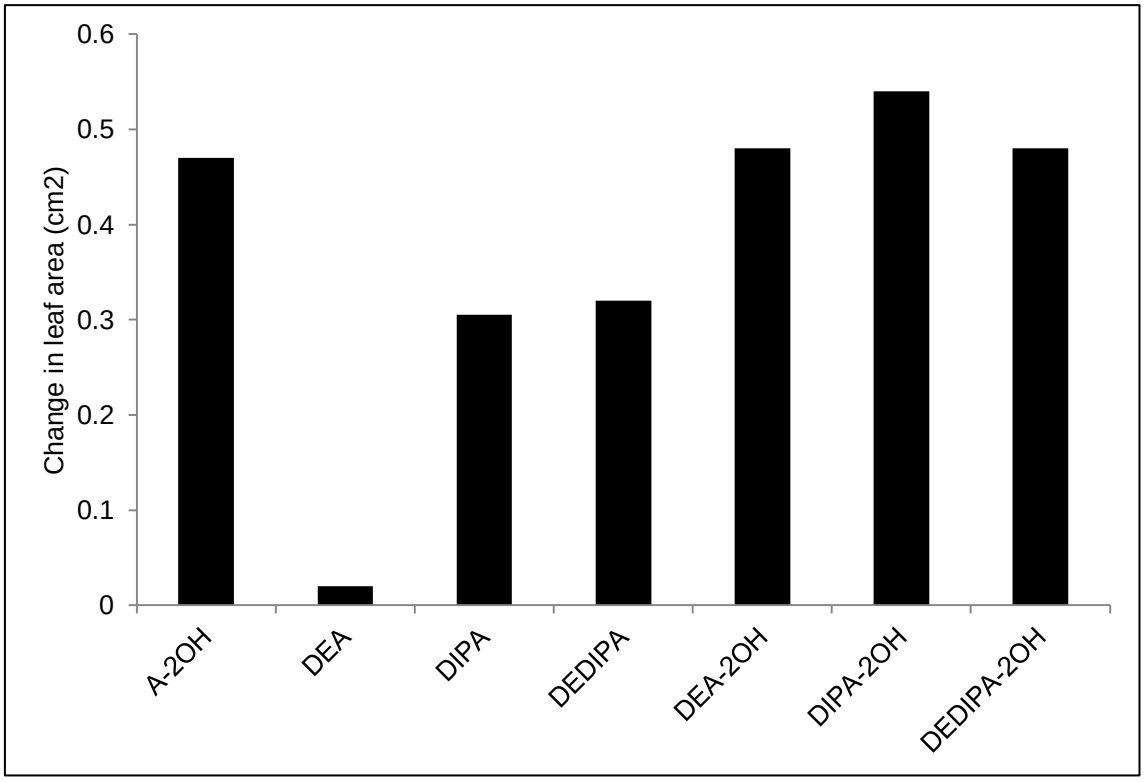

Fig. 6. *Lemna minor* growth inhibition test: change in leaf area after 7 days of exposure to atrazine derivatives: 2-hydroxy-atrazine (A-2OH), desethyl-atrazine (DEA), desisopropyl-atrazine (DIPA), desethyl-desisopropyl-atrazine (DEDIPA), 2-hydroxy-desethyl-atrazine (DEA-2OH), 2-hydroxy-desisopropyl-atrazine (DIPA-2OH), 2-hydroxy-desethyl-desisopropyl-atrazine (DEDIPA-2OH).

Data from *Daphnia magna* acute immobilisation tests of atrazine are presented in Tab. 4. Geometric sequence of concentrations with common ratio 2.5 was prepared for the test.

Tab. 4. Conditions and results in three acute immobilization tests of atrazine on *Daphnia magna*.

Atrazine concentration mg/l	**Exp. 1**	**Exp. 2**	**Exp. 3**
	N mortality (mean ± SD)		
0	4 0 (0 ± 0)	4 0 (0 ± 0)	7 0 (0 ± 0)
1.0 x 10^{-2}	4 0 (0 ± 0)	4 0 (0 ± 0)	7 0 (0 ± 0)
2.5 x 10^{-2}	4 1 (0.25 ± 0.5)	4 0 (0 ± 0)	7 0 (0 ± 0)
6.3 x 10^{-2}	4 1 (0.25 ± 0.5)	4 0 (0 ± 0)	7 0 (0 ± 0)
1.6 x 10^{-1}	4 2 (0.5 ± 0.58)	4 1 (0.25 ± 0.5)	7 0 (0 ± 0)
3.9 x 10^{-1}	4 4 (1 ± 0)	4 1 (0.25 ± 0.5)	7 0 (0 ± 0)
9.8 x 10^{-1}	4 2 (0.5 ± 0.58)	4 2 (0.5 ± 0.58)	7 0 (0 ± 0)
2.4	4 4 (1 ± 0)	4 1 (0.25 ± 0.5)	6 0 (0 ± 0)
6.1	4 2 (0.5 ± 0.58)	4 0 (0 ± 0)	7 0 (0 ± 0)

The results of Kruskal-Wallis statistical analyses on *Daphnia magna* are summarized in Tab. 5.

Tab. 5. Summary of Kruskal-Wallis statistical analyses of *Daphnia magna* acute immobilisation tests.

Experiment No.	**N**	**df**	**H**	**p**
1	36	8	17.28	0.0273
2	36	8	8.58	0.3789
3	62	8	0	1

The positive dose-response relationship of atrazine is significant only for experiment 1 (Kruskal-Wallis: H(36)=17.28; df=8; p=0.0273) while experiment 2 (Kruskal-Wallis: H(36)=8.58; df=8; p=0.3789) and 3 (Kruskal-Wallis: H(62)=0; df=8; p=1) show no significant differences (Tab. 4.). No individuals died in experiment 3. Altogether, atrazine most likely poses no acute toxicity to *Daphnia magna*.

3.2 The occurrence of atrazine in rivers of Vltava river basin

Before the restriction of atrazine use in 2005, atrazine was repeatedly found in most rivers of the Vltava river basin. Among the most polluted were the rivers Sázava, Želivka, Radbuza and their affluents (e.g. the brooks Sedlický, Martinický and Točnický, and the rivers Trnava and Poleňka). In the Sázava River (Fig. 7 and 8), concentrations of atrazine fluctuated in the range from 10 ng/l to almost 1800 ng/l. In the Želivka River (Fig. 9), concentrations in the range from 10 ng/l to 170 ng/l were found with one peak of 380 ng/l in summer 2003. In the Radbuza River (Fig. 10), even higher amounts of atrazine were measured in comparison with Sázava River, specifically in the range from 10 ng/l to almost 2200 ng/l. Since 2005, atrazine concentrations have been gradually decreasing in all investigated water bodies. Nowadays, values of atrazine concentration are in nanograms or tens of nanograms per liter. Apart from atrazine, several atrazine derivatives, for example DEA, DIPA or hydroxyatrazine, can be found in similar concentrations in Vltava river basin.

Higher values of atrazine concentration are measured in smaller streams or in up-river parts of larger streams (compare Fig. 7 and 8). Furthermore, peaks of considerably higher concentrations in certain parts of years can be found in all mentioned rivers (Fig. 7, 8, 9 and 10).

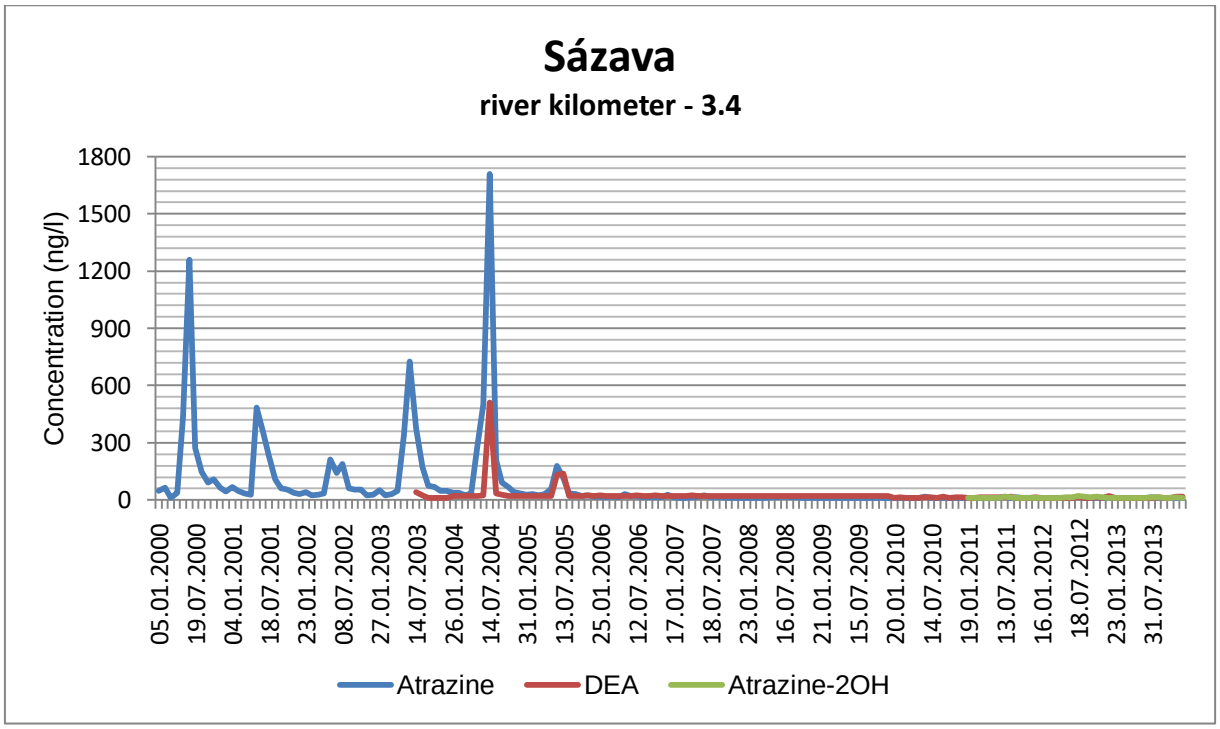

Fig. 7. The occurrence of atrazine and its derivatives in Sázava River (r. kilometer 3.4).

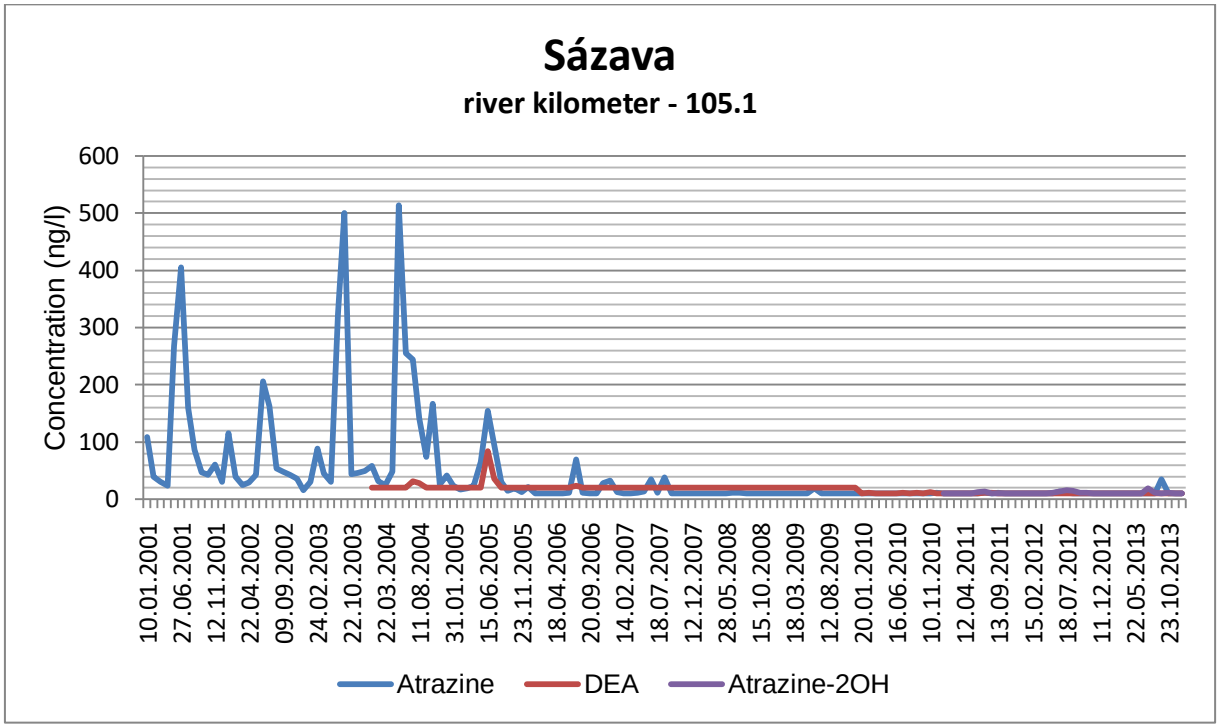

Fig. 8. The occurrence of atrazine and its derivatives in Sázava River (r. kilometer 105.1).

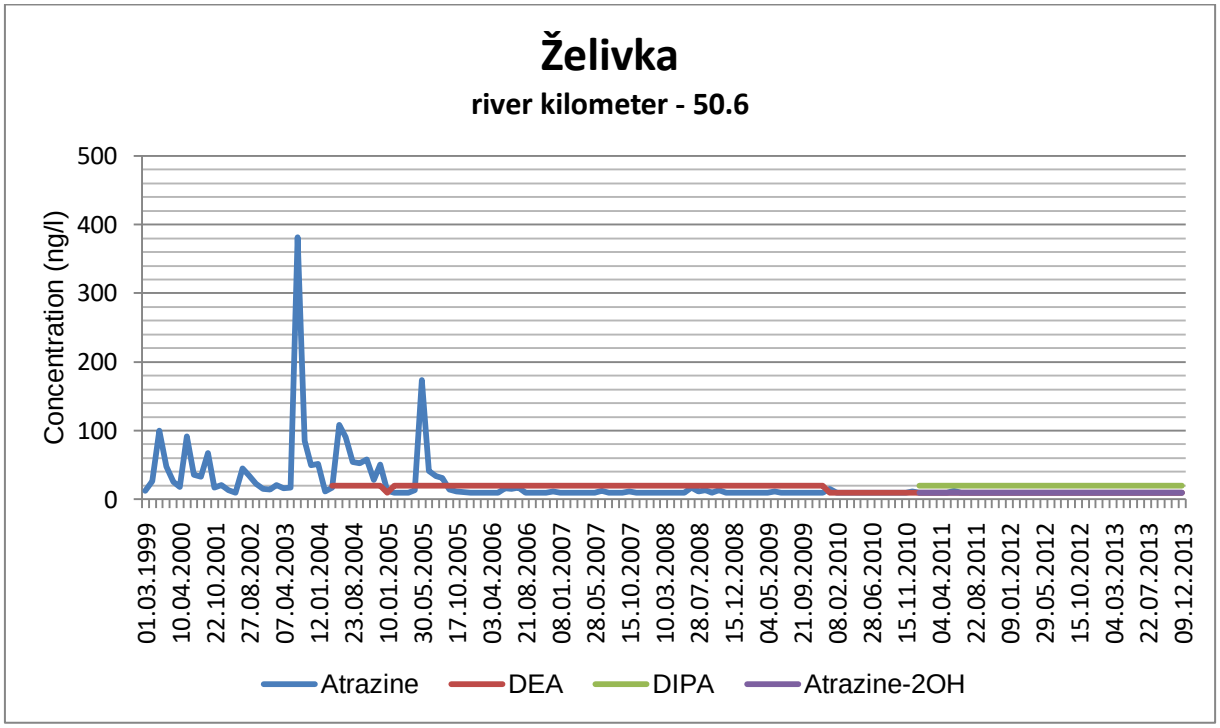

Fig. 9. The occurrence of atrazine and its derivatives in Želivka River (r. kilometer 50.6).

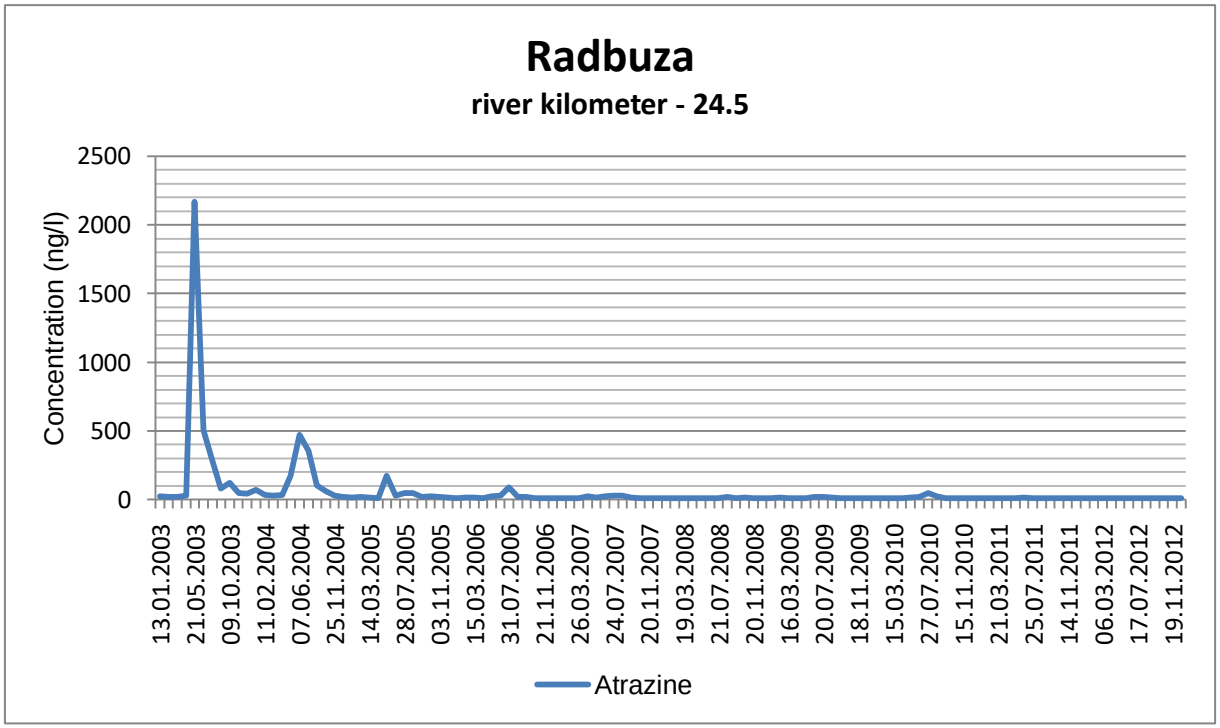

Fig. 10. The occurrence of atrazine in Radbuza River (r. kilometer 24.5).

4 Discussion

Though atrazine is banned in EU from 2005, it is still used in most parts of the world. Therefore its occurrence in the soil and aquatic environment and pathways of its degradation remain in the focus of many scientific groups.

Atrazine does not absorb sun radiation reaching the earth surface; therefore it cannot react in a direct photoinitiated reaction. Other photoinduced mechanisms may lead to its transformation.

Degradation of atrazine by photo-Fenton system, i.e. irradiation in the presence of the combination of Fe(II) + H_2O_2 in the reaction mixture, was studied by many groups in the past decades. It was demonstrated that this type of homogeneous photocatalysis is an effective method of atrazine removal from polluted water samples (De Laat et al., 1999; Canle et al., 2005; Farré et al., 2005; Kassinos et al., 2009). The photo-Fenton system has one significant disadvantage – a large amount of hydrogen peroxide must be continuously added for the reaction to proceed. The process may be considered as a possible way of a waste water treatment; however, its significance for natural processes in polluted river waters is limited since the hydrogen peroxide concentrations measured in surface waters, from micromoles per litre to tens of micromoles per litre (Draper & Crosby, 1983; Cooper et al., 1988) do not reach values necessary for the process.

In this thesis, another approach was adopted: I do not focus on how atrazine can be removed from a pollutant discharge locations but on a possible mechanisms that can contribute to its transformations in an aquatic river and lake environment. A system Fe(III)/UV/air was applied for atrazine degradation. As known from previous investigations of the influence of Fe concentration on the rate of atrazine degradation (Klementová & Hamsová, 2000), the rate of photoinduced atrazine transformation strongly depends on the Fe concentration. For this study which is targeted on intermediates produced in the reaction and their possible toxicity to water organisms, relatively high concentration of Fe (ensuring high reaction rate) has been chosen. For concentration of Fe 3.2 x 10^{-5} mol/l, the degradation process can be characterised with the first rate kinetic constant 0.120 min^{-1}. More than 95% of initial atrazine concentration was removed after 40 minutes of irradiation. These results are in agreement with a study by Du et al. (2009) which studied degradation of atrazine in a similar system and investigated the effect of air presence on photocatalytic process.

In Fe(III)/UV/air system, the production of oxidative species, i.e. OH• radicals, is only via photodecomposition of Fe^{3+} in the hydrated form (Derbalah et al., 2004). Addition of hydrogen peroxide (Huston & Pignatello, 1999; Du et al., 2009) or application of light of wavelength below 300 nm (De Laat et al., 1999) can accelerate the process of atrazine degradation. However, hydrogen peroxide occurs just in trace amounts in natural waters (10^{-5} mol/l - Draper & Crosby, 1983; Cooper et al., 1988) and the majority of sun radiation reaching the Earth´s surface constitutes of VIS and long-wave UV spectrum. Therefore, the Fe(III)/UV/air system can only take place in greater extent in natural waters during sunny days (Derbalah et al., 2004).

Degradation of atrazine by homogeneous photocatalysis leads to the formation of a series of intermediates and final products as shown in Fig. 1. The results of LC-MS analysis revealed significant amounts of the following products of atrazine degradation: desethyl-atrazine, desisopropyl-atrazine, desethyl-desisopropyl-atrazine, hydroxyderivatives of these products and atrazine itself. Cyanuric acid, which is considered to be the final product of atrazine photodegradation, was also present among degradation products but in a very low amount. Evgenidou & Fytianos (2002) obtained the same products, except for cyanuric acid. Formation of cyanuric acid as the final product of atrazine photocatalytic degradation was reported e.g. by Minero et al. (1992). Based on my experiments and those of other authors, e.g. Evgenidou & Fytianos (2002), it seems that formation of this compound strongly depends on the experimental condition.

Results from *Lemna minor* growth inhibition test showed significant inhibition of *Lemna* growth in the presence of atrazine. The value of EC_{50} for atrazine was calculated as 128.4 μg/l, which is similar to 153 μg/l reported by Fairchild et al. (1997). Toxicity of the mixture of atrazine degradation products was not proved because no dose-response trend was observed. However, there was a substantial difference in *Lemna* growth for individual atrazine derivatives, from which desethyl-atrazine and desisopropyl-atrazine seem to be more toxic than others. Therefore, a decrease in toxicity after atrazine degradation has been proven, but some of atrazine products can be equally toxic as atrazine itself. Similar observation was made for example by Tchounwou et al. (2000) who studied toxicity of several pesticides, including atrazine and its derivatives; in this case the most toxic from all atrazine derivatives was desethyl-desisopropyl-atrazine. The decrease in toxicity after atrazine degradation has been observed in a study by Klementová et al. (2015) in which toxicity of atrazine degradation products to a green alga *Raphidocelis subcapitata* was tested. Since atrazine is a herbicide which inhibits photosynthesis by binding to the QB-binding niche on the D1 protein of the photosystem II complex (Weed Society of America, 1994), the sensitivity of *Lemna minor* (vascular plant) or *Raphidocelis subcapitata* (green alga) to atrazine is not surprising.

Overall, results from *Daphnia magna* acute immobilization test did not show any toxic effect of atrazine to *Daphnia magna* in the studied range of atrazine concentrations. Palma et al. (2008) estimated EC_{50} of atrazine for *Daphnia magna* as 35.5 mg/l. A similar value was reported in a study by Tchounwou et al. (2000) using Microtox. Such high value of atrazine concentration equal to atrazine solubility point is not commonly found in natural environment, thus, atrazine apparently poses no acute toxicity to water crustaceans and other microorganisms in their natural habitats. Moreover, no cytotoxicity of atrazine to gill cells was reported in rainbow trout (Klementová et al., 2015) which corresponds with *in vivo* ecotoxicity data from US EPA Ecotox Database (US Environmental Protection Agency, 2014) where EC_{50} of atrazine for rainbow trout in acute tests exceeded 10 mg/l. All these data imply that atrazine pose no acute toxicity to organisms that are not photosynthetically active. Nevertheless, it is important to bear in mind that acute toxicity is not the only threat for animals in the aquatic environment. They spend all their lives in the polluted environment; thus, the cumulative effects of acutely negligible doses may represent a momentous risk factor.

The survey of atrazine occurrence in rivers from Vltava River basin showed that the most polluted rivers were the rivers Sázava, Želivka and Radbuza which are surrounded by agricultural land to a large extent. Before atrazine restriction in 2005, atrazine concentration ranged from hundreds to thousands ng/l. Larger concentrations were measured in up-stream parts of rivers and in their smaller affluents. There were also peaks of significantly higher concentration of atrazine in certain periods of a year. Those were probably periods of rain after atrazine application as suggests a study by Scribner et al. (2005), but we lack supportive meteorological data.

After atrazine restriction, its concentration has been gradually decreasing, and nowadays, the measured values are in nanograms or tens of nanograms per litre. Apart from atrazine, several atrazine derivatives, for example DEA, DIPA or hydroxyatrazine, can be found in similar concentrations (nanograms per litre) in the Vltava River basin. A study by Loos et al. (2009) investigating the occurrence of atrazine in European rivers revealed its presence in 68% of sampled European rivers. The maximum detected concentrations of atrazine and its derivative desethyl-atrazine were 46 ng/l and 80 ng/l, resp. The average detected concentrations were around 10 ng/l for both atrazine and desethyl-atrazine. Thus, atrazine can be found in European natural waters even nowadays, more than five years after atrazine restriction. However, values of atrazine concentration are far below its limit for drinking water, which is 0.1 μg/l (European Commission Directive, 2004), so atrazine should not pose any important environmental risk in European countries in the present day.

5 Conclusions

Degradation of atrazine by homogeneous Fe photocatalysis can be characterised by the pseudo-first order reaction rate constant of 0.120 min^{-1} for the Fe concentration 3.2×10^{-5} mol/l. Homogeneous photocatalysis under these conditions led to the formation of various products, namely desethyl-atrazine, desisopropyl-atrazine, desethyl-desisopropyl-atrazine and hydroxyderivatives of the dealkylated products. The formation of cyanuric acid, the final product of photocatalytic degradation, was also observed.

Toxicity assessments showed significant toxicity of atrazine to *Lemna minor* with EC_{50} equal to 128.4 μg/l. Toxicity of the irradiated mixture of atrazine containing its degradation products was not confirmed. However, there are considerable differences

in *Lemna* growth for individual atrazine derivatives when tested independently as pure substance solutions. Acute toxicity of atrazine to *Daphnia magna* was not proven.

Data from Vltava River basin revealed that several rivers, specifically Sázava River, Želivka River, and Radbuza River contained the highest atrazine concentrations in the past. The values ranged from hundreds to thousands ng/l. Since atrazine restriction in 2005, there has been a gradual decrease in atrazine concentration in affected rivers. Nowadays, on average two orders of magnitude lower amounts of atrazine and its derivatives can be found within Vltava River basin.

6 References

Baek Y. W., An Y. J. (2011) Microbial toxicity of metal oxide nanoparticles (CuO, NiO, ZnO and Sb_2O_3) to *Escherichia coli, Bacillus subtilis*, and *Streptococcus aureus*. *Sci. Total. Environ*. **409**, 1603-1608.

Bogaerts P., Bohatier J., Bonnemoy F. (2001) Use of the ciliated protozoan *Tetrahymena pyriformis* for the assessment of toxicity and quantitative structure – activity relationships of xenobiotics: Comparison with the Microtox test. *Ecotox. Environ. Safe.* **49**, 293-301.

Bulich A. A. (1979) Use of luminiscent bacteria for determining toxicity in aquatic environment. Aquat. Toxicol. Second Conference. ASTM STP667. American Society for Testing and Materials, Philadephia, 171-180.

Canle L. M., Fernández M. I., Santaballa J. A. (2005) Developments in the mechanism of photodegradation of triazine-based pesticides. *J. Phys. Org. Chem*. **18**, 148-155.

Cooper W. J., Zika R. D., Petasne R. G., Plane J. M. C. (1988) Photochemical formation of hydrogen peroxide in natural waters exposed to sunlight. *Environ. Sci. Technol*. **22**, 1156-1160.

De Laat J., Gallard H., Ancelin S., Legube B. (1999) Comparative study of the oxidation of atrazine and acetone by H_2O_2/UV, Fe(III)/UV, Fe(III)/H_2O_2/UV and Fe(II) or Fe(III)/H_2O_2. *Chemosphere* **39**, 2693-2706.

Derbalah A. S., Nakatani N., Sakugawa H. (2004) Photocatalytic removal of fenitrothion in pure and natural waters by photo-Fenton reaction. *Chemosphere* **57**, 635-644.

Draper W. M., Crosby D. G. (1983) The photochemical generation of hydrogen peroxide in natural waters. *Arch. Environ. Con. Tox.* **12**, 121-126.

Du Y., Su Y., Lei L., Zhang X. (2009) Role of oxygen in the degradation of atrazine by UV/Fe(III) process. *J. Photoch. Photobio. A.* **208**, 7-12.

European Commission. Decision 2004/248/EC: Commission Decision of 10 March 2004 concerning the non-inclusion of atrazine in Annex I to Council Directive 91/414/EEC and the withdrawal of authorisations for plant protection products containing this active substance. Available from: [http://eur-lex.europa.eu/smartapi/cgi/sga_doc?smartapi!celexapi!prod!CELEXnumdoc&lg=EN&numdoc=32004D0248&model=guichett]. Accessed: [2016_08_21].

Evgenidou E. and Fytianos K. (2002) Photodegradation of triazine herbicides in aqueous solutions and natural waters. *J. Agric. Food Chem*. **50**, 6423 – 6427.

Fairchild J. F., Ruessler D. S., Haverland P. S., CARLSON A. R. (1997) Comparative Sensitivity of *Selenastrum capricornutum* and *Lemna minor* to Sixteen Herbicides. *Arch. Environ. Con. Tox.* **32**, 353-357.

Fairchild J. F., Ruessler D. S., Carlson A. R. (1998) Comparative sensitivity of five species of macrophytes and six species of algae to atrazine, metribuzin, alachlor, and metolachlor. *Environ. Toxicol. Chem.* **17**, 1830-1834.

Farré M. J., Franch M. I., Malato S., Ayllón J. A., Peral J., Doménech X. (2005) Degradation of some biorecalcitrant pesticides by homogeneous and heterogeneous photocatalytic ozonation. *Chemosphere*. **58**, 1127-1133.

Fenoll J., Hellín P., Martínez C. M., Flores P., Navarro S. (2012) Semiconductor-sensitized photodegradation of s-triazine and chloroacetanilide herbicides in leaching water using TiO_2 and ZnO as catalyst under natural sunlight. *J. Photoch. Photobio. A.* **238**, 81-87.

Hayes T. B., Collins A., Lee M., Mendoza M., Noriega N., Stuart A. A., Vonk A. (2002) Hermaphroditic, demasculinized frogs following exposure to the herbicide, atrazine, at ecologically relevant doses. *Proc. Nat. Acad. Sciences (USA)* **99**, 5476-5480.

Hopenhayn-Rich C., Stump M. L., Browning S. R. (2002) Regional assessment of atrazine exposure and incidence of breast and ovarian cancers in Kentucky. *Arch. Environ. Contam. Toxicol.* **42**, 127-136.

Huston P. L. and Pignatello J. J. (1999) Degradation of selected pesticide active ingredients and commercial formulations in water by the photo-assisted Fenton reaction. *Wat. Res.* **33**, 1238-1246.

Kassinos D., Varnava N., Michael C., Piera P. (2009) Homogeneous oxidation of aqueous solutions of atrazine and fenitrothion through dark and photo-Fenton reactions. *Chemosphere* **74**, 866-872.

Klementová Š., Hamsová K. (2000) Catalysis and sensitization in photochemical degradation of triazines. *Res. J. Chem. Environ.* **4**, 7-12.

Klementová Š. (2011) A critical view of the photoinitiated degradation of herbicides. Herbicides - Properties, Synthesis and Control of Weeds. Mohammed Nagnib Abd El-Ghany Hasaneen. Rijeka: Intech, 297-314.

Klementová Š., Rábová-Toušová Z., Bláha L., Kahoun D., Šimek P., Keltnerová L., Zlámal M. (2015) Photodegradation of atrazine on TiO_2 – products toxicity assessment. *Open. J. Appl. Sci.* **5**, 14-21.

Konstantinou I. K. and Albanis T. A. (2003) Photocatalytic transformation of pesticides in aqueous titanium dioxide suspensions using artificial and solar light: intermediates and degradation pathways. *Appl. Catal. B-Environ.* **42**, 319-335.

Kross B. C., Vergara A., Raue L. E. (1992) Toxicity assessment of atrazine, alachlor, and carbofuran and their respective environmental metabolites using Microtox. *J. Toxicol. Env. Health* **37**, 149-159.

Land Stewardship Project & Pesticide Action Network North America (2010) The Syngenta Corporation & Atrazine. The Cost to the Land, People & Democracy. A report. Available from: [http://www.panna.org/sites/default/files/AtrazineReportBig2010.pdf]. Accessed: [2016_08_08].

Lapertot M., Ebrahimi S., Oller I., Maldonado M. I., Gernjak W., Malato S., Pulgarín C. (2008) Evaluating Microtox© as a tool for biodegradability assessment of partially treated solutions of pesticides using Fe^{3+} and TiO_2 solar photoassisted processes. *Ecotox. Environ. Safe.* **69**, 546-555.

Loos R., Gawlik B. M., Locoro G., Rimaviciute E., Contini S., Bidoglio G. (2009) EU-wide survey of polar organic persistent pollutants in European river waters. *Environ. Pollut.* **157**, 561-568.

Ma J., Wang S., Wang P., Ma L., Chen X., Xu R. (2006) Toxicity assessment of 40 herbicides to the green alga *Raphidocelis subcapitata*. *Ecotox. Environ. Safe.* **63**, 456-462.

Malato S. and Aguera A. (2004) Detoxification of pesticide in water using solar photocatalysis. *Acs. Sym. Ser*. **863**, 113-126.

Mayhew D. (1986) Twenty four month combined oral toxicity and oncogenicity study in rats utilizing Atrazine Technical. Ciba-Geigy Study No. 410-1102. EPA MRID 40629302. Available from:

[https://archive.epa.gov/pesticides/chemicalsearch/chemical/foia/web/pdf/080803/080803-092.pdf]. Accessed: [2016_08_14].

Mills P. K. and Yang R. (2006) Regression analysis of pesticide use and breast cancer incidence in California Latinas. *J. Environ. Health* **68**, 15-22.

Minero C., Pramauro E., Pelizzetti E. (1992) Photosensitized transformations of atrazine under simulated sunlight in aqueous humid acid solution. *Chemosphere* **24**, 1597-1606.

Morseth S. (1998) Chronic (12 – 24 month) study in rats with atrazine technical. Covance Laboratories Inc. Study ♯ 2386-108. EPA MRID 44544701. Available from: [https://archive.epa.gov/pesticides/chemicalsearch/chemical/foia/web/pdf/080803/080803-254.pdf]. Accessed: [2016_08_14].

Muir K., Rattanamongkolgul S., Smallman-Raynor M., Thomas M., Downer S., Jenkinson C. (2004) Breast cancer incidence and its possible spatial association with pesticide application in two counties of England. *Public Health* **118**, 513-520.

O´Connor J., McCormick G., Green J. (1987) Atrazine technical: Chronic toxicity study in dogs. Ciba-Geigy Study No. 852008. EPA MRID 40431301. Page No. 4. Available from: [https://www3.epa.gov/pesticides/chem_search/cleared_reviews/csr_PC-080803_11-Jun-96_243.pdf]. Accessed: [2016_08_14].

OECD (2002) OECD Guidelines for the Testing of Chemicals, Guideline 221. *Lemna* sp. Growth Inhibition Test. Available from: [http://www.oecd-ilibrary.org/environment/test-no-221-lemna-sp-growth-inhabition-test_9789264016194-en;jsessionid=34s3quagr3op3.x-oecd-live-03]. Accessed: [2016_10_15].

OECD (2004) OECD Guidelines for the Testing of Chemicals, Guideline 202. Daphnia sp. Acute Immobilisation Test. Available from: [http://www.oecd-ilibrary.org/environment/test-no-202-daphnia-sp-acute-immobilisation-test_9789264069947-en]. Accessed: [2016_10_15].

Olsberg C. (2007) Atrazine: Chemical Summary. U.S. EPA: Toxicity and Exposure Assessment for Children's Health. Available from: [https://archive.epa.gov/region5/teach/web/pdf/atrazine_summary.pdf]. Accessed: [2016_08_08].

Palma P., Palma V. L., Fernandes R. M., Soares A. M. V. M., Barbosa I. R. (2008) Acute Toxicity of Atrazine, Endosulfan Sulphate and Chlorpyrifos to *Vibrio fischeri*, *Thamnocephalus platyurus* and *Daphnia magna*, Relative to Their Concentrations in Surface Waters from the Alentejo Region of Portugal. *Bull. Environ. Contam. Toxicol.* **81**, 485-489.

Rusiecki J. A., De Roos A., Lee W. J., Dosemeci M., Lubin J., Hoppin J. A., Blair A., Alavanja M. C. (2004) Cancer incidence among pesticide applicators exposed to atrazine in the Agricultural Health Study. *JNCI* **96**, 1375-1382.

Saas J. B. and Colangelo A. (2006) European Union Bans Atrazine, While the United States Negotiates Continued Use. *Int. J. Occup. Environ. Health* **12**, 260-267.

Sanderson J. T., Seinen W., Giesy J. P., Van Den Berg M. (2000) 2-Chloro-s-triazine herbicides induce aromatase (CYP19) activity in H295R human adrenocortical carcinoma cells: A novel mechanism for estrogenicity?. *Toxicol. Sci.* **54**, 121-127.

Scribner E. A., Thurman E. M., Goolsby D. A., Battaglin W. A. (2005) Summary of significant results from studies of triazine herbicides and their degradation products in surface water, ground water, and precipitation in the midwestern United States during the 1990s: Scientific investigations report 2005-5094. Reston, Virginia: U.S. Geological Survey, 1-27.

Tchounwou P. B., Wilson B., Ishaque A., Ransome R., Huang M. J., Leszczynski J. (2000) Toxicity Assessment of Atrazine and Related Triazine Compounds in the Microtox Assay, and Computational Modelling for Their Structure-Activity Relationship. *Int. J. Mol. Sci*. **1**, 63-74.

Thorpe N. and Shirmohammadi A. (2005) Herbicides and nitrates in groundwater of Maryland and childhood cancers: A geographic information systems approach. *J. Environ. Sci. Health, Part C* **23**, 261-278.

Tixier C., Sancelme M., Aït-Aïssa S., Widehem P., Bonnemoy F., Cuer A., Trufaut N., Veschambre H. (2002) Biotransformation of Phenylurea Herbicides by a Soil Bacterial Strain, *Arthrobacter*, sp. N 2: Structure Ecotoxicity and Fate of Diuron Metabolite with Soil Fungi. *Chemosphere* **46**, 519-526.

US Environmental Protection Agency (2014) ECOTOX User Guide: ECOTOXicology Database System. Version 4.0. Available from [http://cfpub.epa.gov/ecotox]. Accessed: [2016_12_03].

Van Leeuwen J. A., Waltner-Toews D., Abernathy T., Smit B., Shoukri M. (1999) Associations between stomach cancer incidence and drinking water contamination with atrazine and nitrate in Ontario (Canada) agroecosystems, 1987 – 1991. *Int. J. Epidemiol.* **28**, 836-840.

Vargas M. H. (2000) ED50plus v1.0. Available from: [http://www.sciencegateway.org/protocols/cellbio/drug/data/readme.txt]. Accessed: [2016_11_26].

Weed Society of America (1994) Herbicide Handbook, 6th ed. Champaign, IL, USA.

Wetzel L. T., Luempert L. G., Breckenridge C. B., Tisdel M. O., Stevens J. T., Thakur A. K., Extrom P. J., Elridge J. C. (1994) Chronic effects of atrazine on estrus and mammary tumor formation in female Sprague-Dawley and Fischer 344 rats. *J. Toxicol. Environ. Health* **43**, 169-182.

7 Supplementary materials

Appendix I: Reoxidation of ferrous ions to ferric ions during homogeneous photocatalysis.

organic molecule + hυ → organic molecule*
organic molecule* + O_2 → organic molecule$^{\bullet+}$ + $O_2^{\bullet-}$
$O_2^{\bullet-}$ + H^+ → $HO_2^{\bullet}$
$HO_2^{\bullet}$ → $O_2^{\bullet-}$ + H^+
$HO_2^{\bullet}$ + Fe^{2+} + H^+ → Fe^{3+} + H_2O_2
$HO_2^{\bullet}$ + Fe^{3+} → Fe^{2+} + O_2 + H^+
Fe^{2+} + H_2O_2 → Fe^{3+} + $OH^{\bullet}$ + OH^-

Two reactive species, HO_2• and H_2O_2, are responsible for the reoxidation of ferrous ions to ferric ions.

Appendix I: Swedish Standard *Lemna* growth medium (SIS).

Stock solution No.	Substance	Concentration in stock solution (g/l)	Concentration in prepared medium (mg/l)	Prepared medium	
				Element	Concentration (mg/l)
I	$NaNO_3$	8.50	85	Na ; N	32 ; 14
	KH_2PO_4	1.34	13.4	K ; P	6.0 ; 2.4
II	$MgSO_4.7H_2O$	15	75	Mg ; S	7.4 ; 9.8
III	$CaCl_2.2H_2O$	7.2	36	Ca ; Cl	9.8 ; 17.5
IV	Na_2CO_3	4.00	20	C	2.3
V	H_3BO_3	1.0	1.00	B	0.17
	$MnCl_2.4H_2O$	0.20	0.20	Mn	0.056
	$Na_2MoO_4.2H_2O$	0.010	0.010	Mo	0.0040
	$ZnSO_4.7H_2O$	0.050	0.050	Zn	0.011
	$CuSO_4.5H_2O$	0.0050	0.0050	Cu	0.0013
	$Co(NO_3)_2.6H_2O$	0.010	0.010	Co	0.0020
VI	$FeCl_3.6H_2O$	0.17	0.84	Fe	0.17
	Na_2-EDTA.$2H_2O$	0.28	1.40	-	-
VII	MOPS (buffer)	490	490	-	-

To prepare 1 liter of SIS medium, the following parts are added to 900 ml of ultrapure water:

- 10 ml of stock solution I
- 5 ml of stock solution II

- 5 ml of stock solution III
- 5 ml of stock solution IV
- 1 ml of stock solution V
- 5 ml of stock solution VI
- 1 ml of stock solution VII

The pH is adjusted to 6.5 ± 0.2 with either 0.1 or 1 M HCl or NaOH, and the volume is adjusted to 1 litre with ultrapure water.