Monitoring of methotrexate chlorination in water

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\textbf{A B S T R A C T}

Anti-cancer drugs are an important class of pharmaceutical products. Methotrexate (MTX) is a folic acid antagonist used in high doses as antimetabolite in anti-cancer treatment as well as in low doses for the treatment of rheumatoid arthritis and adults’ psoriasis. In the past, several anti-cancer drugs, including methotrexate, have been found in the environment. Their presence in water, especially if used for the production of drinking water, is even in low concentrations of particular interest, due to the risk to retrieve them in the consumed water and their high activity and grave effects. But prior to usage as drinking water, raw waters are treated and chlorination is a common practice in several countries. As such a treatment can lead to the formation of organochlorine in water, the study of the fate of MTX during chlorination in a batch trial was carried out. The reaction was monitored by dissolved organic carbon (DOC) and by fluorescence and UV spectroscopy. Investigation of by-products formed was done with liquid chromatography/mass spectrometry (LC/MS). Under the given experimental conditions, Methotrexate was eliminated rapidly (\(t_{1/2}\) around 21 min). However, DOC elimination was incomplete. Monitoring with LC-MS showed the formation of a monochlorinated transformation product of MTX.

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1. Introduction

In the last decade the presence of pharmaceuticals, ranging from nanograms to a few micrograms per liter, has been reported in the aquatic cycle including surface water, wastewater and groundwater (Besse and Garric, 2008; Buerge et al., 2006; Kasprzyk-Hordern et al., 2008; López-Serna et al., 2012; Osorio et al., 2012; Petrovic et al., 2012; Ratola et al., 2012; Roberts and Thomas, 2006; Verlicchi et al., 2012) and, to a
lesser extent, drinking water (De Jongh et al., 2012; Mompelat et al., 2011; Wang et al., 2011a). Advances in analytical instruments have been a key factor driving their increased detection (Ferrer and Thurman, 2012; Grabic et al., 2012; Gros et al., 2012).

As for other micro-pollutants, their presence in environmental water, even at these very low concentrations, has raised particular interest. It points out the need to verify the efficacy of drinking water treatment processes for the removal of such compounds (Stackelberg et al., 2004; Westerhoff et al., 2005).

Drinking water treatment consists of several steps including filtration, flocculation, sedimentation and disinfection. Some treatment facilities also include ion exchange and adsorption onto activated carbon. Depending on the country, disinfection (chlorination, ozonation, UV radiation) is generally applied before the water enters the distribution system as drinking water to ensure elimination of potentially dangerous microbes (Gibs et al., 2007; Stackelberg et al., 2004). Ozonation and UV radiation are considered as powerful and effective disinfectant respectively. Contrary to ozonation and UV treatment whose remanence is very short, chlorination by treatment with chlorine, chlorine dioxide and sometimes chloramines is more often used because of its effectiveness in the treatment plant and its lasting presence and activity in the distribution network, although formation of harmful transformation products could be observed (Cantor et al., 1998; Hamidin et al., 2008; Meier et al., 1883).

Among various classes of pharmaceuticals, anti-cancer drugs are of particular environmental concern because they are potentially carcinogenic, mutagenic and genotoxic, even at low concentrations (Zounkova et al., 2007) and reveal low biodegradability (Baumann and Preiss, 2001; Buerge et al., 2006; Straub, 2010). Methotrexate (MTX) is an analogue of folic acid and inhibits the enzyme Dihydrofolate reductase. It is used in chemotherapy at high doses and at low doses in the treatment of some autoimmune diseases like rheumatoid arthritis, adult psoriasis or ectopic pregnancy. With intravenous administration, 80–90% of the administered dose is excreted unchanged in the urine within 24 h (Drug Bank). It enters the environment via urban wastewaters (Castiglioni et al., 2006, 2005; Catastini et al., 2008), hospital wastewaters (Aherne et al., 1985; Yin et al., 2010) and can be detected even in drinking water (Aherne et al., 1985).

Though the effect of chlorination has been investigated for a number of pharmaceutical products in wastewater (Bedner and MacCrehan, 2006; Hey et al., 2012; Lee and von Gunten, 2010; Li and Zhang, 2012), surface water (Meyer et al., 2002; Shah et al., 2006; Wang et al., 2011b) and pure water (Li et al., 2011; Maeh, 2010; Quintana et al., 2010; Rodil et al., 2012; Soufan et al., 2012), anti-cancer drugs in general and MTX in particular have received very low attention despite their high activity, possible promotion of cancer and teratogenic risk. The only anti-cancer drug yet investigated is cyclophosphamide (Besse et al., 2012; Huber et al., 2005; Kümmerer and Al-Ahmad, 2010; Mompelat et al., 2011).

Experimental toxicity testing of identified transformation products (TP) is often difficult, since many of them are not available commercially. Computer models calculating quantitative structure activity relationship (QSAR) are important tools to overcome this limitation. Once structure elucidation of any TP has been performed, these structures can be investigated using QSAR programs in order to predict the toxic potential of TPs for different toxicological endpoints and other environmental parameters. A set of programs for predicting biodegradation should be applied in order to take into account that the available programs might have individual strengths because of different algorithms and training sets.

The main aim of this study was to monitor the fate of MTX during chlorination (by using spectroscopic methods) with regard to the possible formation of transformation products (by LC/MS).

2. Materials and methods

2.1. General methodology

Chlorination was performed during 5 h at 21 ± 3°C with initial pH of 8.6 (decreasing to pH 7.6 during reaction due to hydrochloric acid production). Experiments were carried out in a 100 mL reactor. Working concentration of MTX was 1 mg/L in pure water. Chlorine was added as sodium hypochlorite to ensure a molar ratio MTX:Cl2 of 1:100. The resulting mixture was stirred during 15–20 s to achieve a homogenous solution. DOC (NF EN 1484), residual chlorine, and pH were measured to follow the general progress of the chlorination. Samples were taken and measured by UV-spectrophotometry in order to simply follow the kinetics of MTX removal. The relative MTX concentration variation was assessed by fluorescence after photooxidation of the chlorinated sample. Finally, LC/MS was used for a preliminary monitoring of possibly formed transformation products.

2.2. Material

For basic measurements, pH was measured with an electrode (pHenomenal® pH 1000 L). A DPD comparator disk kit CIFEC was used for residual chlorine quantification. DOC was measured following chemical oxidation with sodium persulfate using a TOC-meter (OI Analytical 1010).

Qualitative assessment of MTX degradation was followed by UV-spectrophotometry (Lambda 35 Perkin Elmer) using a 100 mm quartz circulation cell connected with a closed loop circuit. Scan speed of wavelength range (200–400 nm with step width of 1 nm and a lamp change at 326 nm) was fixed at 1920 nm/min. A spectrum was acquired every minute.

Fluorescence spectra were measured with a Xenius spectrofluorometer (Safas, Monaco) equipped with a 1 cm quartz cell. Fluorescence was measured at 462 nm with an excitation wavelength of 380 nm. The photomultiplier (PM) voltage was generally set at 700 V and moved to 600 and 500 V according to the signal saturation.

Photooxidation followed by fluorescence measurement was used to assess the concentration of MTX during the chlorination. The photooxidation was performed by using the OX150 device of Secomam (Ales, France) equipped with a low pressure mercury lamp emitting mainly at 185 and 254 nm and permitting direct photolysis of molecule. For this purpose