Chapter 4

Triazine Herbicides in the Environment

Sarka Klementova and Lucie Keltnerova

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/60858

Abstract

This chapter is a review of literature concerning the fate of chloro-s-triazine herbicides, particularly atrazine, in the environment. It addresses the distribution of such herbicides and their metabolites in the soil and in water bodies, including the conditions that affect their transport mechanisms. The biodegradation pathways regarding the microbial degradation are presented as well as modification mechanisms of the compounds in plants capable of tolerating their action. Studies on the influence of the compounds on animal and human physiological processes and health, that is distribution of atrazine in the animal organisms, effects on the regulatory platform in the liver, possible carcinogenesis and endocrine disruption risks are assessed. Toxicity tests used for evaluation of the toxicity of the compounds are critically reviewed. Possible methods for atrazine degradation, including advanced oxidation procedures (AOP techniques), are outlined.

Keywords: atrazine, s-triazine, fate in the environment, influence on animal and human physiological procesess, toxicity tests, methods of degradation

1. Introduction

Since the 1960s, the use of chemicals to control weed emergence and growth has steadily increased mainly in areas of land utilised for agriculture [1,2], but also along roads and railways [3] as well as in urban areas [4].

Many of the applied herbicides are compounds that are relatively water soluble and can thus be transported to bodies of surface water or leach into ground water resources [5].

Among herbicides, the chloro-s-triazine derivatives, and atrazine in particular, are the most heavily used worldwide, and are often therefore, detected in rivers, lakes and groundwater [6]. The main representants of the triazine herbicides are shown in Figure 1.

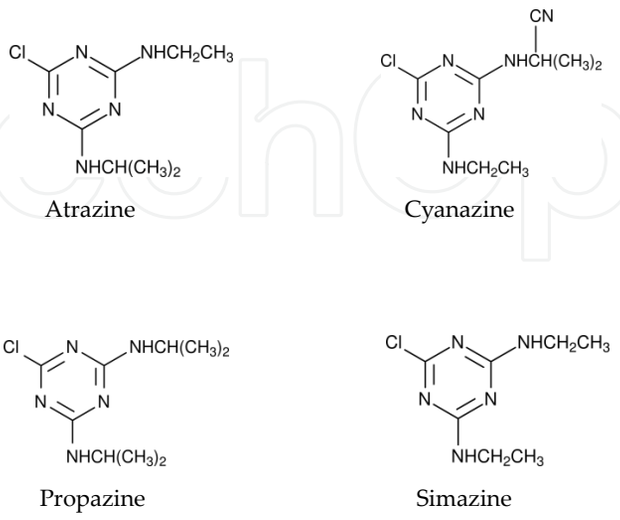

Figure 1. The main triazine herbicides.

Triazine herbicides belong to a category classified as persistent organic compounds [7] since they resist biological and chemical degradation.

Their persistence in natural bodies of water has led to a search of a way to degrade them into environmentally compatible compounds. The following techniques have been evaluated: photocatalytic decomposition on semiconductors [8-10], other advanced oxidation processes (AOPs) [10,11-13], homogeneous photocatalysis [14,15], photosensitised reactions [16,17] or photolysis by high energy UV radiation [11,16,18]. For major sources of pollution, that is wastewater from agricultural industries and pesticide manufacturers, in which the concentration of pesticides may reach levels as high as several hundreds of mg/dm^3, technologies for degradation that are low cost and relatively simple to implement have been proposed and developed [19]. Since degradation methods do not lead generally to full mineralisation of these herbicides, attention should also be paid to the toxicity tests of the resulting mixtures.

Herbicides, toxic by definition to some plant species, may display toxic effects to other species after short- or long-term exposure. The transformation products of herbicides represent an issue of emerging importance since in some cases they pose a greater threat to the environment than the parent molecules [20].

Low-level contamination of drinking water supplies is perceived as a serious potential health problem since many herbicides have been suspected of functioning as potential endocrine disruptors, that is substances interfering with the body´s endocrine system and resulting in adverse developmental, reproductive, neurological and immunological effects in both humans and wildlife [21,22]. Potential carcinogenic effects have been investigated in the laboratory as well as epidemiological studies [21-23].

http://dx.doi.org/10.5772/60858

This chapter is a review of recent literature concerning the fate of atrazine in the environment, the influence of atrazine on animal and human physiological processes, toxicity tests for triazine herbicides and methods used for atrazine degradation.

2. The fate of atrazine in the environment

Atrazine, a white crystalline compound, is a selective herbicide. Its solubility in water is 33 mg/dm^3 at 22°C, pH=7 [24]. Atrazine is usually applied in a water spray at a concentration of 2.2 to 4.5 kg ha^{-1} [25] before the weed emerges.

Atrazine inhibits the photosynthesis of most plants. Its mode of action involves competitive inhibition for plastoquinone binding to the Q_B protein (32-kDa protein) in photosystem II, thus disabling the electron transport chain in the light-dependent reactions of photosynthesis [26,27].

Atrazine has been shown to have a high affinity for soil organic matter, its sorption correlating positively with organic carbon content [28]. The study also revealed that the sorption of atrazine on organic soil matter lowers its availibility to the biota.

Atrazine can be transported through the soil. Two distinct processes contribute to this transport: transport through the soil matrix,which is a slow process, and movement through large pores, which is much faster [29]. Correira et al. [30] evaluated the mobility of atrazine on Ultisol red clay soils, typical for humid moderate and tropic regions and concluded that the soils have a low sorption capacity for atrazine, which results in a high potential for leaching and runoff.

Many groups have studied the influence of organic matter in soil on atrazine sorption. Ben Hur et al. [31] examined the effects of dissolved organic matter (DOM) on atrazine sorption in soil. He reported that the higher the content of soluble organic matter the higher the atrazine affinity to the soil solid phase.

The role of humic substances in atrazine sorption on soils has been investigated in several studies. Several mechanisms of interactions of atrazine with humic organic matter have been hypothesised, such as proton transfer, electron transfer, hydrophobic interactions between humic material and atrazine, but no explicitely conclusive data have resulted mainly due to the large heterogeneity in humic materials [32,33].

In soils, atrazine degradation proceeds mainly via the microbial activity of soil microorganisms. The resulting hydroxylated and dealkylated intermediates can even be mineralised by some microorganisms to carbon dioxide. The sequence of such degradation as proposed by Sadowsky et al. [34] and de Souza et al. [35] is demonstrated in Figure 2.

Atrazine is tolerated by some plants. The detoxification of atrazine occurs through hydrolysis, non-enzymatic hydroxylation, enzyme-mediated N-dealkylation or conjugation with cystein or glutathion [36].

The ability of atrazine to undergo hydrolytic modification is connected with C_4 plants. C_4 plants are characterised by their initial incorporation of CO_2 into an anion of a 4-carbon organic acid; the anion is transported into specialised cells in which CO_2 is regenerated and enters the conventional C_3 pathway of the Calvin cycle. The ability of C_4 plants to perform hydrolytic modification has been shown to be closely correlated with their atrazine tolerance.

The results of Chang et al. [37] demonstrated that poplar (*Populus deltoides*) cuttings could absorb atrazine and metabolise it through hydroxylation and dealkylation into less toxic compounds.

In a growth chamber study, Lin et al. [38] examined the uptake and conversion of ^{14}C-atrazine by several grass species to examine efficacy of the so-called vegetative buffer strips. Multispecies vegetative buffer strips have been recommended as a potentially cost-effective conservation practice to reduce non-point source pollution of adjacent waterways. The study was conducted to compare atrazine degradation profiles in soil rhizospheres from different grasses and correlate the rates and degradation profiles with microbial activity.

The plants treated included seven grasses: orchardgrass (*Dactylis glomerata* L.), tall fescue (*Festuca arundinacea* Schreb), smooth bromegrass (*Bromus inermis* Leyss.), switchgrass (*Panicum virgatum)*, Illinois bundle flower (*Desmanthus illinoensis*), perennial ryegrass (*Lolium perenne* L.) and eastern gamagrass (*Tripsacum dactyloides*). All of the plant species significantly enhanced atrazine degradation, with eastern gamagrass showing the highest capability for promoting the biodegradation of atrazine in the rhizosphere (more than 90% of atrazine was degraded in the plant´s rhizosphere compared with 24% in the control). N-dealkylation of atrazine was strongly correlated with increased enzymatic activity in β-glucosidase and dehydrogenase, which are microbial parameters used for the assessment of soil quality.

Results suggested that the efficacy of vegetative buffer strips in removing herbicides from surface runoff is related to the ability of plant species to promote rapid herbicide degradation. The authors also concluded that the microbial parameters widely used for the assessment of soil quality are promising tools for evaluating the overall degradation potential of various vegetative buffer designs for atrazine-soil remediation.

Atrazine uptake by green algae and diatoms was investigated by Tang et al. [39]. To inhibit the photosynthetic process of freshwater algae, atrazine must be absorbed intracellularly, the sorption is a prerequisite for its action at the chloroplast membrane.Tang et al. determined atrazine bioconcentration and uptake for eight freshwater green algae and diatoms. The results show that atrazine uptake was extremely rapid in all species examined, with nearly 90% of total uptake occurring within the first hour of exposure. Within each division, different species had different bioconcentration capacities, yet the accumulation of atrazine was consistently higher in green algae (5.43–12.73 ng/mg) compared to that in diatoms (0.33–1.69 ng/mg). Atrazine concentrations in the algal cells were much higher than in the medium, although the total amount of atrazine taken up by algae was small relative to the total atrazine in solution (1–3%). The ability of algal cells to accumulate atrazine was highly correlated with algal cell biovolume and surface area, and a strong relationship was observed between sensitivity to atrazine and bioconcentration, cell biovolume and surface area. In general, higher bioconcentration factors were associated with increased atrazine sensitivity.

http://dx.doi.org/10.5772/60858

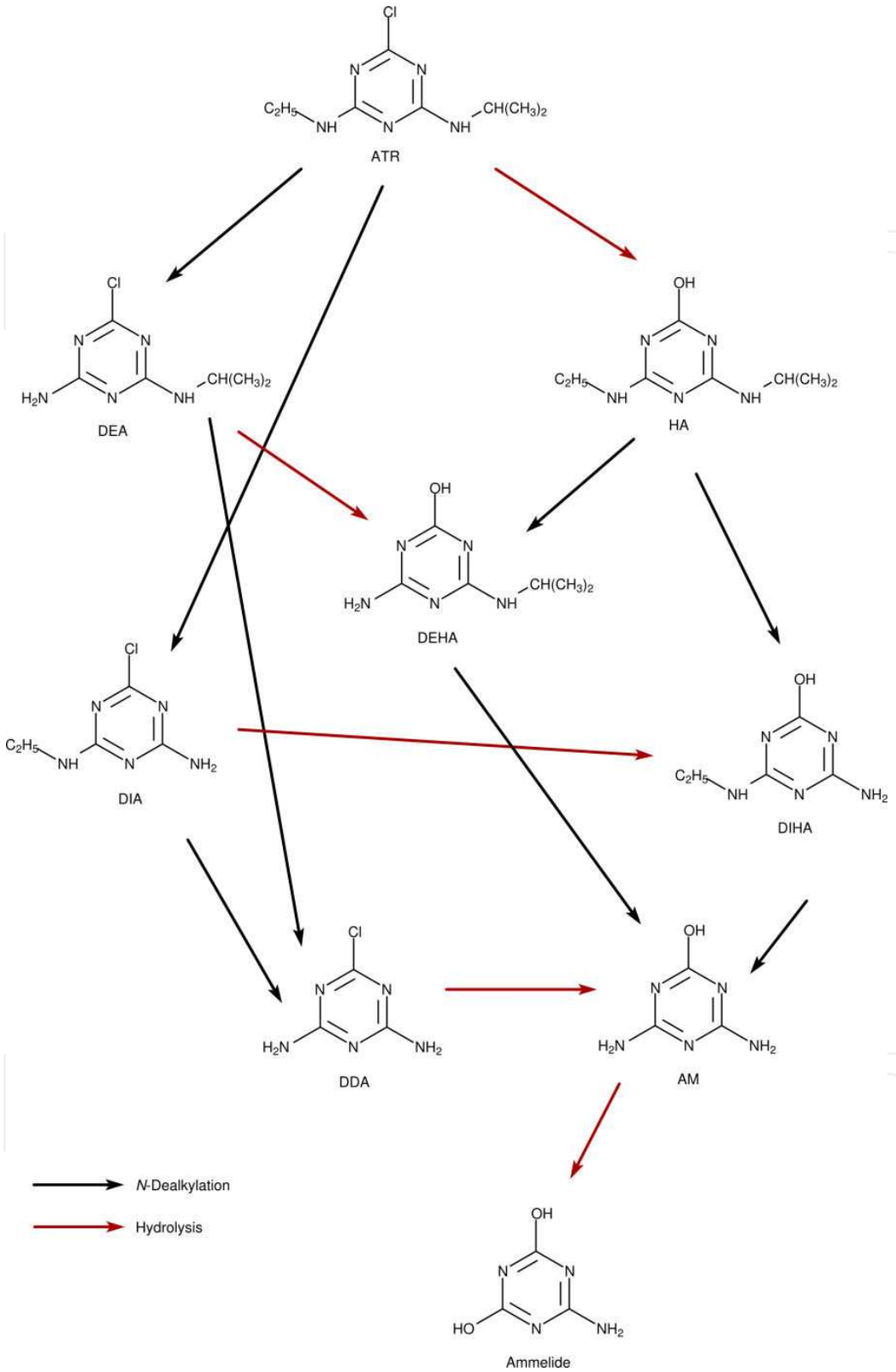

Figure 2. The major atrazine degradation pathways according to Sadowsky et al. [34] and de Souza et al. [35]. ATR – atrazine, DEA – desethyl atrazine, HA – hydroxyatrazine, DIA – desisopropyl atrazine, DIHA – desisopropyl hydroxyatrazine, DDA – didealkyl atrazine, AM – ammeline.

Triazine herbicides are considerably soluble in water and can therefore leach to ground waters or be washed to surface waters, which is why they are among the most often detected xenobiotics in aquatic ecosystems as shown by many studies such as those summarised by Scribner et al. [40].

The study conclusively shows that not only triazine herbicide themselves are found in significant amounts but also that their degradation products have been repeatedly detected; due to the persistence of the compounds, their concentrations in surface waters in agricultural areas remained elevated not only soon after application, but throughout the subsequent summer and into the autumn. Atrazine was shown to occur at the highest frequency (82.1%) and in the highest detectable concentration with a mean of 1.36 μg/L, whereas others were detected at significantly lower frequencies as well as concentrations, for example cyanazine was detected in only 47.2% of samples with a mean detectable concentration of 0.61 μg/L.

The study summarises results from monitoring both surface water bodies, such as reservoirs and streams, and ground water; the findings can be outlined as follows.

2.1. Reservoirs and streams

To ensure more accurate geographic and seasonal interpretation of the triazine and triazine metabolites sampling data, 147 stations in the Midwestern US were sampled in 1989, on 53 of them the sampling was conducted during the 1990s and 2002.

The data revealed that high concentrations of herbicides and their degradation products are mobilised with rainfall, then transported to streams with runoff. The majority of this transportation takes place during the first rainfall and runoff after the application of herbicides. Subsequent runoff tends to produce lesser peaks in concentration. Because of these flushes, the detections of herbicides in larger Midwestern streams tends to be seasonal with higher percentages of detections in spring and early summer and lower percentages in autumn and winter. Measurable amounts of atrazine occurred in 91% of the pre-application samples, providing an indication of its persistence in surface waters.

Three-dimensional images of the distribution of atrazine concentrations in Perry Lake during 1992 showed that recently applied atrazine is mixed with atrazine applied the previous year as water moved through the reservoir (Figure 3). Relations between atrazine and the degradation product, desethyl atrazine, indicated whether the atrazine in the reservoir had been flushed off the fields immediately prior to sampling or whether it remained present from the preceding year. Changes in atrazine concentrations in the reservoir resulted from several factors, including herbicide application, which fuelled and reset the system, and precipitation, which drove the system by flushing atrazine into the reservoir and determining the residence time of water in the reservoir. Concentrations varied between the main inflow and the public water supply intakes located at the opposite ends of the reservoir. The concentration range in the outflow varied much less than concentrations in the upstream parts of the reservoir because of mixing.

The frequency of occurrence, concentrations (both annual average and peak) and quantities of pesticides in streams or reservoir outflows is related to the magnitude of the herbicide used in

http://dx.doi.org/10.5772/60858

the upstream drainage areas. In most cases, the annual loss of herbicides to streams represents about 5% of the amount applied. Pesticide losses are also affected by soil type, climate, land cover, other basin characteristics and management or application techniques.

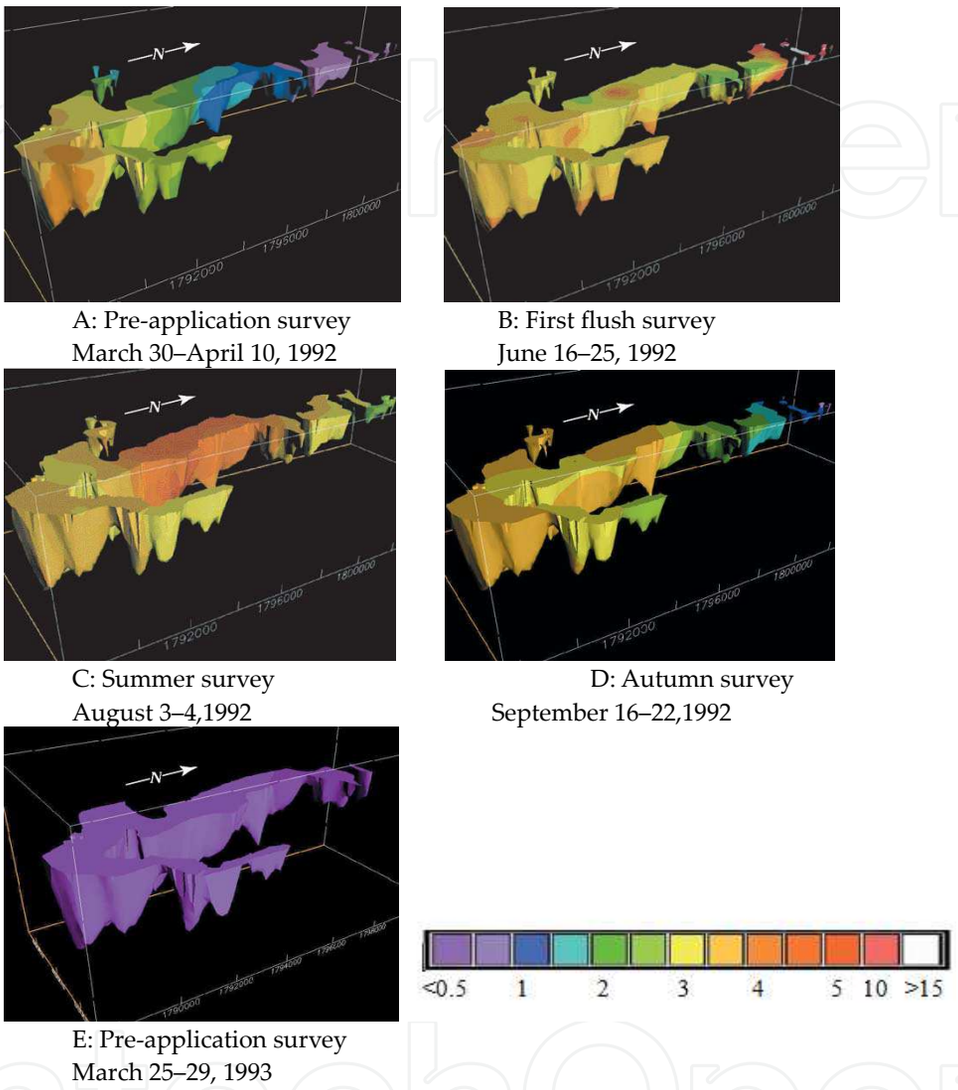

Figure 3. Three-dimensional computer images of atrazine concentrations in Perry Lake, northeastern Kansas as presented by Fallon et al. [41].

Triazine concentrations on the coloured scale are in μg/l.

2.2. Ground water

The study summarises an investigation of samples collected from 131 municipal wells in Iowa from 1995 to 1998. It compares the occurrence of herbicide degradation products with the occurrence of their parent compounds in ground water. An important finding of this study was the high frequency with which degradation products were detected in ground water. During 1995, more than one herbicide compound was detected in samples from 44

of the wells. Atrazine is the only herbicide for which the parent compound was found more often than any of its degradation products. This could be because of the greater environmental stability of atrazine compared to the other s-triazine parent compounds under investigation (simazine, propazine, cyanazine). Aquifer types presumed to have the most rapid recharge rates were those most likely to contain detectable concentrations of herbicides, indicating that groundwater age could be an important factor in explaining these variations in herbicide contamination.

As a continuation of the Iowa study, groundwater wells were sampled and analysed for 21 herbicides and 24 herbicide degradation products during 2001. The frequency of detection increased from 17%when only herbicide parent compounds were detected to 53% when both herbicide parent compounds and degradation products were found.

The groundwater samples collected during these studies consistently revealed that triazine herbicide degradation products were found more frequently than their parent herbicide; the frequency of herbicide detection was, in part, affected by a more sensitive analytical method. The study accentuates that groundwater age could be an important factor in explaining variations in herbicide contamination.

3. Influence of atrazine on animal and human physiological processes and health

The environmental pollution caused by pesticides has become a widespread problem. The high use of these chemicals has caused an increasing concern over their acute and chronic health effects. Persistent pollutants can contaminate organisms at all trophic levels, and may cause severe damage to the organisms through metabolites contained in contaminated bodies of water. The patterns of accumulation and effect of xenobiotics depend on the organism itself, on the properties of the compound, on the quantity of the compound present in the particular part of environment, and – last but not least – on the balance between bioassimilation and metabolic plus excretion rates [42].

Laboratory studies are usually based on tests of LD_{50} (lethal dose) values. An LD_{50} value represents the individual dose required to kill 50 % of a population of the animals tested (e.g. rats, mice, fish). LD_{50} values gives a measure of acute toxicity, thus determining the threshold concentration of the xenobiotic to classify acute hazards of the substance for the purposes of legislation.

To evaluate the impact of a pollutant on an organism the detection of biological effects is needed; such detection involves identifying morphological, molecular, biochemical, or physiological biomarkers [43].

Orally ingested atrazine is readily absorbed by the gastrointestinal tract. Experiments have shown that 20% of the dose administered orally to rats was excreted in feces within 72 h; the remaining 80% was detected in the bloodstream. After 72 h, 65% of the dose was eliminated in urine and the remaining 15% was detected in tissues such as the brain, liver,

kidneys, and lungs [44]. High concentrations of atrazine were found in the liver and kidneys of male mice [45]. This bioaccumulation is probably due to the ability of the substance to interact with the phospholipid components of biomembranes. The interaction prevents the excretion of atrazine until the substance undergoes a degradation leading to a higher solubility in aqueous solutions.

Since the liver is one of the target organs for atrazine, effect on the regulatory platform in the mechanism of liver homeostasis have been investigated. Kalmar and Greensmith [46] described the adaptation mechanism of the response to cellular stress, especially the oxidative stress, which is based on an activation of expression of heat shock proteins (HSPs); this mechanism helps a wide range of organisms from bacteria to mammals to survive environmental challenges and adapt to them. It may underlie the defence abilities of organisms to resist the small-molecule inducers of the heat shock response (HSR). This adaptation mechanism may be connected with so-called gap junctional intercellular communication and its role in the combat of liver-toxic compounds. As shown by Vinken et al. [47], gap junctions, that is aggregates of intercellular channels that permit the direct cell–cell tranfer of ions and small molecules, play a central role in the development of tissues as well as in the so-called bystander effect messaging cell death. They have also been shown to be the platform of communication between hepatocytes. The deletarious effects of toxic compound on the gap junctions are often accompanied by the triggering of oxidative stress in the tissue followed by the heat shock response.

A comprehensive survey of investigations on the health risks of triazine herbicides with respect to carcenogenesis is provided in a review by Jowa and Howd [21]. They collected the results of more than 200 studies on animals (rats, mice, dogs) as well as of human epidemiological studies on exposed populations, including occupational and residential risks. The data on occupational risk came from employees who underwent exposure during manufacturing; the residential risks were assessed from the data on the use of contaminted water and pesticide usage in corn crop production. The survey can be summarised as follows:

- The significant amount of research into the mechanism of mammary tumor formation in rats has investigated triazine effects on the estrus cycle. Both the *in vivo* and *in vitro* data suggest that atrazine and simazine disrupt ovarian cycling and that this mechanism can induce mammary tumors in the SD rat strain (SD rat = Sprague-Dowley rat, an outbred of alpino rat used extensively in medical research). This alteration of the estrous state through hormonal induction and estrogen mediated responses is associated with the incidence of mammary tumors in SD rats.

- Another suggested mechanism for chloro-s-triazine-mediated carcenogenicity is that it may be due to increased levels of estrogen and lower testosterone from the effect of chlorotriazine on aromatase activity. However, the induction of aromatase activity has not been established as a consistent effect. Moreover, long term reproductive studies have not provided particular evidence of feminisation of male rats (an expected concomitant of aromatase induction that would be demonstrated as developmental changes or mating success). The increase of pituitary tumors in rats is thought to be related to the mechanisme of estrogen mediated responses.

- Effects observed in experimental animal toxicity tests are assumed to be relevant to humans unless there is adequate evidence to the contrary. In the case of rat mammary tumors induced by atrazine and related chloro-s-triazine compounds, including metabolites, there is ample contrary evidence, since reproductive cycling in rodents is drastically different from that of humans. Differences between rodents and humans also exist in reproductive senescence. Thus, examination of the mechanistic basis for carcinogenicity of chloro-s-triazines has led to the deduction that the tumorigenic mechanism in rats is not relevant to humans.
- The epidemiologic studies have not indicated association of chloro-s-triazines with mammary carcinogenesis in humans. Evidence of assiociation with the occurence of other cancers is rather weak.
- There is little evidence that atrazine and its congeners are mutagenic. Putative human carcinogenic activity of triazines mediated through endocrine disruption has not been established by the observation of any direct binding to estrogen receptors or a competitive inhibition involving these receptors; there is the exception of some suggestive evidence that atrazine may interact with the GPR30 estrogen receptor (currently denoted as GPER) which is an integral membrane protein with a high affinity for estradiol though not for other endogeneous estrogens. There is no evidence that chloro-s-triazines behave like estrogens in their interaction with estrogen-sensitive tissues. Also, there was no increase in prolactin release with atrazine exposure.
- Hormonally active xenobiotics including atrazine have been identified as endocrine disrupting chemicals. These chemicals exert hormone-like activity in vertebrates and exposure to these compounds may induce both short- and long-term deleterious effects including functional alterations that contribute to decreased reproduction and fitness.There is clear evidence that the chloro-s-triazines have an endocrine influence, and that this influence is likely to be relevant to humans. Changes in circulating endocrine hormones have been observed regardless of rat strains in relation to all the triazines and it is a basis for assuming this to be a relevant endpoint for human risk assessment.

4. Toxicity tests for triazine herbicides

Water pollution consisting of toxic compounds represents a major cause of the failure of biological treatment plants, resulting in noncompliance with discharge permit limits. Experimental studies using nonspecific models at laboratory level are extensively used to predict the potential hazards of chemical and industrial waste regarding the environment, especially aquatic systems. These toxicity evaluation models have the advantage of generating quickly reproducible data at relatively low cost. The criteria of toxicity generally taken into consideration are death or changes in mobility, reproduction, growth, physiological functions, behaviour and genetic information.

Microbial tests have been widely used in toxicity screening procedures due to factors such as short exposure time, ease of handling and reproduciblity. In 1979, Bulich [48] proposed a

http://dx.doi.org/10.5772/60858

specific test for rapid assessment of the toxicity of aquatic samples using the light emitting bacterium *Vibrio fischeri* (former *Photobacterium phosphoreum*). This non-pathogenic marine bacteria emits light as part of cellular respiration, the light can be measured as luminescence. *Vibrio fischeri* have demonstrated high sensitivity across a wide variety of toxic substances. The organism´s response to toxicity is observed as a change in luminescence. In 1982, the system was developed commercially under the trade mark Microtox™ (Berckman Instruments Inc.) This test is now widely accepted as a standard bioassay [49] used also for chloro-s-triazine herbicide [1,50]. During the test, the 'effective concentration' (EC_x) is detected; the EC_x is an analogue to lethal dose LD_x, and represents the concentration at which the light emitted by the microorganisms is reduced by a specific percentage. Usually, EC_{10}, EC_{20} or EC_{50} is determined [1]. Kross et al. [1] determined the EC_{10}, EC_{20} and EC_{50} values for atrazine, desethyl atrazine and desisopropyl atrazine, the values are summarised in Table 1. The data show that the lowest concentrations necessary for a toxic effect are connected with atrazine, the concentration being almost an order of magnitude less than the toxic concentrations of the atrazine metabolites. Of the two atrazine metabolites, desisopropyl atrazine was found to be more toxic.

Nevertheless, as concluded by Lapertot et al. [51], Microtox© is basically inappropriate for surveying the toxicity of herbicides such as atrazine because *V. fischeri* is not a photosynthetic organism, and is not therefore by definition properly sensitive to herbicides, which are toxic in the short term, specifically to photosynthetic species.

	Compound		
	Atrazine	**Desethyl atrazine**	**Desisopropyl atrazine**
EC_{10}			
5 min	13.0 (5-20)	70.0 (37-100)	44.0 (22-75)
15 min	14.4 (0-32)	101.0 (75-125)	63.0 (43-92)
30 min	17.5 (0-37)	134 (120-160)	75.0 (56-102)
EC_{20}			
5 min	25.8 (15-35)	180.0 (130-220)	107.0 (74-155)
15 min	22.6 (7-37)	193.0 (160-220)	109.0 (88-155)
30 min	24.0 (5-45)	220.0 (200-250)	116.0 (96-150)
EC_{50}			
5 min	30	670	350
15 min	20	550	300
30 min	10	550	280

Table 1. Concentration of pesticides and metabolites inducing a toxic response in photoluminescent bacteria. Parentheses indicate 95% confidence intervals for concentrations. For EC_{50} data were extracted from a graphical representation. Data from Kross et al. [1].

For the aquatic environment, toxicity tests based on photosensitising aquatic organisms are considered more relevant. One of these organisms is a species of green alga, *Raphidocelis subcapitata*, a sensitive and environmentally relevant model. Klementová et al. [52] performed growth inhibition test in reaction mixtures of atrazine and atrazine degraded in photocatalytic reaction with UV light in the presence of immobilised TiO_2 as photocatalyst. All atrazine samples displayed dose–response relationships and the highest tested concentration (10 mg/l) of the initial atrazine concentraion treatments reached more than 80% inhibition (Figure 4). The study was focused on the comparison of the toxicity of the parent compound solution and the reaction mixtures after different irradiation times (photodegradation progress). The toxicity of samples to algae decreased exponentially with the time of irradiation in the presence of TiO_2 up to 3 h for both IC_{50} and IC_{20} values calculated for growth rate inhibiton and yield (Figure 5). After 3 h of irradiation the decrease in toxicity slowed down, this effect being more pronounced in IC_{20} values.There is a trend towards increasing variability between replicates in atrazine samples irradiated for longer time periods, which is particularly pronounced in samples irradiated for 3 and 5 h (less than 10% of the original amount of atrazine is present in the irradiated mixture), where the samples cause a moderate stimulation in lower concentration treatments (up to 550 μg/l of the initial atrazine concentration).

The results observed in the study for the non-irradiated atrazine samples are comparable with the results of Van der Heever et al. [53] who reported IC_{50} 385 μg/l, even though lower IC_{50} values for the same species were reported by Weiner et al. [54] and Pérez et al. [55] (48.77 μg/l and 196 μg/l, resp.)

The photochemical degradation on semiconducters such as TiO_2 has been established as an efficient tool for its removal from drinking and waste waters [8,9,18,56]. However, the toxicity of the mixtures of photodegradation products arising under distinct irradiation conditions is still subject to current research since there is evidence that degradation products of some compounds may elicit higher toxicity than the parent compound [20,57]. A decrease in the toxicity of atrazine after photocatalytic degradation has been described using Microtox bacterial bioluminiscence assay [18,58]. The data presented by Klementova et al. [52] show a similar trend with a significant decrease in toxicity to algae after 1 h (or longer) periods of irradiation when more than 65% of atrazine is degraded. The results indicate no formation of by-products with equal or higher toxicity to algae than the parent compound atrazine.

A study of a toxicity test was performed on another model relevant to the aquatic environment, RTgill cells [52]. In the study, cell viability was assessed using the combination of three fluorescent dyes that determine different cytotoxic mechanisms: Alamar Blue, which indicates the metabolic activity, carbon fluorescein diacetate acetoxymethyl ester, which is used for monitoring of cell membrane integrity, and neutral red, which indicates the energetic state of a cell and is used as an indirect criterion of the lysosomal membrane integrity. Experiments with fish RTgill cells showed no toxic effects for either atrazine or its reaction mixtures after photocatalytic degradation of atrazine in the presence of TiO_2.

The results of the study seem to correspond with the *in vivo* ecotoxicity data for atrazine reported in the US EPA Ecotox Database in which EC/LC_{50} of atrazine for rainbow trout in acute tests exceeded 10 mg/l [59].

http://dx.doi.org/10.5772/60858

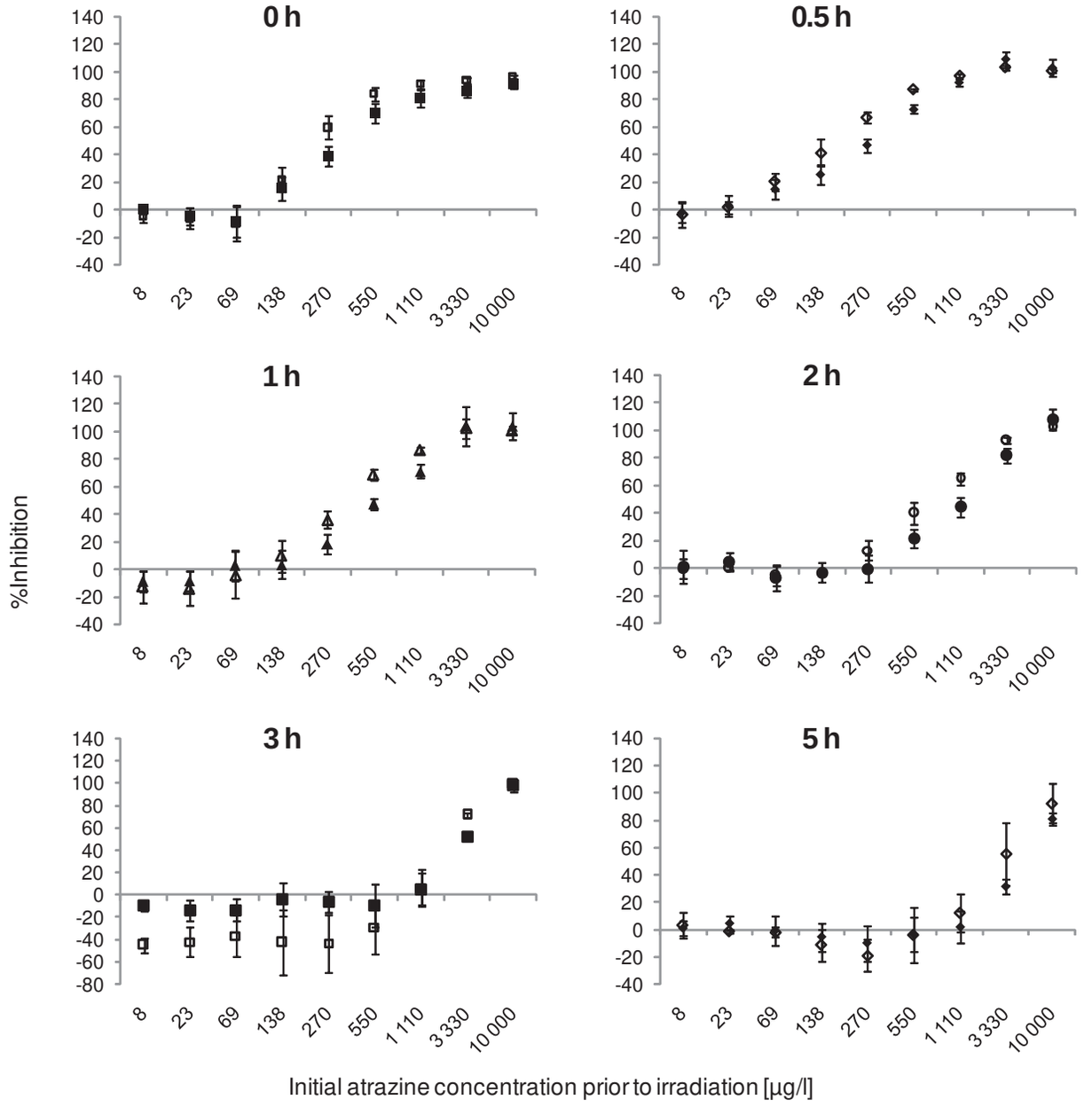

Figure 4. Inhibition of algal growth by atrazine samples with increasing duration of irradiation on TiO_2. The graphs show the initial atrazine concentrations [µg/l] in the non-irradiated sample (*0 h*), which is the theoretical maximum concentration of atrazine itself in the irradiated samples. Closed symbols show growth rate inhibition, open symbols yield inhibition and error bars standard deviation (*N*=5). Figure from Klementová et al. [52].

Wan et al. [60] reported acute LC_{50} values of atrazine 15 and 13 mg/l for 1- and 2–4-day exposures, respectively, for the same species. However, in the study of Waring and Moore [61] atrazine caused a significant reduction in gill Na+K+ATPase activity in Atlantic salmon (*Salmo salar*) smolts at environmentally relevant concentrations (2–10 µg/l). In a study by Prasad et al. [62], atrazine altered the hemocyanin metabolism, hydromineral balance, and gill function in crabs (*Oziotelphusa senex senex*). Exposure to 5 µg/l of atrazine led to osmotic disfunctions in mummichog fish larvae (*Fundulus heteroclitus*) and exposure to 40–80 µg/l resulted in behav-

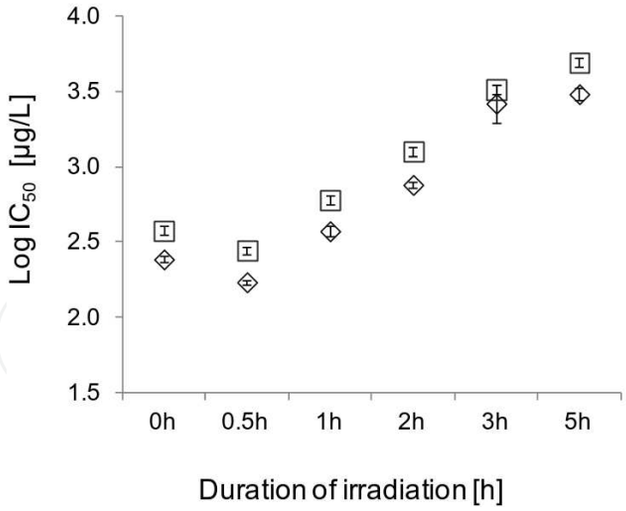

Figure 5. Decrease of atrazine toxicity to green alga *Raphidocelis subcapitata* with the duration of irradiation on TiO_2. The graph shows the logarithms of estimated inhibitory concentrations [µg/l] based on the nominal initial atrazine concentration in the non-irradiated sample (*0 h*), which is the theoretical maximum concentration of atrazine in the irradiated samples. Diamonds show growth rate inhibition and squares yield inhibition. Error bars denote standard deviation of the Hill's model fitting. Figure from Klementová et al. [52].

ioural and growth changes in red drum larvae *Sciaenops ocellatus*) [63]. Therefore, further experiments with fish embryos might provide additional insights into the toxic effects of atrazine and it photodegradation products.

5. Methods for atrazine degradation

The presence of chemically resistant and biorecalcitrant organic comtaminants in freshwater (surface water and ground water) attracts attention to developing technologies promoting easy and cost-effective degradation of these compounds. Among techniques for the treatment and purification of polluted waters, those based on advanced oxidation processes (AOP) are considered most promising. The AOP techniques are characterised by the ´*in situ*´ production of hydroxyl radicals (or other oxidative species. The production of hydroxyl radicals can be achieved in several ways, the most commonly used are Fenton process, photo-Fenton process, photodissociation of ozone or hydrogen peroxide and especially heterogeneous photocatalysis on semiconductors.

Also, some photosensitising reactions combining heterogeneous photocatalysis and photo-sensitisation have been used as AOPs.

Degradation can be achieved by UV photolytic processes that use low-wavelength high-energetic light.

5.1. Homogeneous photocatalysis, Fenton and photo-Fenton reaction

The Fenton reaction is the reaction of a mixture of ferrous ions and hydrogen peroxide providing hydroxyl radicals as shown in Eq. 1.

$$Fe^{2+} + H_2O_2 \rightarrow HO^{\bullet} + Fe^{3+} + OH^{-} \quad (1)$$

In this process, part of the radicals are consumed in the oxidation of ferrous ions, which results in the quick consumption of the added photocatalyst, ferrous ions.

In the photo-Fenton process, the ferrous ions are formed *in situ* photochemically, as shown in Eq. 2, the reaction of the ferrous ions with hydrogen peroxide proceeds as in the Fenton reaction (Eq. 1)

$$Fe^{3+} + H_2O \xrightarrow{h\nu} Fe^{2+} + HO^{\bullet} + H^{+} \quad (2)$$

The efficiency of Fenton type processes is influenced by several operating parameters such as the concentration of hydrogen peroxide, pH and the intensity of UV radiation. The main advantage of the photo-Fenton process is its ability to use sunlight for photochemical activation, thus avoiding the high costs of UV lamps. The disadvantages of the process are the low pH values required (to avoid iron precipitation) and the necessity of iron removal after the treatment. These processes have been used efficiently for different classes of pollutants including atrazine [64].

The photocatalytic action of metals such as ferric, copper and manganese ions on the degradation of triazine herbicides without the addition of hydrogen peroxide was also investigated [65]. The study revealed that cupric and manganese (II) ions exhibited only small activities, and only in high concentrations ($1*10^{-3}$ mol/l), whereas ferric ions positively affect the degradation at concentrations as low as $1.5*10^{-6}$ mol/l, and with increasing concentration of these ions the degradation rate significantly increased. The dependence of the rate constants of the degradation on the initial ferric ions concentration based on the data presented by Klementova [14] is given in Table 2. In all reaction mixtures of s-triazine studied, that is atrazine, propazine, simazine, significant photoreduction of ferric ions to ferrous ones was observed in spite of the saturation of the mixtures with the air; the steady state concentrations of ferrous ions were established in less than 10 min of irradiation and reached 22%, 70% and 85% of the initial concentration of added ferric ions for atrazine, propazine and simazine, respectively. This supports the conclusion that photoreduced metal ions act as a catalycally active form in the homogeneous photocatalytic degradation of triazines [14,65].

Initial concentration of added ferric ions (mol/l)	**Rate constant (min^{-1})**
$1.5*10^{-6}$	$3.0*10^{-4}$
$1.0*10^{-5}$	$9.0*10^{-4}$
$6.6*10^{-5}$	$1.7*10^{-3}$
$1.0*10^{-4}$	$4.8*10^{-3}$
$1.6*10^{-4}$	$1.3*10^{-2}$

Table 2. Dependence of the rate constant of atrazine in homogeneous photocatalytic degradation on the concentration of the ferric ions added.

5.2. O_3/UV, H_2O_2/UV techniques

Degradation techniques involving combination of H_2O_2/UV or O_3/UV often combined with photocatalysis in homogenenous as well as heterogeneous arrangement were applied to promote the degradation and mineralisation of many organic biorecalcitrant compounds including triazine herbicides [66-70].

5.3. Heterogeneous photocatalysis on semiconductors

Semiconductor photocatalysis uses solid catalytic systems while the substrate to be degraded is dissolved or dissipated in the solution (or gaseous phase) around the catalyst. Five distinct steps in the process of degradation of a reactant are, therefore, distinguished:

a. The transfer of liquid or gaseous phase reactant to the catalytic surface by the diffusion

b. The adsorption of the reactant on the catalyst surface

c. The reaction of the adsorbed molecules

d. The desorption of products

e. The removal of products from the interface region by diffusion

The photocatalytic reaction occurs in the stage in which the reactant is adsorbed by the catalyst surface; the activation of the reaction is triggered by incident photons.

Proper activation by irradiation depends on using an appropriate wavelength range corresponding to the band gap energy of the particular semiconductor (Figure 6).

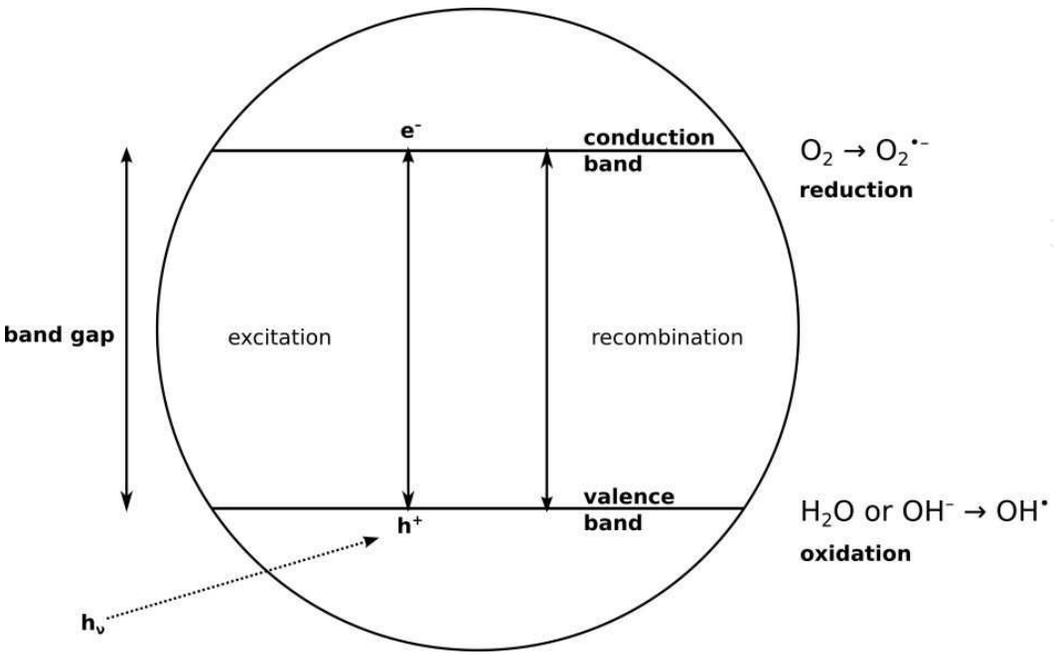

Figure 6. Scheme of oxidation species production in photocatalysis on semiconductors.

The activation generates a pair of charge carriers, a hole and an electron; the charge carriers then react either with water molecules (), or with the dissolved oxygen (), in a series of reaction leading to production of hydroxyl radicals as shown in Eqs. 3–8.

$$h^+ + H_2O \rightarrow HO^\bullet + H^+ \quad (3)$$

$$h^+ + OH^- \rightarrow HO^\bullet \quad (4)$$

$$O_2 + e^- \rightarrow O_2^{\bullet -} \quad (5)$$

$$O_2^{\bullet -} + H^+ \rightarrow HO_2^\bullet \quad (6)$$

$$2HO_2^\bullet \rightarrow H_2O_2 + O_2 \quad (7)$$

$$H_2O_2 + O_2^{\bullet -} \rightarrow HO^\bullet + O_2 + OH^- \quad (8)$$

Various metals oxides were used in semiconductor photocatlytic reactions. The most frequently used is TiO_2 [15,71, 72] but also other materials such as ZnO, CeO_2, WO_3, as well as semiconductor composites, doped or modified semiconductors [73-76].

The disadvantage of the photocalatysis on semiconductors lies in the fact, that though many catalysts were proposed, it is generally admitted that only TiO_2 gives reasonable results in pollutant degradation; unfortunately, photons required for overcoming the energy gap and thus initiating the photocatalytic process in TiO_2 must have a wavelength of less than 385 nm, which practically excludes the sun as a low-cost energy source since this radiation represents only about 5% of the sunlight ultraviolet and visible range reaching the earth's surface.

Doping a semiconductor with precious metals such as gold, platinum, silver or palladium may reduce the band gap but increases the cost of the catalyst.

Another option is a binding of sensitiser molecules on the catalyst. An example is a study of Granados-Oliveros [76] in which porphyrin derivatives with different metal centers were adsorbed on TiO_2 surface. Their delocalised macrocyclic structure, strong absorption in the visible region and excited state energy enabling electron transfer to the catalyst conduction band seem to make them very attractive for investigation though less likely candidates for large-scale usage.

Photocatalytic degradation of s-triazine herbicides on TiO_2 has been studied by many authors [15,52,71, 77-79]; atrazine was found to be degraded mainly to desethyl atrazine and desiso-

propyl atrazine; the hydroxy derivatives of these compounds as well as of the parent compound atrazine were present in the reaction mixtures. It means that photocatalytic degradation leads to the same compounds that have been distinguished as metabolites of atrazine biodegradation.

5.4. Photolysis

Photolysis means direct photochemical degradation with short-wavelength radiation which has enough energy to break bonds in a molecule.

The reaction includes only one reactant, the molecule that undergoes photolysis; therefore the reaction follows the first order kinetics.

To achieve a photolytic decomposition, highly energetic radiation is necessary, usually radiation of 254 nm is used.

The photolytic degradation of triazines has been studied by several authors [11,80,81]. The studies were focused on the kinetics of the degradation; analyses of products revealed that dechlorination is the main degradation pathway. Klementova and Piskova [80] reported on the basis of an analysis of dissolved organic carbon (DOC) that photolytic degradradation leads to the partial mineralisation of atrazine – about 20 % of DOC was mineralised in 90 min of irradiation at the intensity of $1.22*10^{-5}$ einstein/min.

6. Conclusions

Chloro-s-triazine herbicides, namely atrazine, have been shown to be distributed in the soil and water environment. Their biodegradation is slow and leads to dealkylated and hydroxylated derivatives, which – though seemingly have less deleterious effects on organisms – persist in the environment even longer than the parent compounds.

The harmful effects of triazine compounds on the health of humans appear to lie in their action as endocrine disruptors; evidence of possible carcenogenic or mutagenic effects on humans is rather weak but cannot been conclusively excluded.

AOP photochemical methods have proven to be a promising tool in disposing of these pollutant in contaminated bodies of water.

Acknowledgements

I would like to thank my son David Klement for his help with reproducing cited formulas and figures in this manuscript.

Author details

Sarka Klementova* and Lucie Keltnerova

*Address all correspondence to: sklement@jcu.cz

Faculty of Science University of South Bohemia, Ceske Budějovice, Czech Republic

References

[1] Kross BC, Vergara A, Raue LE. Toxicity Assessment of Atrazine, Alachlor, and Carbofuran and their Respective Environmental Metabolites Using Microtox. *Journal of Toxicology and Environmental Health* 1992; 37, 149–159.

[2] Minero C, Pramauro E, Pelizzetti E. Photosensitized Transformations of Atrazine under Simulated Sunlight in Aqueous Humic Acid Solution. *Chemospere* 1992; 24 (11), 1597–1606.

[3] Torstenson L. Use of Herbicides on Railway Tracks in Sweden. *Pesticide Outlook* 2001; 12, 16–21.

[4] Lindner W, Rohermel J, Taschenbrecker E, Wohner G. Algicide Combination. *Patent No 6117817(US).* http://www.google.com.ar/patents/US6117817 (accessed 16 January 2015)

[5] D'Itri FM, Wolfson LG (editors). Rural Ground Water Contamination. Lewis Publishers, Chelsea, MI; 1987.

[6] Graymore M, Stagnitti R, Allison G. Impacts of Atrazine in Aquatic Ecosystems. *Environment International* 2001; 26, 483–495.

[7] Ritter L, Solomin KR, Forget J, Stemeroff M. O'Leary C. Persistent Organic Pollutants. *International Program on Chemical Safety. UN Envirnmental Program* 1996. http://www.who.int/ipcs/assessment/en/pcs_95_39_2004_05_13.pdf (accessed 10 January 2015)

[8] Konstantinou IK, Albanis TA. Photocatalytic Transformation of Pesticides in Aquesous Titanium Dioxide Suspensions Using Artificial and Solar Light: Intermediates and Degradation Pathways. *Applied Catalysis B: Environmental* 2003; 42, 319–335.

[9] Fenoll J, Hellín P, Martínez CM, Flores P, Navarro S. Semiconductor-Sensitized Photodegradation of S-Triazine and Chloroacetanilide Herbicides in Leaching Water Using TiO_2 and ZnO as Catalyst under Natural Sunlight. *Journal of Photochemistry and Photobiology A: Chemistry* 2012; 238, 81–87.

[10] Qin C, Yang S, Sun C, Zhan M, Wang R, Cai H, Zhou J. Investigation of the Effects of Humic Acid on H_2O_2 on the Photocatalytic Degradation of Atrazine Assisted By Microwave. *Frontiers of Environmental Science and Engineering* 2010; 4 (3), 301–328.

[11] Bianchi CL, Pirola C, Ragani V, Selli E. Mechanism and Efficiency of Atrazine Degradation under Combined Oxidation Processes. *Applied Catalysis B: Environmental* 2006; 64 (1–2), 131–138. http://www.sciencedirect.com/science/article/pii/S0926337305004078 (accessed 30 January 2015)

[12] Grcic I, Koprivanac N, Vujevic D, Papic S. Removal of Atrazine from Simulated Groundwater by AOTs. *Journal of Advanced Oxidation Technologies* 2008; 11 (1), 91–96.

[13] Horakova M, Klementova S, Kriz P, Balakrishna SK, Spatenka P, Golovko O, Hajkova P, Exnar P. The synergistic Effect of Advanced Oxidation Processes to Eliminate Resistant Chemical Compounds. *Surface and Coatings Technology* 2014; 241, 154–158.

[14] Klementova S. A Critical View of the Photoinitiated Degradation of Herbicides. In: Naguib Abd El-Ghany Hasaneen (ed.) Herbicides – Properties, Synthesis and Control of Weeds. Rijeka: InTech; 2012. pp. 297–314.

[15] Klementova S, Zlamal M. Photochemical Degradation of Triazine Herbicides – Comparison of Homogeneous and Heterogeneous Photocatalysis. *Photochemical and Photobiological Sciences* 2013; 12, 660–663.

[16] Canle LM, Fernández MI, Santaballa JA. Developments in the Mechanism of Photoderadation of Triazine-Based Pesticides. *Journal of Physical Organic Chemistry* 2005; 18, 148–155.

[17] Dojlido J, Best GA. Chemistry of Water and Water Pollution. Ellis Horwood Limited, Chichester; 1993.

[18] Campanella L, Vitaliano R. Atrazine Toxicity Reduction Following H_2O_2/TiO_2-Photocatalyzed Reaction and Comparison with H_2O_2-Photolytic Reaction. *Annali di Chimica (Rome)* 2007; 97, 123–134.

[19] Malato S, Agüera A. Detoxification of Pesticide in Water Using Solar Photocatalysis. In: Pesticide Decontamination and Detoxification. ACS Symposium Series 863, Washington, DC: American Chemical Society; 2004, 113–126.

[20] Vicente A. Determination of Pesticides and Their Degradation Products in Soil: Critical Review and Comparison of Methods. *Trends in Analytical Chemistry* 2004; 23, 772–789. http://dx.doi.org/10.1016/j.trac.2004.07.008 (accessed 15 January 2015)

[21] Jowa L, Howd R. Should Atrazine and Related Chlorotriazines be Considered Carcinogenic for Human Health Risk Assessment? *Journal of Environmental Science and Health, Part C* 2011; 29, 91–144.

[22] Hayes TB, Collins A, Lee M, Mendoza M, Noriega N, Stuart AA, Vonk A. Hermaphroditic, Demasculinized Frogs Following Exposure to the Herbicide Atrazine, at Eco-

logically Relevant Doses. *Proceedings of the National Academy of Sciences (USA)* 2002; 99 (8), 5476–5480.

[23] Van Leeuwen JA, Waltner-Toews D, Abernathy T, Smit B, Shoukri M. Association between Stomach Cancer Incidence and Drinking Water Contamination with Atrazine and Nitrate in Ontario (Canada) Agroecosystems. *International Journal of Epidemiology* 1999; 28 (5), 836–840.

[24] Tomlin CDS. The Pesticide Manual. BCPC (British Crop Protection Council), Hampshire, UK; 2003.

[25] Mudhoo A., Garg VK. Sorption, Transport and Transformation of Atrazine in Soils, Minerals and Composts: A Review. *Pedosphere* 2011; 21 (1), 11–25.

[26] Velthuys BR. Electron Dependent Competition between Plastoquinone and Inhibitors for Binding to Photosystem II. *Febbs Letters* 1981; 126, 277–282.

[27] Fuert EP, Norman MA. Interactions of Herbicides with Photosynthetic Electron Transport. *Weed Science* 1991; 39, 458–464.

[28] Binet F, Kersanté A, Munier-Lamy C, Le Bayon R-C, Belgy M-J, Shipitalo MJ. Lumbricid Macrofauna Alter Atrazine Mineralization and Sorption in a Silt Loam Soi. *Soil Biology and Biochemistry* 2006; 38 (6), 1255–1263.

[29] Ma L, Selim HM. Atrazine Retention and Transport in Soils. *Reviews of Environmental Contamination and Toxicology* 1996; 145, 129–173.

[30] Correira FV, Macrae A, Guilherme LRG, Langenbach T. Atrazine Sorption and Fate in a Ultisol from Humid Tropical Brazil. *Chemosphere* 2007; 67, 847–854.

[31] Ben-Hur M, Letey J, Farmer Wj, Williams CF, Nelson SD. Soluble and Solid Organic Matter Effects on Atrazine Adsorption in Cultivated Soils. *Soil Science Society of America Journal* 2003; 67, 1140–1146.

[32] Piccolo A., Conte P, Scheunert I, Paci M. Atrazine Interactions with Soil Humic Substances of Different Molecular Structure. *Journal of Environmental Quality* 1998; 27, 1323–1333.

[33] Martin-Neto L, Traghetta DG, Vaz CMP, Crestana S, Sposito G. On the Interaction Mechanism of Atrazine and Hydroxyatrazine with Humic Substances. *Journal of Environmental Quality* 2001; 30, 520–525.

[34] Sadowsky MJ, Tong Z, De Souza M, Wackett LP. ArtzC Is a New Member of Amidohydrolase Protein Superfamily and is Homologous to Other Atrazine Metabolizing Enzyme. *Journal of Bacteriology* 1998; 180, 152–158.

[35] De Souza ML, Newcombe D, Alvey S, Crowley DE, Hay A, Sadowsky MJ, Wackett LP. Molecular Basis of a Bacterial Consortium: Interspecies Catabolism of Atrazine. *Applied Environmental Microbiology* 1998; 64, 178–184.

[36] Lin CH, Lerch RN, Garret HE, George MF. Bioremediation of Atrazine-Contaminated Soil by Forage Grasses: Transformation, Uptake, and Detoxification. *Journal of Environmental Quality* 2008; 37, 196–206.

[37] Chang SW, Lee SJ, Je CH. Phytoremediation of Atrazine by Poplar Trees: Toxicity, Uptake, and Transformation. *Journal of Environmental Science and Health B* 2005; 40, 801–811.

[38] Lin CH, Lerch RN, Kremmer RJ, Garret HE. Stimulated Thizodegradation of Atrazine by Selected Plant Species. *Journal of Environmental Quality* 2011; 40 (4), 1113–1121. http://www.ars.usda.gov/sp2UserFiles/Place/50701000/cswq-0464-lin.pdf (accessed 15 January 2015)

[39] Tang J, Hoagland KD, Siegfried BD. Uptake and Bioconcentration of Atrazine by Selected Freshwater Algae. *Environmental Toxicology and Chemistry* 1998; 17, 1085–1090. http://onlinelibrary.wiley.com/doi/10.1002/etc.5620170614/pdf (accessed 2 February 2015)

[40] Scribner EA, Thurman EM, Goolsby DA, Meyer MT, Battaglin WA, Colpin DW. 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. U.S. Geological Survey, Reston, VA; 2005; 1–27. http://pubs.usgs.gov/sir/2005/5094/pdf/SIR20055094.pdf (accessed 20 January 2015)

[41] Fallon JD, Tierney DP, Thurman EM. Movement of Atrazine and Deethylatrazine through a Midwestern Reservoir. *Journal-American Water Works Association* 2002; 94 (11), 54–65.

[42] Nwani CD, Lakre WS, Nagpure NS, Kumar R, Kushwaha B, Srivastava SK. Toxicity of the Herbicide Atrazine: Effects on Lipid Peroxidation and Activities of Antioxidant Enzymes in the Freshwater Fish *Channa punctatus* (Bloch). *International Journal of Environmental Research and Public Health* 2010; 7 (8), 3298–3312. http://www.mdpi.com/1660-4601/7/8/3298/htm (accessed 20 January 2015).

[43] [43]Campero M, Ollevier F, Stoks R. Ecological Relevance and Sensitivity Depending on the Exposure Time for Two Biomarkers. *Environmental Toxicology* 2007; 22, 625–631.

[44] Campos-Pereira FD, Oliveira CA, Pigos AA, Silva-Zacarin CM, Barbieri R., Spatti EF, Marin-Morales MA, Severi-Aguiar DC. Early Cytotoxic and Genotoxic Effects of Atrazine on Wistar Rat Liver: A Morphological, Immunohistochemical, Biochemical, and Molecular Study. *Ecotoxicology and Environmental Safety* 2012; 78, 170–177. http://www.sciencedirect.com/science/article/pii/S0147651311004180 (accessed 20 January 2015)

[45] Ross MK, Jones TL, Filipov NM. Disposition of the Herbicide 2-chloro-4-(ethylamino)-6-(isopropylamino)-s-triazine (Atrazine) and Its Major Metabolites in Mice: A

Liquid Chromatography/Mass Spectrometry Analysis of Urine, Plasma, and Tissue Levels. *Drug Metabolism and Disposition* 2009; 37, 776–786. http://dmd.aspetjournals.org/content/37/4/776.long (accessed 20 January 2015)

[46] Kalmar B, Greensmith L. Induction of Heat Shock Proteins for Protection against Oxidative Stress. *Advanced Drug Delivery Reviews* 2009; 61, 310–318. http://www.sciencedirect.com/science/article/pii/S0169409X09000416 (accessed 20 January 2015)

[47] Vinken M, Doktorova T, Decrock E, Leybaert L, Vanhaecke T., Rogiers V. Gap Junctional Intercellular Communication as a Target for Liver Toxicity and Carcinogenicity. *Critical Reviews in Biochemistry and Molecular Biology* 2009; 44, 201–222. http://informahealthcare.com/doi/pdf/10.1080/10409230903061215 (accessed 20 January 2015)

[48] Bulich AA. Use of Luminescent Bacteria for Determining Toxicity in Aquatic Environments. In: Marking SS, Kimerle RA (eds.) Aquatic Toxicology, Second Conference ASTM STP 667. American Society for Testing and Materials, Philadelphia, PA; 1979. pp. 98–106.

[49] Parvez S, Venkataraman C, Mukherji S. A review of Advantates of Implementing Luminiscence Inhibition Test (*Vibrio fisheri*) for Acute Toxicity Prediction of Chemicals. *Environment International* 2006; 32 (2), 265–268.

[50] Tchounwou PB, Wilson B, Ishaque A, Ransome R, Ming-Ju Huang, Leszczynski J. Toxicity Assessment of Atrazine and Related Triazine Compounds in the Microtox Assay, and Computational Modeling for Their Structure-Activity Relationship. *International Journal of Molecular Science* 2000; 1 (4), 63–74. http://www.mdpi.com/1422-0067/1/4/63 (accessed 21 January 2015)

[51] Lapertot M, Ebrahimi S, Oller I, Maldonado MI, Gernjak W, Malato S, Pulgarín C. Evaluating Microtox© as a Tool for Biodegradability Assessment of Partially Treated Solutions of Pesticides Using Fe^{3+} and TiO_2 Solar Photoassisted Processes. *Ecotoxicology and Environmental Safety* 2008; 69 (3), 546–555.

[52] Klementova S, Rabova-Tousova, Z, Blaha L, Kahoun D, Simek P, Keltnerova L and Zlamal M. Photodegradation of Atrazine on TiO_2 – Products Toxicity Assessment. *Open Journal of Applied Sciences* 2015; 5, 14–21. http://dx.doi.org/10.4236/ojapps.2015.51002 (accessed 25 January 2015]

[53] Van der Heever JA, Grobbelaar JU. The Use of *Selenastrum capricornutum* Growth Potential as a Measure of Toxicity of a Few Selected Compounds. *Water SA* 1996; 22, 183–191.

[54] Weiner JA, DeLorenzo ME, Fulton MH. Relationship between Uptake Capacity and Differential Toxicity of the Herbicide Atrazine in Selected Microalgal Species. *Aquatic Toxicology* 2004; 68, 121–128.

[55] Pérez J, Domingues I, Soares AMVM, Loureiro S. Growth Rate of *Pseudokirchneriella subcapitata* Exposed to Herbicides Found in Surface Waters in the Alqueva Reservoir

(Portugal): A Bottom-Up Approach Using Binary Mixtures. *Ecotoxicology* 2011; 20, 1167–1175.

[56] Lekkerkerker-Teunissen K, Benotti MJ, Snyder SA, Van Dijk HC. Transformation of Atrazine, Carbamazepine, Diclofenac and Sulfamethoxazole by Low and Medium Pressure UV and UV/H_2O_2 Treatment. *Separation and Purification Technology* 2012; 96, 33–43.

[57] Andreu V, Picó Y. Determination of Pesticides and their Degradation Products in Soil: Critical Review and Comparison of Methods. *TrAC Trends in Analytical Chemistry* 2004; 23, 772–789.

[58] Lapertot M, Ebrahimi S, Dazio S, Rubinelli A, Pulgarin C. Photo-Fenton and Biological Integrated Process for Degradation of a Mixture of Pesticides. *Journal of Photochemistry and Photobiology A: Chemistry* 2007; 186 (1), 34–40.

[59] US Environmental Protection Agency (2014) ECOTOX User Guide: ECOTOXicology Database System. Version 4.0. http://cfpub.epa.gov/ecotox (accessed 10 January 2015)

[60] Wan MT, Buday C, Schroeder G, Kuo J, Pasternak J. Toxicity to *Daphnia magna*, *Hyalella azteca*, *Oncorhynchus kisutch*, *Oncorhynchus mykiss*, *Oncorhynchus tshawytscha*, and *Rana catesbeiana* of Atrazine, Metolachlor, Simazine, and Their Formulated Products. *Bulletin of Environmental Contamination and Toxicology* 2006; 76, 52–58. http://dx.doi.org/10.1007/s00128-005-0888-4 (accessed 20 January 2015)

[61] Waring CP, Moore A. The Effect of Atrazine on *Atlantic salmon* (*Salmo salar*) Smolts in Fresh Water and after Sea Water Transfer. *Aquatic Toxicology* 2004; 66, 93–104. http://dx.doi.org/10.1016/j.aquatox.2003.09.001 (accessed 19 January 2015)

[62] Prasad TAV, Srinivas T, Janardan S, Reddy DC. Atrazine Toxicity on Transport Properties of Hemocyaninin the Crab *Oziotelphusa senex senex*. *Ecotoxicology and Environmental Safety* 1995; 30, 124–126. http://dx.doi.org/10.1006/eesa.1995.1015 (accessed 22 January 2015)

[63] Del Carmen Alvarez M, Fuiman LA. Environmental Levels of Atrazine and Its Degradation Products Impair Survival Skills and Growth of Red Drum Larvae. *Aquatic Toxicology* 2005; 74, 229–241. http://dx.doi.org/10.1016/j.aquatox.2005.05.014 (accessed 23 December 2014)

[64] De Luca A, Dantas RF, Simoes ASM, Toscano IAS, Lofrano G, Cruz A, Esplugas S. Atrazine Removal in Municipal Secondary Effluents by Fenton and Photo-Fenton Treatment. *Chemical Engineering and Technology* 2013; 36 (12), 2155–2162. http://onlinelibrary.wiley.com/doi/10.1002/ceat.201300135/pdf (accessed 28 January 2015)

[65] Klementova S, Hamsova K. Catalysis and Sensitization in Photochemical Degradation of Triazines. *Research Journal of Chemistry and Environment* 2000; 4 (2), 7–12.

[66] Arántegui J, Prado J, Chamarro E, Esplugas S. Kinetics of the UV Degradation of Atrazine in Aqueous Solution in the Presence of Hydrogen Peroxide. *Journal of Photo-*

chemistry and Photobiology A: Chemistry 1995; 88, 65–74. http://ac.els-cdn.com/1010603094039865/1-s2.0-1010603094039865-main.pdf?_tid=99d62272-a6ff-11e4-a42d-00000aab0f01&acdnat=1422457925_475a3bec36c334471900e282cb176446 (accessed 28 January 2015)

[67] De Laat J, Gallard H, Ancelin S, Legube B. 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* 1999; 39 (15), 2693–2706.

[68] Piera E, Calpe J, Brillas E., Doménech X, Peral J. 2,4-Dichlorophenoxyacetic Acid Degradation by Catalysed Ozonation: TiO_2/UVA/O_3 and Fe(II)/UVA/O_3 Systems. *Applied Catalysis B* 2000; 27, 169–177.

[69] Farré MJ, Franch MI, Malato S, Ayllón JA, Peral J, Doménech X. Degradation of Some Biorecalcitrant Pesticides by Homogeneous and Heterogeneous Photocatalytic Ozonation. *Chemosphere* 2005; 58 (8), 1127–1133.

[70] Keishuke Ikehata, Naeimeh Jodeiri Naghashkar, Mohamed Gamal El-Din. Degradation of Aqueous Pharmaceuticals by Ozonation and Advanced Oxidation Processes. A Review. *Ozone. Science and Engineering: The Journal of the International Ozone Association* 2006; 28 (6), 353–414. http://www.tandfonline.com/doi/pdf/10.1080/01919510600985937 (accessed 28 January 2015)

[71] Pelizzetti E, Maurino V, Minero C, Carlin V, Pramauro E, Zebinatti O, Tosato ML. Photocatalytic Degradation of Atrazine and Other S-Triazine Herbicides. *Environmental Science and Technology* 1990; 24, 1559–1565.

[72] Hashimoto K, Irie H, Fujishima A. TiO_2 Photocatalysis: A Historical Overview and Future Prospects. *Japanese Journal of Applied Physics* 2005; 44 (12), 8269–8285. https://www.jsap.or.jp/jsapi/Pdf/Number14/04_JJAP-IRP.pdf (accessed 30 January 2015)

[73] Byrappa K, Subramani AK, Ananda S, Lokanatha Rai KM, Dinesh R, Yoshimura M. Photocatalytic Degradation of Rhodamine B Dye Using Hydrothermally Synthesized ZnO. *Bulletin of Material Science* 2006; 29 (5), 433–438. http://www.ias.ac.in/matersci/bmsoct2006/433.pdf (accessed 30 January 2015)

[74] Yongging Zha, Shaoyang Zhang, Hui Pang. Preparation, Characterization and Photocatalytic Activity of CeO_2 Nanocrystalline Using Ammonium Bicarbonate as Precipitant. *Material Letters* 2007; 61 (8–9), 1863–1866. http://www.sciencedirect.com/science/article/pii/S0167577X06009633 (accessed 30 January 2015)

[75] Guo Y, Quan X, Lu N, Zhao H, Chen S. High Photocatalytic Capability of Self-Assembled Nanoporous WO_3 with Preferential Orientation of (002) Planes. *Environmental Science and Technology* 2007; 41 (12), 4422–4427.

[76] Granados-Oliveros G, Páez-Mozo EA, Martínez-Ortega F, Ferronato C, Chovelon JM. Degradation of Atrazine Using Metalloporphyrins Supported on TiO_2 under Visible Light Irradiation. *Applied Catalysis B: Environmental* 2009; 89 (3–4), 448–454.

[77] Penuela GA, Barceló D. Comparative Photodegradation Study of Atrazine and Desethylatrazine in Water Samples Containing Titanium Dioxide/Hydrogen Peroxide and Ferric Chloride/Hydrogen Peroxide. *Journal of AOAC Internatioal* 2000; 83 (1), 53–60.

[78] Héquet V, Gonzales C, Le Cloirec P. Photochemical Processes for Atrazine Degradation: Methodological Approach. *Water Research* 2001; 35 (18), 4253–4260. http://www.sciencedirect.com/science/article/pii/S004313540100166X (accessed 30 January 2015)

[79] Konstantinou IK, Sakellarides TM, Sakkas VA, Albanis TA. Photocatalytic Degradation of Selected S-Triazine Herbicides and Organophosphorus Pesticides over Aqueous TiO_2 Suspensions. *Environmental Science and Technology* 2001; 35, 398–405.

[80] Klementova S, Piskova V. UV Photodegradation of Triazines Atrazine, Simazine and Propazine and of Atrazine Metabolites Desethylatrazine and Desisopropylatrazine. *Research Journal of Chemistry and Environment* 2005; 9 (4), 20–23.

[81] Prosen H, Zupancic-Kralj L. Evaluation of Photolysis and Hydrolysis of Atrazine and Its First Degradation Products in the Presence of Humic Acid. Environmental Pollution 2005; 133 (3), 517–529. http://www.sciencedirect.com/science/article/pii/S0269749104002775