Effect of wastewater chlorination on endocrine disruptor removal

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ABSTRACT

Endocrine disrupting chemicals (EDCs) are compounds of mainly anthropogenic origin that interfere with the endocrine system of animals and humans thus causing a series of disorders. Wastewater treatment plants are one of the major routes for transporting such chemicals to the water courses. In the context of this study, several chlorination batch tests were performed in order to assess the effectiveness of chlorination to remove bisphenol A (BPA), triclosan (TCS), nonylphenol (NP) and its ethoxylates (NP1EO and NP2EO) from secondary effluent. According to the results, an appreciable removal of NP, BPA and TCS to the order of 60–84% was observed as an effect of moderate chlorination doses. This was not the case for NP1EO and NP2EO as even at high chlorine doses, removal efficiencies were lower (37% for NP1EO and 52% for NP2EO). Removal efficiencies of NP, BPA and TCS are practically independent of contact time, although this was not the case for NP1EO and NP2EO. Based on toxicity experiments, it is anticipated that following chlorination of the target chemicals, production of more toxic metabolites is taking place. Therefore the effectiveness of chlorination to remove EDCs is questionable and more research is needed to guarantee safe wastewater reuse.

Key words | chlorination, endocrine disruptors, toxicity, wastewater reuse

INTRODUCTION

Endocrine disrupting chemicals (EDCs) are compounds of mainly anthropogenic origin that interfere with the endocrine system of animals and humans thus causing a series of disorders. Among EDCs, nonylphenol (NP) and its polyethoxylates (NP1EO and NP2EO), bisphenol A (BPA) and triclosan (TCS) are of significant interest due to their persistent detection in raw and treated wastewater (Nakada et al. 2006; Stasinakis et al. 2008a).

The presence of NP in the aquatic environment is mainly associated with the use of nonylphenol polyethoxylates surfactants (NPnEO) with various chain lengths. In contrast to higher NPnEO, their degradation by-products such as NP, nonylphenol monoethoxylate (NP1EO) and nonylphenol diethoxylate (NP2EO) have been reported to exhibit significant estrogenic potential (Nice et al. 2000; Vetillard & Bailhache 2006). Due to its importance, NP has been defined as a priority substance in Annex X of the Water Framework Directive (2000/60/EC) and an annual average threshold value of 0.3 μg/l and a maximum allowable concentration of 2 μg/l have been set by European Directive 2008/105/EC for all European surface water bodies. Recently a threshold concentration of 2 μg/l for effluent NP concentration has been set in Greece, under the Joint Ministerial Decision 354/8-3-2011, in order to allow for wastewater reuse for Wastewater Treatment Plants (WWTPs) with a population equivalent greater than 100,000. BPA is widely used for the production of polycarbonate, flame retardants and epoxy resins. The estrogenic potential of BPA is also well known (Welshons et al. 2006). TCS serves as an antimicrobial and preservative agent and it is widely used in personal care products (soaps, toothpastes, shaving creams, etc.). The occurrence of all the aforementioned compounds in wastewater and the aquatic environment is well documented (Bester 2005; Li et al. 2004; Wu et al. 2007; Stasinakis et al. 2008a).
The removal of the aforementioned EDCs during biological wastewater treatment is relatively high. More specifically, removal efficiencies greater than 85–90% have been reported for BPA, TCS, NP1EO and NP2EO, whereas the respective results for NP are rather contradictory (Nakada et al. 2006; Gonzalez et al. 2007; Ying & Kookana 2007; Stasinakis et al. 2008a). Appreciable removal efficiencies of these compounds have also been reported as an effect of chlorination (Hu et al. 2002; Greyshock & Vikesland 2006; Korshin et al. 2006; Thurman 2006). In these studies, a series of chlorination by-products of these compounds have also been identified. Nevertheless all these studies were focused on pure water or potable water chlorination rather than wastewater chlorination. Besides their high removal during wastewater treatment, in some cases the effluent concentrations of these organic compounds are still appreciable. Especially in the case of wastewater reuse and by taking into consideration the strict threshold values which have been set in Greek legislation for organic compounds such as NP in treated effluent, there is a need to assess additional processes to further decrease the concentrations of such compounds.

In view of the above, the aim of this study is to examine the removal efficiency of these EDCs through wastewater chlorination as well as to assess the effect of operating parameters such as chlorine dose and contact time in chlorination performance.

**Materials and Methods**

**Chemicals**

Methanol (MeOH) and ethyl acetate were of HPLC (high-performance liquid chromatography) grade (Merck, Germany) and were used as received. BSTFA + 1% TMCS solution (Supelco, USA) and pyridine (Carlo Erba-SDS, France) were used for silylation. Analytical standards of NP, NP1EO, NP2EO and TCS were supplied by Dr Ehrenstorfer (Germany). BPA was purchased from Fluka (Switzerland), whereas the deuterated BPA-d16, which was used as surrogate, was purchased from Sigma-Aldrich (USA). All compounds were used without further purification (minimum purity >99%). Stock solutions of individual compounds were prepared in methanol at 1,000 mg/l and kept at −18 °C. HPLC grade water was prepared in the laboratory using a Milli-Q/Milli-RO Millipore system (USA). Ultra pure HCl (32%) was used for acidification of the samples (Merck, Germany).

**Chlorination batch tests**

Several chlorination batch tests (in triplicates) have been performed in 11 reactors, where liquid chlorine in the form of NaOCl was added to secondary treated wastewater samples collected from the Psyttalia Sewage Treatment Works (PSTW). Based on the experimental protocol in order to test different Ct products (C represents residual chlorine concentration and t chlorination contact time), chlorinated wastewater samples were collected after 5, 10, 20, 30 and 60 min contact time. The initial chlorine dose in all experiments was equal to 11 mgCl₂/l. After sampling and before analyses, residual chlorine was inactivated through sodium sulfite addition (40 mg Na₂SO₃/l) and accordingly was being analysed to confirm zero residual chlorine values. Prior to sodium sulfite addition, determination of residual chlorine concentration was also taking place. Based on the experimental results, residual chlorine concentrations were equal to 8.05 mgCl₂/l, 8 mgCl₂/l, 7.5 mgCl₂/l, 6 mgCl₂/l and 4.87 mgCl₂/l at a chlorination contact time of 5, 10, 20, 30 and 60 min respectively. Therefore Ct values examined were equal to 40 mgCl₂/l . min, 80 mgCl₂/l . min, 150 mgCl₂/l . min, 180 mgCl₂/l . min and 292 mgCl₂/l . min.

**Toxicity tests**

Toxicity experiments were also performed in order to assess the toxicity potential of the target EDCs and their metabolites generated during chlorination. The acute bioluminescence method was carried out according to standard operational procedures (ISO/DIS 11348-2). In order to account only for the toxicity of the target compounds and their metabolites and not for the organic by-products developed due to chlorination of the organic matter of secondary treated wastewater samples, toxicity experiments were performed with pure water samples spiked with a mixture of the target compounds and chlorinated at several Ct doses. The bioluminescent bacterium used in the toxicity testing was *Vibrio fischeri*, a marine bacterial strain. Bioluminescence was measured in a luminometer (LUMInox, Dr Lange GmbH, Berlin, Germany). The dilutions required to achieve a 20% and 50% reduction of luminescent activity of a pre-culture of *Vibrio fischeri*, were determined for all water chlorinated samples tested and were expressed as GL₂₀ and GL₅₀ values respectively. Furthermore the effective concentrations causing a 20% (EC20) and 50% (EC50) luminescence inhibition were determined.
Analytical methods

For the determination of the target compounds, wastewater samples before and after chlorination were analysed using a chromatographic method developed by Samaras et al. (2011). The developed procedure included solid phase extraction, while for the qualitative and quantitative analysis, an Agilent Gas Chromatograph 7890A connected to an Agilent 5975C Mass Selective Detector (MSD) was used. Wastewater characteristics (pH, chemical oxygen demand, total suspended solids, turbidity) were determined according to Standard Methods (APHA 1992).

RESULTS AND DISCUSSION

The effect of chlorination on the removal of target compounds was evaluated for a range of \( \text{Ct} \) doses between 40 and 292 mgCl\(_2\)/l.min (\( C \) represents residual chlorine concentration and \( t \) chlorination contact time). The results regarding the five EDCs are presented in Figures 1–4.

According to the results, an appreciable removal of the target compounds was recorded as an effect of chlorination. More specifically for \( \text{Ct} \) values between 40 and 292 mgCl\(_2\)/l.min, removal efficiencies were equal to 60–74% for NP, 75–80% for BPA and 77–84% for TCS (Figures 1–3). Based on these removal efficiencies it can be stated that the threshold value of 2 \( \mu \)g/l, which has been set in Greek legislation for NP for wastewater reuse, can be achieved through chlorination of the secondary effluent even at low chlorination doses in the order of 40 mgCl\(_2\)/l.min. The same \( \text{Ct} \) dose is adequate to achieve an appreciable removal of TCS and BPA. As chlorination doses in the order of 40 mgCl\(_2\)/l.min are typically sufficient to meet the criteria defined in the Greek legislation for restricted irrigation and industrial reuse of treated wastewater, it is postulated that the application of a typical tertiary treatment followed by chlorination is adequate to meet the 2 \( \mu \)g/l limit value for NP as well. It is anticipated that this can be achieved for NP concentrations in secondary effluent no greater than 5 \( \mu \)g/l, which seems to be the case for most of the WWTPs in Greece (Stasinakis et al. 2008a).
The majority of the total removal of the target compounds (82% for NP, 92% for TCS and 98% for BPA) took place at a chlorination dose of 40 mgCl₂/l·min which corresponds to a contact time of 5 min. Thus it can be stated that contact time is not a critical parameter for the design of the chlorination process when the oxidation of such chemicals is the primary goal. The almost instant oxidation of NP, BPA and TCS after chlorine addition can be attributed to the immediate electrophilic attack of chlorine to the phenolic ring of these compounds rather than to the other functional groups. These results are in good agreement with the results reported by other researchers (Ying et al. 2002; Korshin et al. 2006). However, Rule et al. (2005) reported that besides TCS’s almost instant degradation, its removal follows first order kinetics with respect to chlorine dose, a trend that was not obtained in our study.

In contrast to the aforementioned compounds, chlorination removal of nonylphenol ethoxylates (NP1EO and NP2EO) was not significant and was highly dependent on Ct dose. Based on the results, for Ct values between 40 and 292 mgCl₂/l·min, removal efficiencies were equal to 14–37% and 22–52% for NP1EO and NP2EO respectively. These results are in good agreement with the results reported by Thurman (2006). Furthermore only 37–42% of the total removal of NP1EO and NP2EO took place at a chlorination dose of 40 mgCl₂/l·min, which corresponds to a contact time of 5 min (Figure 4). Both NP1EO and NP2EO chlorination experiments exhibited an exponential dependence on the Ct dose. The equations describing the effect of Ct on NP1EO and NP2EO concentrations during chlorination are presented in Figure 4 and are the following:

\[ [\text{NP1EO}] = 3,550 \times e^{-0.002Ct} \]
\[ [\text{NP2EO}] = 1,488 \times e^{-0.003Ct} \]

Based on the above it can be stated that in order to achieve a satisfactory removal for nonylphenol ethoxylates, significantly high chlorination doses should be applied (much higher than those required for disinfection purposes even in the case of unrestricted agricultural wastewater reuse and urban wastewater reuse).

In addition to chlorination experiments, toxicity tests have been also performed in order to assess the toxicity potential of the target EDCs and their metabolites produced during chlorination. The results of these tests are summarized in Table 1. Based on the results it is concluded that toxicity is increased with chlorination dose and although at a dose of 40 mgCl₂/l·min toxicity is moderate, the further increase of chlorination dose resulted in a significant increase of the toxicity of the samples. If we take into consideration that by applying a low chlorination dose of 40 mgCl₂/l·min, almost all NP, BPA and TCS were removed, it can be stated that by increasing either the chlorine dose or the contact time, toxicity is increased due to the formation of degradation by-products with a higher toxic potential (50–100 times more toxic than prior to chlorination). This argument is consistent with studies regarding the production of toxic metabolites due to chlorine oxidation of NP, BPA and TCS (Ying et al. 2002; Rule et al. 2005). Based on these studies, for a chlorination contact time of 60 min, oxidation of TCS resulted in the production of 2,4,6 trichlorophenol and chloroform, whereas for chlorination doses in the order of 90 mgCl₂/l·min, significant chlorinated by-products were produced (tetrachloro-BPA, trichloro-BPA, 2,4,6 trichlorophenol). Therefore it seems possible that the high toxicity measured for chlorination doses above 80 mgCl₂/l·min is associated with the production of such chlorinated by-products.

In order to assess whether toxicity refers to all EDCs used to the aforementioned toxicity experiments, two additional toxicity experiments were performed, one with NP and one with BPA as the sole target compound. Both experiments were performed with pure water samples spiked with the reference EDCs and being chlorinated at a Ct dose of 300 mgCl₂/l·min. Based on the results of these experiments, GL50 values were equal to 43 and 37 for NP and BPA respectively; values very similar to those recorded at the toxicity experiment which was performed on the mixture of all EDCs. Furthermore EC20 values were equal to 17 and 6 ng/l for NP and BPA respectively; values very similar to those recorded at the toxicity experiment which was performed on the mixture of all EDCs. Furthermore EC20 values were equal to 17 and 6 ng/l for NP and BPA respectively; values very similar to those recorded at the toxicity experiment which was performed on the mixture of all EDCs. Furthermore EC20 values were equal to 17 and 6 ng/l for NP and BPA respectively; values very similar to those recorded at the toxicity experiment which was performed on the mixture of all EDCs.
almost two orders of magnitude lower than the value of 3.51 mg/l calculated by Stasinakis et al. (2008b). This significant difference is related to the different samples used in these studies. In our study, chlorinated samples of pure water spiked with NP were used, whereas Stasinakis et al. (2008b) used non-chlorinated samples. Therefore it seems that the significant increase of toxicity is related primarily to the by-products produced through chlorination rather than due to the presence of the target compounds.

CONCLUSIONS

Several chlorination batch tests were performed in order to assess the effectiveness of chlorination to remove the target EDCs from secondary effluent. According to the results, chlorination leads to an appreciable removal of NP, BPA and TCS to the order of 60–74%, 75–80% and 77–84% respectively. Removal efficiencies are practically independent of contact time, as 5 min seems to be enough for high removal of the target compounds. Therefore it is stated that a moderate chlorination dose of 40 mg Cl₂/l.min is adequate to provide for high NP, BPA and TCS removal. This was not the case, however, for NP1EO and NP2EO, as even at chlorine doses of 292 mg Cl₂/l.min, removal efficiencies were relatively low (37% for NP1EO and 52% for NP2EO). For both NP1EO and NP2EO, chlorination experiments exhibited an exponential dependence on the Ct dose. Therefore it can be postulated that in order to achieve a satisfactory removal of nonylphenol ethoxylates, significantly high chlorination Ct doses should be applied, with values higher than those required to guarantee proper disinfection for unrestricted agricultural wastewater reuse and urban wastewater reuse. Based on toxicity experiments, it is anticipated that following chlorination of the target chemicals, production of more toxic metabolites is taking place. Therefore the effectiveness of chlorination to remove EDCs is questionable and more research is needed to guarantee safe wastewater reuse.

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REFERENCES


Welshons, W., Nagel, S. & Vom Saal, F. 2006 Large effects from small exposures III, endocrine mechanisms mediating effects
of bisphenol a at levels of human exposure. *Endocrinology* **147** (6), 56–69.


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