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Determination of biocides and pesticides by on-line solid phase extraction coupled with mass spectrometry and their behaviour in wastewater and surface water

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Incomplete removal of biocides and pesticides during wastewater treatment.

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ABSTRACT

This study focused on the input of hydrophilic biocides into the aquatic environment and on the efficiency of their removal in conventional wastewater treatment by a mass flux analysis. A fully automated method consisting of on-line solid phase extraction coupled to LC-ESI-MS/MS was developed and validated for the simultaneous trace determination of different biocidal compounds (1,2-benzisothiazoline-3-one (BIT), 3-Iodo-2-propynylbutyl-carbamate (IPBC), irgarol 1051 and 2-N-octyl-4-isothiazolinone (octhilinone, OIT), carbendazim, diazinon, diuron, isoproturon, mecoprop, terbutryn and terbutylazine) and pharmaceuticals (diclofenac and sulfamethoxazole) in wastewater and surface water. In the tertiary effluent, the highest average concentrations were determined for mecoprop (1010 ng/L) which was at comparable levels as the pharmaceuticals diclofenac (690 ng/L) and sulfamethoxazole (140 ng/L) but 1–2 orders of magnitude higher than the other biocidal compounds. Average eliminations for all compounds were usually below 50%. During rain events, increased residual amounts of biocidal contaminants are discharged to receiving surface waters.

1. Introduction

Biocides and pesticides[1] are substances that are intended to destroy, prevent the action of, or otherwise exert a controlling effect on a target organism. Pesticides are legally defined as chemicals applied (mainly agricultural use) for protecting plants whereas biocides are legally classified as those chemicals applied for all other purposes (only urban-use) (BPD, 1998). In the EU, biocides are regulated by the Biocidal Product Directive 98/8/EC (BPD, 1998) which divides the biocides into four main groups (I. disinfectants, II. preservatives, III. pest control, and IV. other biocidal products) which are further sub-classified into 23 product-types, containing approximately 955 identified substances and 372 notified substances (Bürgi et al., 2009). Biocidal substances for which applications are regulated in other guidelines (e.g., pesticides in agriculture, pharmaceuticals) are not classified as biocides. However, there is also a need for clarification towards classification of biocidal products in different guidelines. For example, the use of biocidal products in bituminous roof sealing membranes (e.g., mecoprop) does not fall under the biocide directive although use and impact are comparable to the use of biocides in paints, facades, or roofing foils (Bucheli et al., 1998a, 1998b).

Reports from Denmark suggest that the amounts of pesticides used in agriculture are comparable to the use of urban biocides (Lassen et al., 2001). The annual Swiss consumption in 2007 was estimated to be 7500 t/year with a total of 277 active substances (Bürgi et al., 2009). Only about 30 of these substances are applied in amounts over 5000 kg/year corresponding to more than 95% of the total use.

Based on these statistics, significant input of biocides to the aquatic environment can be anticipated, mainly through rain water and wastewater. Biocides used for facades and roof paintings can leach during rainfall events and reach significant levels in roof runoff and eventually enter surface waters (Jungnickel et al., 2008; Burkhardt et al., 2009; Schoknecht et al., 2009). The occurrence of biocides in wastewater and surface waters has been reported including: biocides of the isothiazolinone type (used as in-can and film preservatives for paints and cosmetics) (Madsen et al., 2001; Rafoth et al., 2007); diuron and irgarol (Konstantinou and Albanis, 2004). If wastewater treatment plants (WWTPs) have limited effectiveness in removing biocides from waste streams, they may act as point sources to the aquatic environment. Hence, it is important to understand the behaviour of these compounds in WWTPs by mass flux studies, which

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[1] The term pesticide is used for the active ingredient of the pesticide product.

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could reveal the impact of urban sources of discharge of biocidal compounds into surface waters, especially during rain events when inputs from combined sewer overflows (CSOs) are significant. However, only a few mass flux studies have been performed in full-scale WWTPs such as those for the fungicide carbendazim (used for film preservation for paints) (Kupper et al., 2006), for ortho-phenylphenol (Jonkers et al., 2009), for triclosan and triclorcarban (Lindstrom et al., 2002; Singer et al., 2002; Heidler et al., 2006; Heidler and Halden, 2007) as well as for quaternary ammonia compounds (Clara et al., 2007; Martinez-Carballo et al., 2007).

The focus of the present study was to assess the input of hydrophilic biocides into the aquatic environment and to investigate the efficiency of removal of these compounds in conventional wastewater treatment by a mass flux analysis. Based on the prioritization of Bürgi et al. (2009) where water pollution risks by biocides were evaluated with regard to potential environmental emissions as well as on environmental behaviour and ecotoxicological effects, 16 hydrophilic analytes and 8 transformation products were selected for this study taking into account their sources and input pathways (Table 1). The following analytes were selected: i) compounds used only as biocides such as 1,2-benzisothiazoline-3-one (BIT), N,N-Dimethyl-N-phenylsulfamide (DMSA) the hydrolysis product of dichlofluanid, 3-Iodo-2-propynylbutylcarbamate (IPBC), irgarol 1051 and its descyclopropyl transformation product and 2-N-octyl-4-isothiazolinone (Octhilinone, OIT); and ii) compounds used in urban and agricultural areas such as carbendazim, diazinon, diuron and its desmonomethyl transformation product DCPMU, isoproturon and its desmonomethyl transformation product, mecoprop, terbutryn and terbutylazine. Additionally, agricultural and wastewater tracers were included. Atrazine and its desethyl and hydroxy transformation products, N,N-dimethyl-N-methylphenylsulfamide (DMST) the hydrolysis product of tolylfluanid, and sulcotrione were used as agricultural tracers. Caffeine, the human pharmaceuticals diclofenac and sulfamethoxazole as well as its human metabolite N4-acetysulfamethoxazole, were included as wastewater tracers.

For the simultaneous determination of the polar contaminants present at trace concentrations, a fully automated method was developed for analyzing different pesticides, biocides and pharmaceuticals in wastewater and surface water. The method consisted of on-line solid phase extraction coupled to liquid chromatography and tandem mass spectrometry using positive and negative electrospray ionization (SPE-LC-ESI-MS/MS).

Several on-line enrichment methods for the determination of pesticides (Castro et al., 2000; Pocurull et al., 2000; Hernandez et al., 2001; Sancho et al., 2004; Stoob et al., 2005; Marin et al., 2006; Kuster et al., 2008; Viglino et al., 2008), and pharmaceuticals (Stoob et al., 2005; Pozo et al., 2006; Choi et al., 2007; Segura et al., 2007a, 2007b; Postigo et al., 2008) in the aquatic environment have been reported. However, to our knowledge no on-line methods for the simultaneous determination of biocides, pesticides, and pharmaceuticals have been published.

The objectives of the present study were: i) the development of an on-line SPE-LC-ESI-MS/MS method for biocidal compounds, wastewater and agricultural tracers; and ii) mass flux studies in a wastewater treatment plant.

2. Materials and methods

2.1. Chemicals and reagents

Unlabelled standards for diclofenac and sulfamethoxazole were purchased from Sigma–Aldrich (Buchs, Switzerland) and caffeine was obtained from Fluka Chemicals (Buchs, Switzerland). Atrazine, desethylatrazine, isoproturon, and mecoprop were purchased from Riedel de Haën (Seelze, Germany). Diazinon, DMSA, 2-hydroxyatrazine, carbendazim, diuron, IPBC (iodocarb), irgarol 1051, octhilinone (OIT), terbutryn and terbutylazine were obtained from Dr. Ehrenstorfer (Augsburg, Germany). Sulcotrione was kindly supplied by Zeneca Agrochemicals (Berkshire, UK). Descyclopropyl-irgarol was synthesized at request by ASCA GmbH, Berlin, Germany. BIT was supplied by THOR GmbH, Speyer, Germany. The isotope labelled standards (surrogates) D_5-atrazine, D_5-2-hydroxyatrazine, D_4-carbendazim, D_9-caffeine, D_4-diclofenac, D_6-diuron, D_9-irgarol, D_6-isoproturon, D_6-mecoprop, D_5-terbutryn, D_5-terbutylazine were purchased from Dr. Ehrenstorfer (Augsburg, Germany). D_{10}-diazinon was purchased from Cambridge Isotope Laboratories (Andover, MA, USA). D_{17}-octhilinone, D_5-acetylsulfamethoxazole, and D_4-sulfamethoxazole were purchased from Toronto Research Chemicals (North York, ON, Canada). D_3-sulcotrione was supplied at request from Solvias, Basel, Switzerland and $^{15}N_3$-desethylatrazine was kindly supplied by Novartis, Basel, Switzerland.

Stock solutions of all compounds and surrogate standards were prepared in methanol, ethanol, or acetonitrile with concentrations of 1 μg/μL. Methanol mixture solutions for the different analytes were prepared in concentrations of 0.01, 0.1 and 1 ng/μL. Surrogate standard mixture solutions in methanol contained 1 ng/μL of each substance.

HPLC-grade methanol and water were purchased from Scharlau (Barcelona, Spain). All other chemicals were purchased from Merck (Darmstadt, Germany).

2.2. Online SPE-LC

The instrumental set-up was similar to the one reported earlier and consisted of a tri-directional autosampler (HTC PAL, CTC Analytics, Zwingen, Switzerland), a dispenser syringe, a sample loop of 20 mL, three LC pumps, two six-port valves, and an on-line extraction cartridge (Stoob et al., 2005). The HPLC pump system consisted of a binary pump (load pump, Surveyor LC, Finnigan), a quaternary low pressure mixing gradient pump (elution pump, Rheos 2200, Flux instruments, Switzerland) for the SPE elution and the methanol gradient, and an isocratic pump (Rheos, 2000; Flux instruments, Switzerland) for the water gradient and a column oven (Portmann Instruments AG, Biel-Benken, Switzerland).

The on-line SPE procedure consists of three main steps: loading; enrichment; and elution. The 20 mL loop was loaded with the dispenser syringe by dual injection of 10 mL samples. For sample enrichment a Strata-X extraction cartridge (a functionalized polymeric sorbent with N-vinylpyrrolidone functional groups, 20 mm × 2.1 mm I.D., 33 μm particle size, Phenomenex, Brechbühler AG, Schlieren, Switzerland) and two six-port valves were used. The sample was loaded with a flow rate of 2 mL/min and subsequently eluted in the back-flush mode with a flow rate of 40 μL/min.

Sharp elution profiles were achieved using the back-flush mode and methanol amended with 0.1% formic acid. In order to re-establish the initial conditions for the LC, the methanol/0.1% formic acid SPE eluate was diluted with water and 0.1% formic acid by an additional pump with an active mixer (Portmann Instruments AG, Biel, Switzerland) with a low volume (15 μL) mixing chamber. This procedure allows for refocusing of the eluted analytes on the analytical column. The addition of formic acid resulted in sharper SPE elution profiles compared to neutral solutions. Methanol with 0.1% formic acid allowed complete elution in 7 min at a flow rate of 40 μL/min resulting in a total elution volume of 280 μL.

To prevent cross-contamination, the sample loop and the extraction cartridge were flushed with acetonitrile after every extraction and conditioned with water and 0.1% formic acid prior to enrichment of the next sample. Instrumental blanks consisting of deionized water and injected following highly concentrated samples showed maximum carryover rates of less than 1%.

Separation was achieved using a 50 × 2 mm Xbridge C_{18} column (Waters, Baden-Dättwil, Switzerland) equipped with a 10 × 2 mm pre-column containing the same sorbent. Optimal separation was achieved at 30 °C with a total flow rate of 300 μL/min. Solvent A was water acidified with 0.1% formic acid and solvent B was methanol acidified with 0.1% formic acid. The gradient was initiated with 10% B for 5 min, followed by a 3 min linear gradient to 50% B, a 10 min linear gradient to 60% B, and another 3 min gradient to 90% B. Afterwards the column was washed with 90% B for 2 min. Initial conditions were re-established in 0.1 min, and the column was equilibrated for 3 min prior to the next analysis. The total chromatographic run time for one sample including on-line SPE and LC-MS/MS was 26 min.

2.3. Tandem mass spectrometry

The LC was coupled with an electrospray probe (ESI) to a TSQ Quantum Ultra triple quadrupole MS (Thermo Scientific, San Jose, CA, USA), operated under unit resolution in the selected reaction monitoring (SRM) mode.

Analyses were performed in the positive or negative mode during the same run (Table 1). For all analytes, protonated ($[M+H]^+$) or deprotonated ($[M-H]^-$) molecular ions were selected as precursor ions. Specific and intense product ions of each target analyte were used for quantification, and a secondary product ion was used as a qualifier ion for confirmatory purposes. Nitrogen was used as the sheath gas (50 arbitrary units) and as auxiliary gas (10 arbitrary units), and argon was used as the collision gas (1.5 mTorr).

Analyses were performed at a spray voltage of +3800 V (positive mode)/−3000 V (negative mode), a capillary temperature of 350 °C, scan time of 0.01 s (positive mode)/0.05 s (negative mode), and a scan width of 0.1 *m/z*. For the