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Evolution of toxicity during melamine photocatalysis with TiO_2 suspensions

A. Bozzi [a], M. Dhananjeyan [a], I. Guasaquillo [a], S. Parra [a], C. Pulgarin [b], C. Weins [c], J. Kiwi [a,*]

[a] *Laboratory of Photonics and Interfaces, Institute of Molecular Chemistry and Biology, Swiss Federal Institute of Technology, 1015 Lausanne, Switzerland*
[b] *Laboratory of Biological Engineering, ENAC, Swiss Federal Institute of Technology, 1015 Lausanne, Switzerland*
[c] *Max Planck Institute for Computer Science, Stuhlsatzhausweg 85, 66123 Saarbrucken, Germany*

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Abstract

The fate of melamine has been studied under UV light irradiation (medium pressure mercury arc) in the presence of TiO_2 and H_2O_2. The increase in the concentration of aromatic and aliphatic photo-products were determined in solution concomitantly with the decrease observed for the melamine concentration. The main intermediate products occurring during photodegradation were identified by liquid chromatography coupled mass spectrometry (LC-MS) with the MS detection in positive and negative ions. The oxidation of melamine under UV-irradiation in the presence of H_2O_2 proceeds step by step leading to ammeline, ammelide, and finally to cyanuric acid. The mineralization of melamine was not observed due to the formation of cyanuric acid as intermediate photo-product. The adsorption of melamine on TiO_2 was found to vary with the pH of the suspension increasing at pH $>$ 5 due to the charge-exchange transfer between the adsorbent and the adsorbate. Two different bioassays using *Vibrio fischeri and Daphnia magna* have been used to test the evolution of toxicity during the UV/TiO_2/H_2O_2 treatment. The toxicity of the initial melamine solution seems to increase due to the intermediates generated in solution.

Keywords: Photocatalysis; Melamine; Melamine adsorption; Melamine ionization; Aromatic photo-products; Toxicity evolution; Oxidation processes

1. Introduction

Liebig, who obtained it from fusion of KSCN and NH_4Cl, first described melamine in 1834. But only 100 years later did melamine find application in the production of melamine–formaldehyde resins and is used widely today in resins, adhesives, and plastics polymeric material. The *s*-triazine ring is very stable and cleaves only under drastic conditions like heating above 600 °C or fusion with alkali compounds.

Photocatalytic mineralization of organic compounds by Advanced Oxidation Technologies (AOTs) is a field that has grown in importance during the last decade [1]. This field aims at the mineralization of contaminants to CO_2, H_2O and inorganic compounds. Photocatalytic decomposition of toxic and non-biodegradable organic compounds using TiO_2 photocatalysis is an important component in the field of AOTs [2–4]. Recent studies in our laboratory have addressed the use of TiO_2 semiconductor suspensions to mineralize a variety of organic compounds containing one, two, or three aromatic rings compounds like anthraquinone-sulfonates [5,6] and azo-dye textile dyes [7,8].

Many years ago, the degradation of atrazine, an *s*-triazine type herbicide, was shown to lead cyanuric acid under UV/H_2O_2/TiO_2 treatment [9]. The cyanuric acid end product could not be degraded by photolysis, photocatalysis, or common oxidants with a high redox potential in solution. This study addresses the (a) degradation of the cyanuric acid on TiO_2 suspension under light, (b) the detection of the long-lived intermediates during the photo-oxidation, and (c) the evolution of toxicity in the melamine–TiO_2 solution during the photocatalysis. Bacteria is able to transform the cyanuric acid in a biuret which then is converted into urea yielding at a later stage CO_2 and ammonia [10].

Determining the toxicity in the course of oxidative degradation processes is carried out by nowadays by several bioassay procedures since toxicity can be measured by a diversity of biological sensors. To increase the confidence in the evaluation of the toxicity, different biological bacteria

* Corresponding author. Tel.: +41-21-693-3621; fax: +41-21-693-3621.
E-mail address: john.kiwi@epfl.ch (J. Kiwi).

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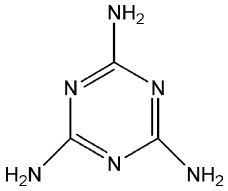
are used. This approach will be used during this study to assess the toxicity evolution during the photo-oxidation of melamine. Our intention is to treat melamine under drastic photo-oxidative conditions and to assess: (a) the nature of the photo-produced intermediates and (b) the evolution of the toxicity of the melamine solution during the photocatalysis.

2. Experimental section

2.1. Materials

Melamine (2,4,6-triamino-1,3,5-triazine) and other chemicals were ACS grade and obtained from Fluka, Buchs, Switzerland. The sample of TiO_2 Degussa P-25 ($50\,m^2/g$, ~80% anatase and ~20% rutile; BET area $55\,m^2/g$) was obtained from Degussa AG, Hanau, Germany. Tri-distilled water was used throughout this study. The structure of melamine (2,4,6-triamino-1,3,5-triazine) is shown below



2.2. Irradiation set-up and analysis of solutions

Melamine solutions were illuminated in cylindrical glass vessels of 70 ml capacity containing 60 ml sample solutions with a medium pressure mercury lamp (400 W). TiO_2 suspensions containing various amounts of the powder and the selected concentration of melamine were stirred within the reaction period. For the determination of CO_2, gas phase aliquots (100 μl) were taken with the gas-tight syringe and injected into a Carlo Erba gas chromatograph provided with a TCD detector. Helium gas was used as carrier gas and Porapak-T as the column packing material. Spectrophotometric analysis of the solutions was carried out with a Hewlett-Packard 8452 diode-array spectrophotometer. The total organic carbon (TOC) was monitored with a Schimadzu 500 total C-analyzer equipped with an ASI automatic sample injector.

2.3. Adsorption of melamine on TiO_2

The adsorption on TiO_2 of melamine at different pH values was determined in the following way: TiO_2 (1.5 g/l) was added to melamine (2.5 mM) and subsequently the individual pH values were adjusted by the addition of either solutions of diluted NaOH or HCl. The suspensions were stirred continuously during the experiment and the temperature maintained at 20 °C to avoid losses due to evaporation. The concentration of melamine in the supernatant solution was determined following spectrophotometrically the melamine peak at $\lambda = 236$ nm after filtering the suspension through a Millipore (22 μm) filter. The amount of melamine adsorbed on TiO_2 was determined by subtracting the melamine concentration from the initial value as determined from the respective peaks in the spectrophotometer. The concentration of C in solution was analyzed by a TOC-500 to determine the amount of C left in the solution. Before analysis each sample was filtered on a 0.22 μm Millipore-Q filters to eliminate the TiO_2 particles.

2.4. Analysis of melamine and aromatic and aliphatic products of the photocatalysis

High-pressure liquid chromatography (HPLC) was carried out with a Varian 9100 LC unit equipped with a 9065-diode array. These modules were piloted with a PC computer with the Varian Star 5.5 software for liquid chromatography. A reverse phase Spherisorb silica column ODS-2 and an aqueous sodium formate /acetonitrile solution were used to run the chromatography in gradient mode. At the melamine absorption peak ($\lambda = 236$ nm), the scanning by the diode array detector revealed the existence of aromatic degradation intermediates different to the melamine peak. The latter species were observed to vary as function of the degradation time as shown below in Section 3.4.

2.5. High-pressure liquid chromatography-mass spectrometry (HPLC-MS) analysis of the photodegradation intermediates

By-products identification was performed by HPLC-MS. The HPLC system was a 1050-Ti-chromatograph (Agilent Technologies, Palo Alto, CA) equipped with a Chromosphere 5B 5 μm, 250 mm × 3 mm column, a 10 mm × 2 mm pre-column, both from Chromopack (Walnut Creek, CA). Samples were injected with a Gilson 234 auto-sampler (Gilson, Middleton, WI) equipped with a 9010 Rheodyne valve and a 5 μl loop, were eluted by a water/methanol 50/50 mixture at 0.6 ml/min. The HPLC system was interfaced to an API 165 mass spectrometer (Applied Bio-systems/MSD Sciex, Foster City, CA) equipped with a turbo-ion spray interface consisting of a nebulizer gas (air) = 1.2 l/min, curtain gas (nitrogen) = 1.2 l/min, turbo-ion spray gas (nitrogen at 300 °C) = 6 l/min, needle voltage = +5000 or −4500 V, orifice voltage = +25 or −30 V, and ring voltage = +200 or −140 V for positive and negative ions analysis respectively. The flow from the HPLC before entering the turbo-ion spray interface was split to allow a flow rate of 200 μl/min.

2.6. Toxicity measurements

2.6.1. Vibrio fischeri bio-assay

The effect of melamine and the intermediates on the luminescence of the strain bacterium *V. fischeri* was carried

out according to German Norms DIN 38 412 part L 34 [11]. Since bacterial bioluminescence is tied to cell respiration, any inhibition of cellular activity (toxicity) results in a decreased rate of respiration and is reflected in a decrease in the observed luminescence. The sample toxicity is determined by measuring the sample G_d. The value of G_d refers to the lowest G value for the population that will under the experimental conditions used hinder <20% of the sample light emission. The latter value found is dimensionless and is always reported taken into account the value of a control sample. The bacterial light emission intensity is followed in by a photo-multiplier in a luminometer equipped with a constant temperature bath. The measurements are carried in salt solution (2% NaCl) since the bacteria is of marine origin. The bacteria was purchased in a freeze-dried state and stored at −20 °C. Immediately before use the bacteria sample was hydrated.

2.6.2. *Daphnia magna* bio-assay

German standard method DIN 38 412 L 30 was used to determine the non-acute poisonous effect of a toxicant to *D. magna* strains in the stipulated dilution limits. This bioassay has been validated for all kind of toxicants in aqueous solution. At different dilutions it measures the non-poisonous effect of the toxicant on the *D. magna* within 24 h. The damage to the *D. magna* is quantified as the loss of mobility, since the death of the bacteria is not easily detected. This toxicity measurements in Table 2 refer to the lowest numerical G ($^*G_L$ value) for the sample, when at least nine *D. magna* still to show mobility in the aqueous solution. The dilution attained is a measure of the experimental result obtained [12]. The dilution water was free from any form of chlorides. All tests were performed in the dark at a constant temperature (20 °C).

2.7. Modeling of melamine species in solution

The modeling of the melamine species in solution was carried out via the Acuchem IV program from the National Bureau of Standards, Gaithersburg, MD. The ionization of melamine as a function of pH was calculated using the pK_a values of melamine and calculating for each pH value the ionized fraction via the ChemEQL program. The program for the ChemEQL version 2.0 was written by B. Mueller, Kastanienbaum, Switzerland.

3. Results and discussions

3.1. Irradiation with a mercury arc of melamine solutions in the presence of TiO_2 suspensions

Mineralization of melamine under the medium pressure mercury lamp in the presence of TiO_2 and H_2O_2 was carried out at the initial unadjusted pH of 6.9. The results are presented in Fig. 1. The CO_2 generated by the melamine solution reached only 2–3 μmol after 5 h irradiation. This is ∼0.1% of the stoichiometric amount of CO_2 expected for full melamine mineralization (22.5 mmol CO_2). The final oxidation step may be achieved after very long times, but we did not follow this approach here since such long degradation periods are unacceptable when using the photocatalytic system employed in this study. Direct photolysis of melamine can be discounted due to the weak light absorption at wavelengths >260 nm.

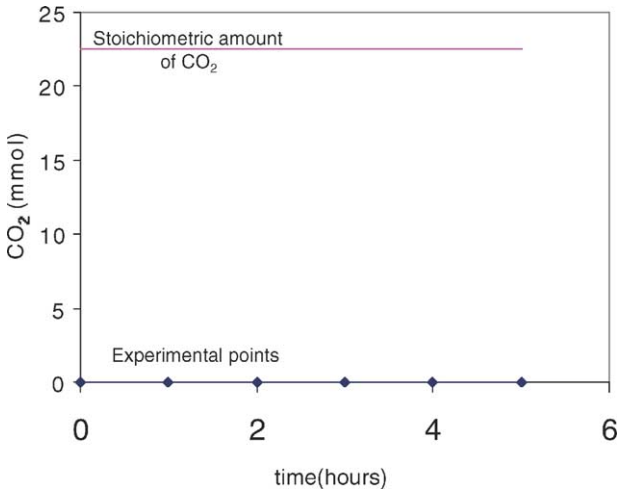


Fig. 1. Photodegradation of melamine (2.5 mM) in aqueous suspension of TiO_2 Degussa P-25 (3.25 g/l) with an initial amount of H_2O_2 (4.25 g/l) under mercury lamp irradiation (400 W). Initial pH 6.9. The experimental points are noted by (◆).

Melamine is not mineralized by adding TiO_2 to the solution under UV light in the presence of H_2O_2. This is seen by the lack of CO_2 evolution reported in Fig. 1. Runs were carried out at pH 6.9, since at this pH no adjustment by acid or base was necessary. No mineralization of melamine was also observed at pH values: 2.5, 5.0, and 9.0. A very small amount of CO_2 (<0.1 mmol) was observed and comes from the C-impurities on the TiO_2 surface during its manufacture or from fixation of residual atmospheric CO_2 under light irradiation [6].

3.2. Adsorption of melamine on TiO_2 and ionic species involved in the surface charge transfer

A brief presentation of some features of the system melamine–TiO_2 is necessary before proceeding to identify of the products of the photocatalysis the toxicity evolution during the pretreatment.

The adsorption of melamine on TiO_2 Degussa P-25 is presented in Fig. 2. At pH > 5, the adsorption was seen to increase considerably attaining at pH 9 about 2 μmol/m^2. The lack of adsorption of melamine (HL^+) on TiO_2 in the acid range at pH < 5 is shown in Fig. 2 and is explained in terms of the repulsion of the melamine surface species HL^+ (Fig. 3) with the $TiOH_2^+$ existing in the acid pH

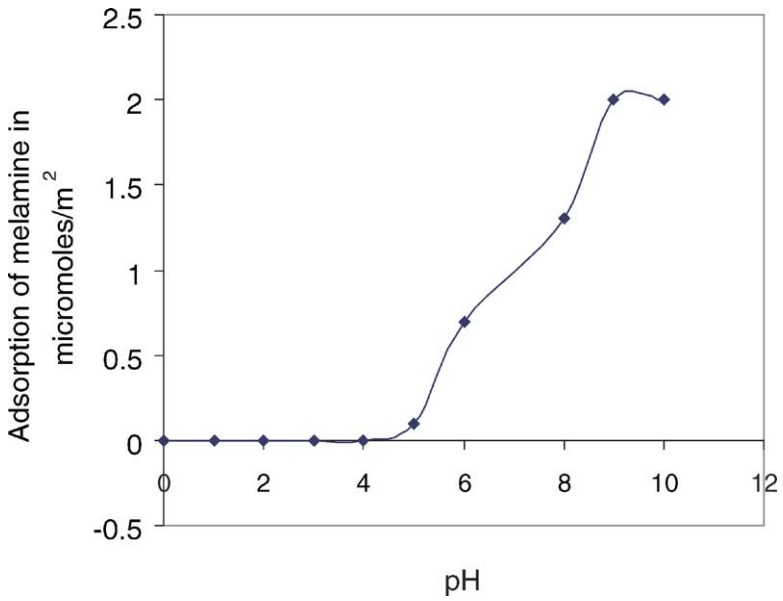


Fig. 2. Adsorption of melamine on TiO_2 Degussa P-25 as a function of pH.

region. Concomitantly, the system presents a lack of attraction of HL^+ with the neutral TiOH species. The adsorption of melamine (HL) on TiO_2 then proceeds through charge exchange interaction at pH values above the isoelectric point (IEP) ~6 for TiO_2 Degussa P-25 (Fig. 2).

Fig. 3 shows the distribution of the melamine ionic species in aqueous solution calculated by a matrix that takes into consideration the values pK_{a_1} 5.05 and $pK_{a_2} \sim 14$ [13]. The pK_{a_1} value is ascribed to the deprotonation of the melamine-NH_2 group. Up to pH 5.05, Fig. 3 shows that the melamine is mostly in the HL^+ form. Melamine is a Lewis base and its conjugate acid is HL^+.

3.3. High-pressure liquid chromatography (HPLC) identification of the photocatalytic intermediates

Fig. 4 shows the decrease as a function of irradiation time in the melamine concentration and the concomitant increase in the concentration of sum of the aromatic photo-products. The melamine HPLC retention time was 3.2 min. The

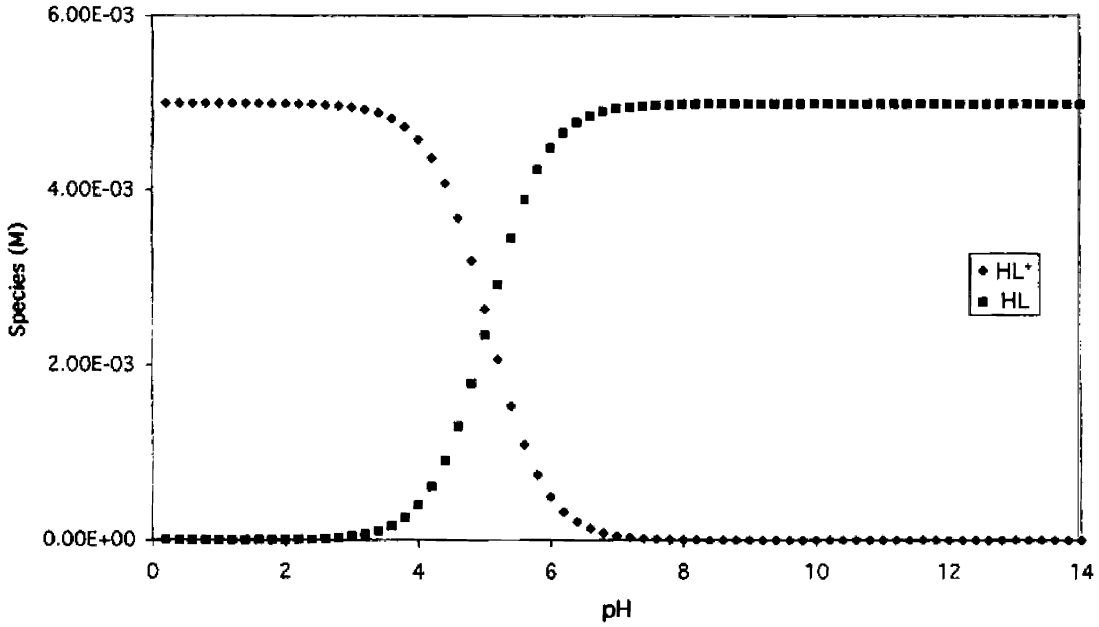


Fig. 3. Distribution of melamine species in solution as a function of pH. For other details see text.

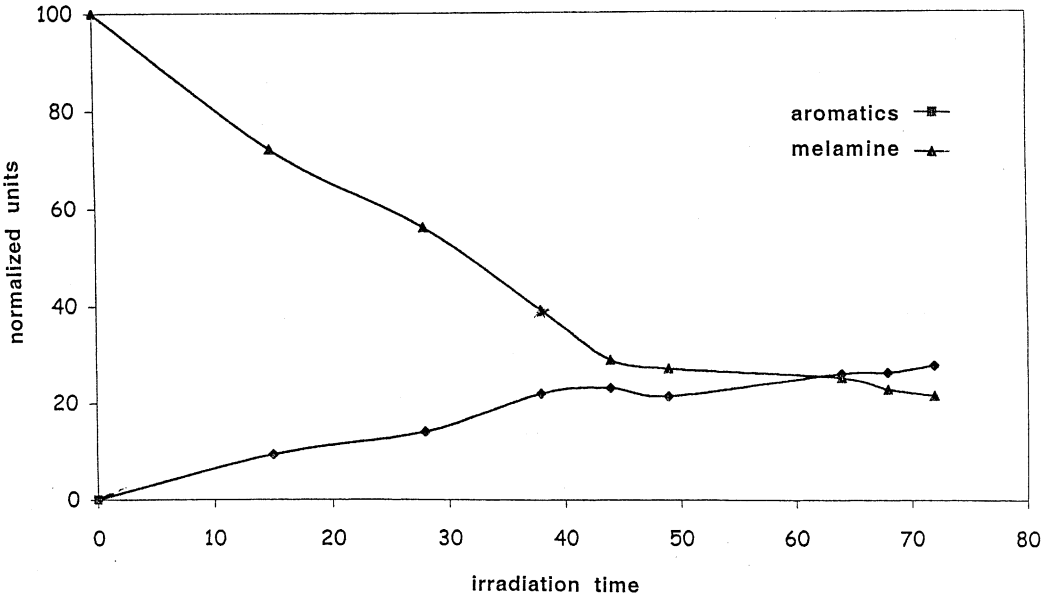


Fig. 4. Concentrations of melamine and aromatic intermediates as found by HPLC during the photocatalysis of a melamine solution with the same make up as in Fig. 1.

aromatic components were seen to grow and reach a plateau >50 min, and were detected by the same approach as used previously in reference [14]. The analysis in the HPLC was carried out immediately after taken the sample to avoid the effect of the oxidation of solution intermediates due to the residual H_2O_2 left in solution.

3.4. HPLC-MS investigation of melamine degradation. Nature and evolution of intermediates

The results in Fig. 5 show that the disappearance of melamine leads to the major intermediate ammeline and low concentrations of ammelide and cyanuric acid as detected by HPLC-MS. Table 1 reports the molecular weight (MW) of the three main intermediates and the fragment ion-mass for melamine, ammeline and ammelide detected as positive ions whereas the cyanuric acid was detected as a negative ion. The oxidation of melamine in Scheme 1 proceeds stepwise with the loss of one, two or three amino groups. At the same time and a corresponding increase in the state of oxidation of melamine is observed due to the oxidative radical attack generated by the TiO_2/H_2O_2 in solution. In this particular experiment, titanium dioxide powder (3.25 g/l) was suspended in 60 ml of melamine solution (3.15 g/l) and

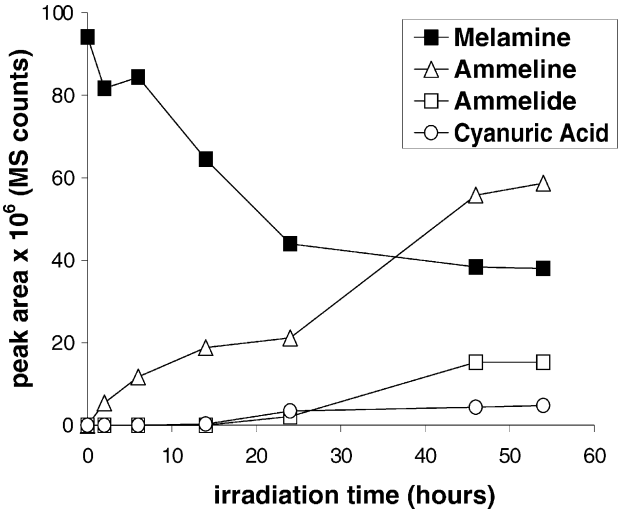
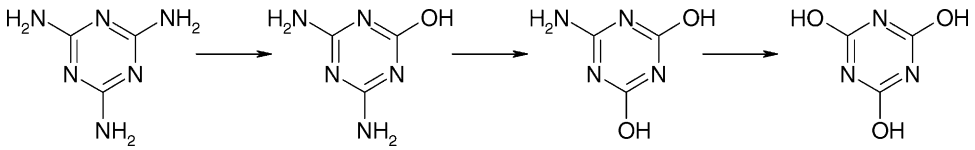


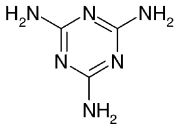
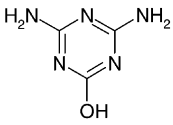
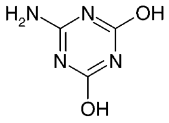
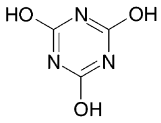
Fig. 5. Melamine decrease and photo-products formation up to 54 h for a solution treated under the same experimental conditions as employed in Fig. 1.

subsequently H_2O_2 (4.25 g/l) was added for samples irradiated for less than 24 h. The same amount of H_2O_2 was added at 24 h intervals for the samples irradiated at longer times (see Fig. 5). The amount of intermediates observed



Scheme 1. Identified intermediates in the photocatalytic degradation of the melamine.

Table 1
Proposed chemical structures, $[M-H]^-$ or $[M+H]^+$ ions and fragmentation masses of the degradation intermediates

By-product	Proposed structure	MW	$[M+H]^+$ and fragment ion masses (relative intensity)	$[M-H]^-$ and fragment ion masses (relative intensity)
Melamine		126	127 (100), 85 (5.7)	
Ammeline		127	128 (100), 86 (5.3)	
Ammelide		128	129 (100), 87 (8.0)	
Cyanuric acid		128		128 (78.5), 97 (100)

in solution was a function of the pH, concentration, and temperature of the different samples tried.

3.5. Toxicity evolution of melamine treated by $UV/H_2O_2/TiO_2$

Table 2 shows the intrinsic toxicology values EC_{50} obtained by *V. fischeri* and *D. magna* at different times during the photocatalysis of melamine under the experimental conditions used in the above Section 3.4. The quantities of TiO_2 and H_2O_2 used during this run were directed to maximize the amount of melamine photo-products during the photocatalysis. From the standpoint of toxicity, melamine is not classified as a health risk [15] and is eliminated unchanged in the urine due to its stability when administered in doses <0.38 mg/kg [16]. This is confirmed by the result shown at time zero in Table 2. The toxicology values reported for 3 and 7 h originate from the H_2O_2 present in solution. To eliminate the residual H_2O_2 with catalase or sulfite would introduce an added compound with its own toxicology. This makes impossible any comparison with the results obtained at different degradation times in Table 2. To use less H_2O_2 avoiding the excess oxidant leads to a much lower photo-production rate of intermediates and would not allow for the efficient production of the intermediates as reported in Table 1. The last three values for the toxicity in Table 2 show that: (a) the toxicity at times 23, 46, and 54 h cannot originate from the H_2O_2 in solution, (b) melamine degradation leading to ammelide and ammeline and possibly other less important photo-products present a higher toxicity than the initial melamine, and (c) the intermediates show different toxicities towards the two micro-organisms since the toxicity values found by the two methods are different.

Table 2
Toxicology of melamine solutions treated by $TiO_2/UV/H_2O_2$ as a function of time

Duration of photo-oxidation (h)	*V. fischeri* G_d[a]	*D. magna* G_L[b]	Content of H_2O_2 (μg/ml)
0	0	0	0
3	320	400	1500
7	640	320	1200
14	64	16	80
23	80	4	0
46	28	8	0
54	16	3	0

[a] G_d refers to the lowest G value for the population that will, under the experimental conditions used, hinder <20% of the sample light emission.
[b] G_L refers to the lowest numerical G ($^*G_L$) value for the sample implies that at least nine *D. magna* still show mobility in the aqueous solution. The dilution attained when reporting this values is a measure of the experimental result.

4. Conclusions

Melamine remains stable in aqueous unless solutions with a high concentration of H_2O_2/TiO_2 are used under UV light to produce some photo-products leading to cyanuric aid. The formation of cyanuric acid prevents the complete mineralization of melamine as observed for atrazine and other *s*-triazines on TiO_2 photocatalysts. The evolution of toxicity was followed by two methods and the results reflect the technique used. The toxicity of the photocatalyzed solutions was higher than the initially found for melamine. This is due to the intermediates generated during photo-oxidation by the $UV/H_2O_2/TiO_2$ treatment. The results reported in this study

suggest that melamine degradation in living tissues may be accompanied by an undesirable increase of toxicity.

Acknowledgements

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