UV degradation of carbofuran insecticide in aqueous solution: identification and toxicity evolution of by-products

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Abstract The paper reports the results of an investigation about the UV degradation of carbofuran, a widely used insecticide in Europe. Specific objectives were the identification of the by-products formed and the evaluation of the toxicity of the irradiated solution compared to that of carbofuran. The experimental results, obtained treating an aqueous carbofuran solution (50 mg/L) by high pressure UV lamp (125 W), show that the insecticide is completely removed within 120 min. Several intermediate by-products have been identified by liquid chromatography-mass spectrometry (LC-MS) as a result of hydroxylation process of the 2,3-dihydro benzofurane ring and other reactions such as the cleavage of the carbamate group, the hydrolysis of ethereal moiety, radical coupling and decarboxylation processes. After 270 min of reaction the identified by-products were completely degraded and COD and TOC removals of 35 and 20% were measured, respectively. Toxicological analyses performed using the comparison procedure of the Microtox™ assay provided interesting clues concerning toxic effects of the photodegradation by-products. The results revealed a substantial increase of the toxicity during the first 15 min proving that photodegradation of organic contaminant could even lead to an increase of the toxicity of treated solution.

Keywords Carbofuran; LC/MS analyses; photodegradation by-products; toxicity tests; UV radiation

Introduction

The application of insecticides on agricultural soils is well established as an effective practice to amplify plant productivity for high production. Consequently, the use of pesticides has steadily increased during recent decades. This has led to the contamination of groundwater and surface water that are the main sources for drinking water. Therefore there is concern regarding insecticide contamination of water resources and also an interest in reliable technologies for their removal from water. Recently, a growing interest has been observed in the area of UV activated processes due to (i) the continuous decrease of treatments costs because of the breakthrough into the market of relatively cheap low-energy UV lamps, (ii) the possibility to avoid, by using non-contact reactors, UV lamp fouling, (iii) the simultaneous use of UV rays and chemical oxidants (e.g., ozone or hydrogen peroxide). Because of their specific technological requirements, UV based treatments are suitable for removing organic pollutants from groundwater resources as they are usually characterized by a low content of suspended solids, low light scattering and aromatic organic compounds and low optical absorption.

UV degradation of organics degradation usually occurs through oxidation reactions. These reactions imply the homolysis of a C-X bond giving rise to an organic radical which reacts with oxygen \( R-X \rightarrow R^* + X^*; R^* + O_2 \rightarrow RO_2 \) or an excitation of the organic molecule by UV light and then an electronic transfer to ground-state molecular oxygen leading to the formation of a radical cation. Obviously, an important parameter that affects the rates of both routes is the excitation wavelength. UV low pressure lamps, having an irradiation at
253.7 nm, beside their good efficiency for water disinfection purposes (EPA 811-R-96-002, 1996), have shown usefulness for degrading substituted aromatics but they are not effective for chlorinated aliphatics removal (Sundstrom et al., 1986). Conversely, the medium- and high-pressure lamps, having a broader emission band, have shown great efficiency in removing chlorinated organics by homolysis of C-Cl bonds (Toy et al., 1990).

In any case, even though organic pollutants degradation by UV based processes can be achieved successfully, the complete oxidation of these contaminants (i.e. the total mineralization of organic carbon to CO₂) is seldom obtained. Accordingly, the formation of several by-products is expected (de Bertrand and Barceló, 1991) and their identification is a crucial step for assessing whether their toxicity is greater or lower than that of parent compounds.

In the present study, the attention has been focused on carbofuran (2,3-dihydro-2,2-dimethyl benzofuran-7-yl methyl carbamate), a broad spectrum insecticide with a high toxicity. Its oral LD₅₀ is 11 mg/kg body weight in rats compared to 8 mg/kg for the extremely toxic parathion and 1,300 mg/kg for atrazine, an herbicide of low moderate toxicity (Kuhr and Dorough, 1976). Carbofuran is widely used in the cultivation of corn, rice, cotton, and other crops as a substitute for organochlorine pesticides because of his greater biodegradability (Worthing, 1988). It is known that carbofuran and its metabolites are highly soluble in water and their stability under certain environmental conditions (the half life in soil is about 150 days) has made serious the risk of surface water as well as ground water contamination (RIZA, 1997).

However, although degradation of carbofuran has been investigated by ozone (Benitez et al., 2002), Fenton’s reagent (Wang and Lemley, 2003), ultrasound (Hua and Pfalzer-Thompson, 2001) and UV light (Bhattacharya et al., 1994), the by-products identification has been seldom reported and a lack of knowledge concerning by-products toxicity still exists. Therefore in the present paper, investigating carbofuran UV degradation in aqueous solutions, two issues have been tackled: (1) the by-products identification during the photochemical treatment with a high pressure mercury vapor lamp; (2) the toxicity evaluation of the irradiated aqueous solutions with respect to the initial solution containing only the parent insecticide.

**Methods**

Carbofuran (Table 1, structure 1) of purity higher than 98% (Riedel-de Haën, Germany) was used as received. Carbofuran working solutions were prepared fresh daily using high purity water obtained by a Milli-Q Gradient A-10 system (Millipore). The obtained concentration was checked by Total organic carbon (TOC) analysis by using a TOC-5050 analyzer (Shimadzu) equipped with a platinum catalyst on alumina spherical support.

Photolysis experiments were conducted in a 1 L cylindrical glass four-necked reactor. Temperature control was achieved by water circulation through the cylindrical glass inner jacket. The light source used was a high-pressure mercury arc lamp (Helios Italquartz, 125 W) fixed at the central axis of the inner jacket. 500 mL of carbofuran solution, magnetically stirred, was irradiated for a total time of 270 min. The progress of the reaction at various times was monitored by withdrawing small aliquots (10 mL) of the reaction mixture that were analyzed by TOC, TN, COD and HPLC-MS. Total nitrogen (TN) analyses were carried out by a TOC-V analyzer equipped with a TNM-1 module (Shimadzu) and a platinum catalyst on alumina spherical support. Chemical Oxygen Demand (COD) were carried out according to the standard official protocols for wastewater (Standard Methods, 1999).

At the scheduled times the residual carbofuran concentration was determined by loop injection LC-MS-MS monitoring the product ion of m/z 165 deriving from the fragmentation of [M+H]⁺ ion of m/z 222. The instrumentation used consisted of a Varian
9012 HPLC system interfaced to an API QSTAR mass spectrometer (Applied Biosystems/MSD Sciex) equipped with a turboionspray interface. Interface conditions (positive ions) were as follows: needle voltage = +5,500 V, declustering potential = +40 V, focusing potential = +120 V and collision energy = 15 V. Samples, injected by a 1074 ADT Valco valve and a 1 μL loop, were eluted at 0.3 mL/min through a Perkin-Elmer C18 column (3 μm, 30 × 4.6 mm) with a solution of water/methanol/50 mM ammonium acetate in methanol 10/85/5 as a mobile phase. Injections were performed in duplicate and concentration values were obtained by using a proper calibration curve.

### Table 1
Proposed chemical structures and elemental compositions of carbofuran degradation products determined during LC-MS analysis of irradiated solutions

<table>
<thead>
<tr>
<th>Compound Number</th>
<th>Compound Structure</th>
<th>Elemental Composition</th>
<th>Measured Mass ([M+H]+)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbofuran (1)</td>
<td><img src="image" alt="Carbofuran" /></td>
<td>C_{12}H_{15}NO_{3}</td>
<td>222.1</td>
</tr>
<tr>
<td>2</td>
<td><img src="image" alt="Compound 2" /></td>
<td>C_{12}H_{15}NO_{4}</td>
<td>238.1</td>
</tr>
<tr>
<td>3</td>
<td><img src="image" alt="Compound 3" /></td>
<td>C_{12}H_{15}NO_{4}</td>
<td>238.1</td>
</tr>
<tr>
<td>4</td>
<td><img src="image" alt="Compound 4" /></td>
<td>C_{10}H_{12}O_{4}</td>
<td>197.1</td>
</tr>
<tr>
<td>5</td>
<td><img src="image" alt="Compound 5" /></td>
<td>C_{12}H_{15}NO_{3}</td>
<td>222.1</td>
</tr>
<tr>
<td>6</td>
<td><img src="image" alt="Compound 6" /></td>
<td>C_{12}H_{17}NO_{4}</td>
<td>240.1</td>
</tr>
<tr>
<td>7</td>
<td><img src="image" alt="Compound 7" /></td>
<td>C_{11}H_{15}NO</td>
<td>178.1</td>
</tr>
<tr>
<td>8</td>
<td><img src="image" alt="Compound 8" /></td>
<td>C_{22}H_{20}NO_{4}</td>
<td>368.1</td>
</tr>
</tbody>
</table>
By-products identification was performed by LC-MS using the above reported instrumentation. Samples injected by a 1074 ADT Valco equipped with a 200 μL loop, were eluted at 0.6 mL/min through a Alltima C18 5 μm, 250 × 3.2 mm column (Alltech) and C18 4 × 2.0 mm precolumn (Phenomenex) with the following gradient: from 80/15/5 (water/methanol/50 mM ammonium acetate in methanol) to 10/85/5 in 15 min, which was then hold for 5 min. The flow from the HPLC-UV was split to allow 200 μL/min to enter into the turboionspray interface. The MS interface conditions were as follows: needle voltage = + 5,500 or −4,200 V, declustering potential = + 40 or −40 V, focusing potential = +120 or −120 V for positive and negative ions analysis, respectively.

The toxicity of the UV treated samples were tested with the Microtox™ test, using a Model 500 analyser and lyophilised cultures of *Vibrio fischeri* NRRL-B-11177, an organism supplied by SDI (Newark, Delaware, USA). In the Microtox™ system, the toxicity of the carbofuran solution was measured with the standard procedure: the inhibition of bacterial light emission was measured in duplicate experiments at 15°C after 15 and 30 min of exposure. The inhibition of the UV treated samples was measured with the comparison procedure comparing the sample with the dilution solution with two readings, after 15 and 30 minutes of incubation, at 15°C. The Microtox® data acquisition software version 6.1 was then used to calculate the EC50 value for the standard procedure and the percent of inhibition expressed in gamma values for the comparison procedure according to Bulich and Isenberg (1981).

**Results and discussion**

In Figure 1 is depicted the residual % of carbofuran, COD and TOC during the UV treatment. Inspection of such a figure reveals that, under the adopted experimental conditions, complete carbofuran removal is accomplished within ca. 120 min. As expected, COD and TOC decays are considerably less steep and after 270 min the observed decreases were 35 and 20%, respectively.

The recorded COD and TOC removals suggest that during the UV treatment, beside direct photolytic degradation, oxidative reactions also take place. In fact, such reactions are known

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**Figure 1** Substrate concentration, TOC and COD decay, all expressed as % with respect to the initial value, during the photodegradation of a 50 ppm carbofuran aqueous solution.
to induce COD reduction and, ultimately, the partial mineralization of organic carbon. Such oxidative reactions were already reported to be active in other studies employing high pressure UV lamps and are likely due to the formation of hydroxyl radicals (HO·) (Legrini et al., 1993). These radicals, which can be generated also by other methods, are known to react with organics at high rates and with poor selectivity leading, ultimately, to a partial substrate mineralization.

The observed complete insecticide disappearance, together with the measured residual TOC and COD, entails the formation of carbofuran degradation by-products. In order to elucidate the chemical structures of such organic by-products, samples withdrawn at growing irradiation times (1, 5, 15, 30, 60, 90, 150, 210, 270 min) were analyzed by LC-MS as described in the experimental section. In addition, TN measurements were also performed in order to assess the fate of organic nitrogen. The results showed that nitrogen remains constant during the whole reaction time. This suggests that during carbofuran photodegradation, nitrogen remains bound to the by-products formed and that no nitrogen-containing volatile products such as NO, NO₂ or ammonia are formed.

In Table 1 are reported the chemical structures of the principal by-products identified as well as the chemical structure of carbofuran. By-products 2–6 are consistent with degradation mechanisms already known in the literature. In fact, by-product 2 (3-hydroxy carbofuran) results from the hydroxylation of the benzylic position (Bhattacharya et al., 1994), which is the preferred site for HO· attack on the carbofuran molecule. This by-product has been also reported to be the main carbofuran metabolite in plants (National Research Council of Canada, 1979). By-product 3, instead, arises from a combination of the hydroxylation process with the cleavage of the carbamate group, the latter mechanism being already observed (Bhattacharya et al., 1994; Bachman and Patterson, 1999). The Fries’ rearrangement, already reported for sunlight degradation of carbofuran in organic solvent (Bhattacharya et al., 1994), generates the by-product 5. By-product 6 is consistent with the hydrolysis of ethereal moiety of 2,3-dihydro furan ring, such a pathway being proposed by Bachman and Patterson (1999) for carbofuran UV degradation.

As for by-products 7 and 8, two new reaction pathways might proposed to justify their formation: a decarboxylation process of the carbamate moiety and a radical coupling reaction between two carbofuran fragments.

In Figure 2 is depicted the evolution of these by-products where it is evident that compounds 2 and 3 are the predominant degradation products. Furthermore, Figure 2 shows that the complete removal of all by-products occurs upon 270 min. It follows that the formation of lower-molecular weight carboxylic acids is likely, as already found during the degradation of other organic pollutants (Mascolo et al., 2001).

Once the chemical structures of the by-products are known, an other important aspect that has been investigated is their toxicity with respect to the parent insecticide. This has been performed by through the Microtox™ assay of carbofuran irradiated solution samples. Firstly, a dose-response curve was determined for the starting carbofuran solution (50 mg/L) adopting the Microtox™ standard protocol. The EC50 value obtained after 15 min of exposure (13.7 ppm) was used for calculating the estimated toxicity of the different reaction mixture samples on the basis of measured carbofuran concentrations. The obtained toxicity of UV treated samples expressed in gamma values was therefore corrected for the estimated toxicity and the resulting difference was attributed to the presence of by-products.

As reported in Figure 3, the highest toxicity was measured for the samples withdrawn after 5 and 15 min of UV irradiation. Such result can be correlated with the concentrations of the by-products 6, 7 and 8, even though the correlation is not statistically significant ($R^2$=0.37–0.45). Finally, the toxicity of the samples disappear 210 min after the UV treatment as for by-products too.
Conclusions

An experimental investigation has been carried out to identify the by-products formed during the UV degradation of a widely used insecticide, i.e. carbofuran. The toxicity of irradiated solution was also measured and compared to that of the parent insecticide. The results indicated that although carbofuran was completely removed within 120 min, eight by-products were formed as a result of hydroxylation, cleavage of the carbamate group.
hydrolysis of ethereal moiety, radical coupling and decarboxylation processes. After 270 minutes of reaction such by-products were completely degraded and COD and TOC removals of 35 and 20% were measured, respectively. Toxicological analyses performed carrying out the comparison procedure of the Microtox™ assay on irradiated mixtures revealed a substantial increase of the toxicity during the first 15 min. This implies that particular attention should be paid when using UV treatments in water contaminated by insecticides because they could also lead to the formation of by-products that are more toxic than the parent compounds.

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**References**


