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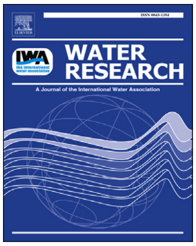

Emerging nitrogenous disinfection byproducts: Transformation of the antidiabetic drug metformin during chlorine disinfection of water

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ABSTRACT

As an environmental contaminant of anthropogenic origin metformin is present in the high ng/L- up to the low μg/L-range in most surface waters. Residues of metformin may lead to the formation of disinfection by-products during chlorine disinfection, when these waters are used for drinking water production. Investigations on the underlying chemical processes occurring during treatment of metformin with sodium hypochlorite in aqueous medium led to the discovery of two hitherto unknown transformation products. Both substances were isolated and characterized by HPLC-DAD, GC-MS, HPLC-ESI-TOF, ^{1}H-NMR and single-crystal X-ray structure determination. The immediate major chlorination product is a cyclic dehydro-1,2,4-triazole-derivate of intense yellow color (**Y**; $C_4H_6ClN_5$). It is a solid chlorimine of limited stability. Rapid formation was observed between 10 °C and 30 °C, as well as between pH 3 and pH 11, in both ultrapure and tap water, even at trace quantities of reactants (ng/L-range for metformin, mg/L-range for free chlorine). While **Y** is degraded within a few hours to days in the presence of light, elevated temperature, organic solvents and matrix constituents within tap water, a secondary degradation product was discovered, which is stable and colorless (**C**; $C_4H_6ClN_3$). This chloroorganic nitrile has a low photolysis rate in ambient day light, while being resistant to heat and not readily degraded in the presence of organic solvents or in the tap water matrix. In addition, the formation of ammonia, dimethylamine and *N*,*N*-dimethylguanidine was verified by cation exchange chromatography.

1. Introduction

Centuries before the advent of modern pharmacology, the guanidine derivate *galegine* had been used for alleviation of the symptoms caused by *diabetes mellitus type 2* (Bailey et al., 2007). The alkaloid occurs in *Galega officinalis*, a member of the *Faboideae* family, which is spread throughout Southern, Eastern and Central Europe. Systematic research revealed its chemical structure (*isoamylene*-guanidine) and consecutively

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led to the synthesis of metformin (*N*,*N*-dimethylbiguanide), today's antidiabetic drug of choice (Witters, 2001; Reitman and Schadt, 2007). Considering an average daily intake of 2 g of metformin hydrochloride by affected people (WHO, 2014) the drug is prescribed at estimated annual quantities exceeding 1100 t within Germany (Mengel, 2011) and 2400 t within the USA (Sargen et al., 2012). Metformin is not metabolized by the human body and consequently found in concentrations of approximately 100 μg/L in influents of wastewater treatment plants (Oosterhuis et al., 2013). The degradation to guanylurea, a dead-end transformation product of biological wastewater treatment, is incomplete. Thus, metformin is released into surface waters, in which it is frequently detectable within the high ng/L-range. Environmental concentrations strongly depend upon the wastewater burden (Trautwein and Kümmerer, 2011; Scheurer et al., 2009). In a recent study, metformin was detected in the German rivers Elbe, Weser and Lake Constance at average concentrations of 472 ng/L, 349 ng/L and 102 ng/L, respectively (Trautwein et al., 2014). A screening of natural samples from the Rhônes-Alpes region in France revealed the presence of metformin at average levels of approx. 100 ng/L in surface waters and 10 ng/L in ground waters. The highest record among the 71 surface water and 70 ground water samples collected at independent sites was 735 ng/L, whereas metformin was detectable in all samples (Vulliet and Cren-Olivé, 2011). An earlier study by Kolpin et al. (2002) performed during 1999 and 2000 detected metformin in 4.8% of 84 samples taken from US streams across 30 states. It was found at an average concentration of 110 ng/L with a maximum record of 150 ng/L. These findings imply that metformin is ubiquitously present in anthropogenically influenced surface waters. While the use of pharmaceuticals generally increases due to expanding populations and age expectancies, diabetes drugs play a prevalent role. The global emergence of *diabetes mellitus type 2* has adopted an epidemic extent with the number of affected people having doubled within the last three decades (Chen et al., 2012).

Investigations on the fate of metformin during water treatment processes by Scheurer et al. (2012) and Ahmad (2012) led to the discovery that aqueous metformin solutions develop an intensive yellow color during treatment with sodium hypochlorite (NaOCl). A strong absorption in the ultra-violet range with a distinct maximum at 385 nm was observed. Despite metformin being a well-investigated environmental contaminant, little information on the reaction of metformin with chlorine or other halogens can be found in literature. Ahmad (2012) reported stoichiometric conversion of metformin in the reaction with NaOCl and the reaction was recommended for the photometric quantification of metformin in wastewater and pharmaceutical preparations. With reference to Marczenko (1976) a reaction pathway leading to the formation of *N*,*N*-dimethylcarbamylurea was suggested, which was described as a “yellow chromophore”. However, the cited source did not contain this information and the suggested reaction pathway was disproved during the course of this study. Mubeen et al. (2010) observed the emergence of a yellow coloration after treatment of aqueous metformin solutions with hydrogen peroxide in strongly alkaline medium. A structural elucidation was not performed.

While metformin is effectively removed by bank filtration (Scheurer et al., 2012), the direct abstraction of surface waters will lead to the presence of residual metformin in the raw water used for drinking water production. It is only partially removed by ozonation and adsorption onto activated carbon (Scheurer et al., 2012). Because a transformation of metformin is likely to take place during the chlorine disinfection, harmful substances may be formed. Metformin residues have been detected in drinking water produced from surface water in the range of 2 ng/L to 61 ng/L (Trautwein et al., 2014). A transformation may also take place during treatment of wastewater or swimming pool water. Components of excreted human body fluids have been identified to significantly contribute to the formation of toxic disinfection by-products during chlorine disinfection of recreational water (Lushi et al., 2014). Considering a daily personal intake of 2 g of metformin hydrochloride (WHO, 2014), it may contribute to the cocktail of precursors transformed during disinfection.

The aim of this study was to characterize the chemical processes taking place during and after chlorination of metformin containing water. Reaction pathways, stoichiometry, as well as the influence of pH, temperature and matrix factors on formation and degradation were investigated. The analyses were performed with regard to answering the question, whether the same reactions take place during chlorine treatment of drinking water. This is the first in-depth characterization of the chemical transformation of metformin in the presence of free active chlorine.

2. Material and methods

2.1. *Chemicals*

Unless explicitly stated differently, all reagents were used directly as commercially available analytical grade from Merck (Darmstadt, Germany) and Sigma–Aldrich (Steinheim, Germany). Organic solvents were purchased from Carl Roth GmbH (Karlsruhe, Germany) and ultrapure water was produced by a laboratory water purification system (Millipore Synergy, Merck).

2.2. *Preparation of the yellow transformation product (Y; $C_4H_6ClN_5$)*

1 g of metformin hydrochloride (corresponding to 0.8 g of free metformin; 6 mmol; 1 equivalent, Eq) was dissolved in 40 mL of demineralized water. Under stirring 10 mL of a NaOCl solution containing 130 g/L of active chlorine (accounting for 1.3 g of elemental chlorine; 18 mmol; 3 Eq) were slowly added during 5 min. Within 1 h the aqueous reaction mixture turned to a dark, orange-brown color while remaining transparent. Moderate gas evolution was observed and the final reaction mixture had a pH of 6. The aqueous reaction mixture was extracted three times with 30 mL of dichloromethane (DCM). The organic phases were pooled, dried over anhydrous sodium sulphate (Na_2SO_4), filtered and evaporated (530 mbar, 35 °C) until approximately 3 mL of a thick, dark-brown liquid remained. Safety note: the solid residue deflagrates at temperatures above 60 °C independently of the presence of