Screening and human health risk assessment of pharmaceuticals and their transformation products in Dutch surface waters and drinking water

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KWR Watercycle Research Institute

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Screening and human health risk assessment of pharmaceuticals and their transformation products in Dutch surface waters and drinking water

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Preface

This report is part of project B111744 Dealing with pharmaceuticals in the water cycle. The contents of this report will be submitted for publication in a peer-reviewed journal. The results described in this report will also be presented briefly in the final report of project B111744 and B111718.

Summary

Numerous studies describe the presence of pharmaceuticals in the water cycle, while their transformation products are usually not included. In the current study 17 common pharmaceuticals and 10 transformation products were monitored in the Dutch waters, including surface waters, pre-treated surface waters, river bank filtrates, two groundwater samples affected by surface water and drinking waters. In these samples, 12 pharmaceuticals and 8 transformation products were observed to be present. Concentrations were generally highest in surface waters, intermediate in treated surface waters and river bank filtrates and lowest or not detected in produced drinking water. However, the concentrations of phenazone and its environmental transformation product AMPH were significantly higher in river bank filtrates, which is likely due to historical contamination that is still present in river bank filtrates. Fairly constant ratios were observed between concentrations of transformation products and parent pharmaceuticals. This might enable prediction of concentrations of transformation products from concentrations of parent pharmaceuticals.

The present study also addresses the toxicological relevance of the observed pharmaceuticals and transformation products. For these compounds, (i) a substance specific provisional guideline value (pGLV) and (ii) a group pGLV for groups of (related) pharmaceuticals and transformation products were derived by assuming an additive mechanism of action within each group. A substantial margin exists between the maximum summed concentrations of these compounds present in different water samples and the derived (group) pGLVs. Based on the results of this limited screening campaign no adverse health effects of the studied compounds are expected in (sources of) drinking water in the Netherlands. The presence of transformation products with similar pharmacological activities and concentration levels as their parents illustrates the relevance of monitoring transformation products, and including these in risk assessment. However, more thorough monitoring yielding information on statistical uncertainty and variability in time and space, and research on possible synergistic effects of low concentration mixtures of compounds belonging to similar pharmacological classes require attention.

Contents

1 Introduction

Numerous studies describe the presence of pharmaceuticals in wastewater and surface water, groundwater and sometimes even drinking water (Monteiro and Boxall, 2010). Current monitoring efforts are focused on parent compounds, while transformation products are usually not included. However, as Escher and Fenner (2011) have shown, transformation products can be relevant for environmental risk assessment. After consumption or application, pharmaceuticals can be transformed in various (environmental) compartments. With regards to the fate of pharmaceuticals, firstly, they can be transformed within the consumer (i.e. human metabolism) by phase I (activation, e.g. oxidation) and phase II (conjugation) transformations (Testa and Kramer, 2008). Secondly, various chemical and biological processes can transform the pharmaceuticals and transformation products during wastewater treatment (e.g. hydrolysis, oxidation, de-conjugation, photodegradation) (Kern et al., 2010). Thirdly, the pharmaceuticals and products can be transformed in the environment by similar processes as those that may occur in the wastewater treatment plant (Escher and Fenner, 2011). Finally, the pharmaceuticals can be transformed when surface water or groundwater is treated to produce drinking water. Especially oxidative techniques such as ozonation, UV/hydrogen peroxide treatment and chlorination will lead to the formation of (oxidized) transformation products (Richardson et al., 2007). Consequently, depending on the properties of the compounds, numerous products can be formed in consumers and the water cycle (i.e. wastewater treatment, the environment and drinking water production).

Transformation products can exert effects by the same mode of action as their parent when the active substructure that triggers the specific mode of action (toxicophore) remains intact with transformation, or can exhibit lower (baseline) toxicity when the toxicophore is lost during transformation. In rare cases, transformation can create new toxicophores, that can lead to (higher) toxicity by the similar or different mode of action (Escher and Fenner, 2011). Several reports are available on the human health risk of exposure to pharmaceuticals via drinking water (Snyder et al., 2008; Bruce et al., 2010; Kumar et al., 2010), but only few take into account the presence of transformation products (Schwab et al., 2005; Cunningham et al., 2010). Selecting transformation products for monitoring and risk assessment is cumbersome. Data on excretion of human metabolites can be found in literature. Additionally there are various models predicting environmental transformation products. Nevertheless , there is only limited knowledge on which environmental products are formed in relevant fractions and tend to persist under various environmental conditions (Kern et al., 2009). Further, analytical standards of transformation products are often not readily available, which hampers identification and quantification.

In the current study common pharmaceuticals and some related transformation products that were available as standards were monitored in surface waters, bank filtrated river water (later referred to as

'river bank filtrate') and drinking water produced from these sources in the Netherlands. The study had two objectives: Firstly, the occurrence of some frequently observed pharmaceuticals and some of their transformation products as well as the ratio of parent pharmaceuticals and products were described and related to the characteristics of the surface water sources and water treatment. Secondly, the toxicological relevance of these pharmaceuticals and transformation products observed in the water cycle was assessed. This leads to a more general approach on how to assess human health risks of parent pharmaceuticals and their transformation products and how provisional drinking water guidelines can be derived.

2 Materials and methods

2.1 Sampling locations

In October 2009, seven surface water samples were taken in the Dutch part of the river Rhine catchment, five samples in the Dutch part of the river Meuse catchment, one sample from Haringvliet (where the Meuse and Rhine confluence) and one from the Drentsche Aa, a small river in the north of the Netherlands. Furthermore 17 samples were taken from source water entering drinking water production plants. Ten of these samples originated from surface water that had been treated by storage in large reservoirs, rapid sand filtration, and dune infiltration. These are later referred to as 'pre-treated surface water'. Five samples were taken from river bank filtrates and two samples were taken from phreatic groundwater that is known to be affected by surface water. Strictly speaking, river bank filtrate and surface water-affected groundwater is 'source water' and not 'treated' process water. However, since this water is affected by historical surface waters, it was compared to current surface water samples in this paper. Finally, the 17 corresponding produced finished waters were sampled as well (Figure 1). The samples used in the present study were obtained from a screening campaign in the Netherlands aimed at evaluating the occurrence and toxicological relevance of drugs of abuse in (sources of) drinking water (van der Aa et al., 2011). This screening was executed by the National Institute for Public Health and the Environment (RIVM) in close cooperation with the joint research programme (BTO) of the Dutch water companies, carried out by KWR Watercycle Research Institute.

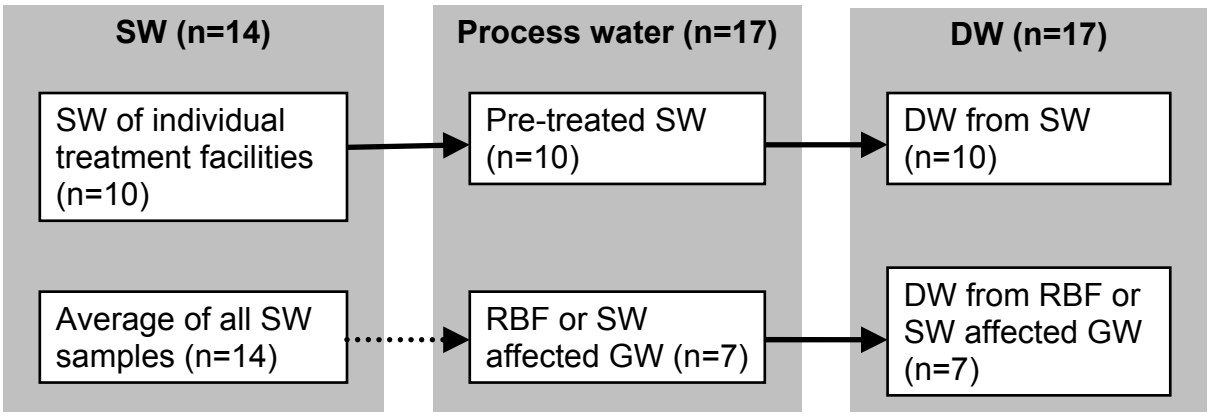

Figure 1: Schematic overview of the 48 samples taken. SW = surface water, RBF = river bank filtrate, GW = groundwater, DW = drinking water. The dotted line between the SW and RBF indicates the indirect linkage between surface water and river bank filtrate that is used as source for drinking water. The 14 SW samples mentioned include the 10 SW samples of the individual treatment facilities.

2.2 Selecting pharmaceuticals and transformation products

The parent pharmaceuticals and their transformation products selected in the present study constitute only a small selection of all pharmaceuticals and products that (can) occur in drinking water sources

(Roig, 2010). The compounds were selected based on occurrence in the environment, knowledge on transformation, availability of standards and available analytical methods. The compounds are listed in the Supplemental Information, Table S1. It is not the intention of the study to cover all (relevant) pharmaceuticals and transformation products.

2.3 Sampling extraction and analysis

Samples were collected in 1000 mL ultra-clean dark green glass bottles and directly stored in the dark at 4°C. Related samples of drinking water and source water were sampled the same day. Processing was done within six weeks after sampling. All samples except drinking water were filtered over a 1.0µm and a 0.2µm PES filter. Deuterated standards (phenazon-d3, carbamazepine-d10, atenolol-d7, fluoxetine-d5 and gemfibrozil-d6) were added at 72 ng/L, the samples were acidified to pH 2.0 with HCl, and eluted over a cartridge with 200 mg (60 µm) of Oasis-HLB SPE sorbent (Waters, Milford, MA, USA) at 10 mL/min. The compounds were extracted from the SPE material by 7.5 mL of acetonitrile, which was concentrated to ~500 µL under a gentle stream of nitrogen. Additionally, because acidic extraction appeared suboptimal for some pharmaceuticals and transformation products, 1000 mL samples from the same locations were also extracted at pH 7.0 using 150 mg of 60 µm Oasis-HLB SPE sorbent, extracted with 8.0 mL of methanol, concentrated to 250 µL under a gentle stream of nitrogen and adjusted to 500 µL with 20/80 methanol/water (v/v).

Subsequently, 500 µL of a 1 mg/L aqueous injection standard solution containing atrazin-d5 (positive ionization) and bentazon-d6 (negative ionization) was added to all extracts. This resulted in a final volume of 1 mL and a concentration factor of 1000. The compounds in the extracts were separated and analyzed with liquid chromatography-high resolution LTQ FT Orbitrap mass spectrometry (Thermo Electron GmbH, Bremen, Germany) with an electrospray interface scanning all masses between 100 and 850 Da. This technique was applied in the positive and negative ion-mode. The compounds were identified with the accurate mass (resolution <5 ppm), the retention time, and the presence and ratio of two product ions (Mezcua et al., 2009). The concentrations were determined with external standard series. External recoveries were used to correct for the extraction efficiency. The average recovery was 72%, ranging from 27% to 115% (Table S1, Supplemental Information). Recoveries in surface water were generally 10% less than recoveries in drinking water and the limit of quantification was below 0.01 µg/L for all compounds. Hydroxycarbamazepine was not included in the external standard series, and no recovery experiments were performed with this compound. The tentative identification and approximate quantification are described in the Supplemental Information.

2.4 Calculating average concentrations when missing data are involved

Calculating average concentrations is cumbersome when compounds are only detected in a selection of all samples analysed. Excluding all samples with concentrations below the quantification limit (negatives) results in an overestimation of the average concentration, while including these negative samples as 'zero' underestimates the average. We therefore applied the log corrected 'half detection limit method' (Haas and Scheff, 1990) where the values below the detection limit are assigned a nominal value that corresponds to the geometric mean between the limit of detection (LOD) and zero (LOD - ln(2) = LOD * 0.31). This method inevitably biases the data but provides a more accurate estimation of the average concentrations than simply ignoring negatives or including them as 'zero'. The method is only applied if the compounds were detected in at least two samples within a defined class.

2.5 Assessing toxicological relevance

The relevance for human health of the compounds present in surface water, pre-treated surface water, river bank filtrate and produced drinking water was determined by deriving a drinking water provisional guideline value (pGLV) and comparing maximum concentration levels present in the samples with this guideline value. Currently, for the compounds selected no statutory drinking water guideline values are available from e.g. European Commission, US EPA or WHO. The general methodology as described by Schriks *et al.* was followed to calculate a pGLV for the compounds selected (Schriks et al., 2010). Briefly, the point of departure for calculating the pGLV for the pharmaceuticals or transformation products was preferably an established Acceptable Daily Intake (ADI) or Tolerable Daily Intake (TDI). If not available, a provisional TDI was derived from the lowest therapeutic daily dose using an uncertainty factor of 100 under the implicit assumption that this dose is equivalent to a lowest observed adverse effect level (LOAEL) (Versteegh et al., 2007). If such a dose was not available, then a provisional TDI was derived based on the lowest chronic no observed (adverse) effect level (NO(A)EL) obtained in rodent studies divided by an uncertainty factor of 1000 (a factor of 10 for animal-to-human extrapolation, a factor of 10 for inter-individual differences and a factor of 10 to extrapolate from subchronic to chronic exposure) (van Leeuwen and Vermeire, 2007; Schriks et al., 2010). Then the pGLV was calculated by allocating a 10% proportion of the ADI or TDI to drinking water to make allowance for exposure from other sources, e.g. food and subsequently by multiplying this proportion by the average weight of an adult (70 kg) and dividing by the average drinking water intake (adults: 2 L per day). After a pGLV was derived for each individual compound, the compounds were grouped based on a common toxicophore or pharmacological mechanism of action and for each group a group pGLV was determined under the assumption of additivity of effects. This group pGLV was set at the level of the lowest pGLV within the group (van der Aa et al., 2011).

To determine the toxicological relevance of the compounds selected, the maximum concentration levels or the sum of these concentrations present in the samples were compared to the derived pGLVs or group

pGLVs, respectively and expressed as a quotient (concentration in water divided by pGLV). Compounds with a quotient of ≥ 1 may be of potential human health concern if the water were to be consumed over a lifetime period. As proposed previously by Schriks *et al*, compounds with a BQ value of > 0.1 in drinking water were identified as those that may warrant further investigation (Schriks et al., 2010). For compounds found in (pre-treated) surface waters and river bank filtrates the threshold for additional assessment is set at an arbitrary value of ≥ 0.2, since these waters are purified in drinking water treatment plants which provides additional protection (Schriks et al., 2010). Compounds in (pre-treated) surface waters and river bank filtrates with a quotient < 0.2 and drinking water with a quotient < 0.1, are presumed to present no appreciable concern to human health.

3 Results and discussion

3.1 Presence and concentrations of pharmaceuticals in water

Table 1 lists the average concentrations of the pharmaceuticals that were detected in one or more samples calculated with the 'half detection limit method' as described in the Materials and Methods section. Additionally maximum concentrations and number of positive observations are listed. Trimethoprim, O-desmethylmetoprolol, fluoxetine, norfluoxetine, paroxetine cyclophosphamide and iphosphamide are not included in the table as their concentrations could not be detected or quantified (concentrations were below the limit of quantification; 0.01 µg/L). Most compounds were observed in surface waters (n=18), fewer in pre-treated surface waters (n=11) and even less compounds were observed in river bank filtrates (n=8). Finally, only three compounds were present in drinking water produced from river bank filtrates (Table 1), while none could be quantified in drinking water produced from surface waters. Apparently, the advanced treatment applied in the production of drinking water from surface water, which includes oxidative treatment (e.g. ozonation, UV-H_2O_2) and active carbon treatment (de Moel et al., 2006), reduces the concentrations of these compounds to levels that can not be quantified.

Table 1: Average concentrations of observed pharmaceuticals (ng/L) in surface waters (SW), in pre-treated waters originating from surface waters (pre-treated SW), in drinking water obtained from surface water (DW-SW), in river bank filtrates (RBF) and in drinking water obtained from river bank filtrates (DW-RBF). Maximum concentrations and number of positive samples per compound are given between brackets. Pharmaceuticals and transformation products from the same pharmacological class are grouped.

Compound	SW (n=14)	Pre-treated SW (n=10)	DW-SW (n=10)	RBF (n=5)	DW-RBF (n=5)
phenazone	9 (25, 6)	6 (21, 3)	- [2]	135 (258, 5)	20 (35, 3)
dimethylaminophenazone	- [2]	- [2]	- [2]	15 (22, 5)	- [2]
propyphenazone	- [2]	- [2]	- [2]	12 (20, 4)	- [2]
1-acetyl-1-methyl-2-phenylhydrazide (AMPH) [1]	16 (66, 8)	7 (19, 5)	- [2]	109 (172, 5)	10 (19, 3)
4-acetylaminoantipyrine (AAA) [1]	76 (176, 10)	28 (124, 4)	- [2]	(20) [3]	- [2]
4-formylaminoantipyrine (FAA) [1]	49 (164, 7)	23 (147, 2)	- [2]	(45) [3]	- [2]
tramadol	51 (107, 12)	19 (53, 7)	- [2]	- [2]	- [2]
O-desmethyltramadol [1]	17 (78, 8)	- [2]	- [2]	- [2]	- [2]
erythromycin-H20 [1]	10 (35, 4)	(17) [3]	- [2]	- [2]	- [2]
clindamycine	5 (16, 2)	- [2]	- [2]	- [2]	- [2]
carbamazepine	59 (121, 12)	29 (50, 8)	- [2]	27 (48, 5)	- [2]
carbamazepine 10,11-epoxide [1]	17 (35, 11)	6 (14, 6)	- [2]	- [2]	- [2]
hydroxycarbamazepine [1,4]	17 (35, 14)	8 (15, 9)	- [2]	7 (11, 5)	1 (3, 3)
oxcarbazepine	(8) [3]	- [2]	- [2]	- [2]	- [2]
atenolol	6 (26, 6)	- [2]	- [2]	- [2]	- [2]
metoprolol	41 (107, 12)	5 (16, 3)	- [2]	- [2]	- [2]
sotalol	31 (99, 9)	- [2]	- [2]	- [2]	- [2]
venlafaxine	21 (59, 11)	5 (13, 3)	- [2]	- [2]	- [2]
O-desmethylvenlafaxine [1]	32 (112, 7)	- [2]	- [2]	- [2]	- [2]
bezafibrate	5 (17, 2)	- [2]	- [2]	- [2]	- [2]

[1] Transformation product; [2] Compound could not be quantified in the sample
[3] If a compound is only observed in one sample, the observed concentration is listed between brackets
[4] No standard of hydroxycarbamazepine was injected so identification is not verified, and concentrations are calculated assuming an equal response of carbamazepine and hydroxycarbamazepine

3.2 Comparing occurrence and concentrations in the different types of water

The differences between concentrations observed in surface waters from the Rhine catchment (n=7) and from the Meuse catchment (n=5) did not exceed a factor 2.5 when compounds were present in both sources (data not shown). Therefore the data of surface waters from both catchments were pooled. The ratios of levels in pre-treated surface waters to those in source surface waters (pre-treated SW / SW) and those in drinking water prepared from riverbank filtrate to those in river bank filtrate (DW-RBF / RBF) were calculated for individual drinking water production locations. The ratios of concentrations in river bank filtrate to those in surface waters (RBF / SW) were calculated using average surface water concentrations, because it is impossible to relate individual surface water samples to individual river bank filtrates.

Concentrations in pre-treated surface waters are generally 10 to 100% of concentrations in the corresponding source waters (Figure 2a). The surface water concentrations of tramadol, venlafaxine and carbamazepine and its transformation products were significantly higher than concentrations in pre-treated surface waters. For the other compounds no significant difference was observed between concentrations in pre-treated surface waters and surface waters. However, the limited observations in treated waters reduce statistical power, so the absence of a statistical difference does not necessarily mean that there is no effect of the water pre-treatment. The pre-treatment of surface waters is diverse; some of these waters sampled are solely stored in large reservoirs, while others are treated with fast sand filtration or infiltrated in dunes. Apparently, these relatively simple treatments already reduce concentrations of (part of the) compounds up to one order of magnitude (Figure 2a). It should, however, be noted that concentrations of pharmaceuticals in river surface waters vary in time (ter Laak et al., 2010). Since treated surface waters originate from surface water collected weeks or months ago, part of the variation might be explained by temporal variations in concentrations. In a single sample, the concentration of 4-formylaminoantipyrine (FAA) in the treated waters largely exceeded the concentrations in surface waters (Figure 2a). This deviation may be caused by an experimental artefact.

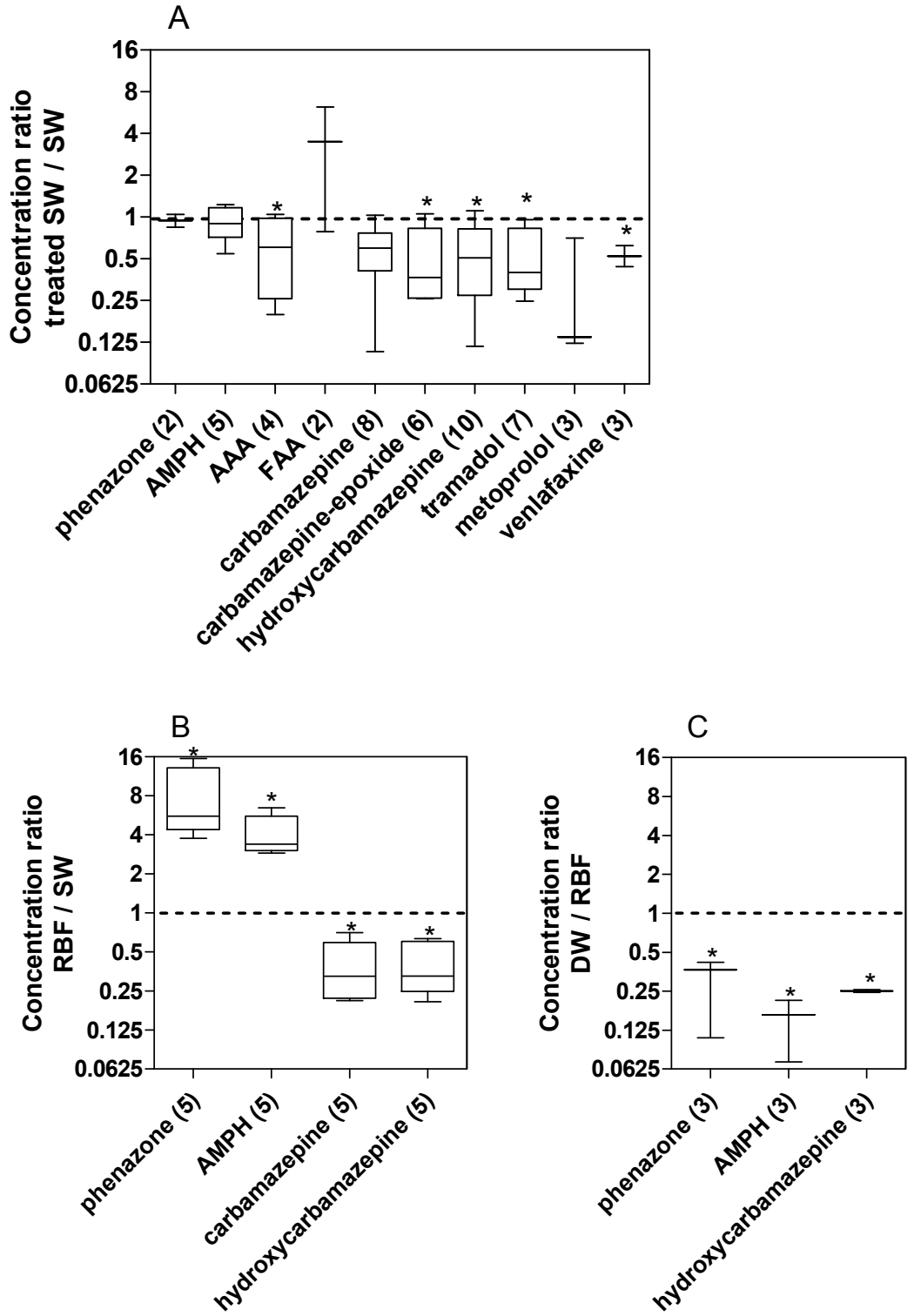

Figure 2a-c: Box-Whisker plots with averages, 25 to 75 percentiles (box) and minimum and maximum values (error bars) of concentration-ratios of the pharmaceuticals and transformation products in the different water sources. Figure 2a shows the ratio of pre-treated surface waters to surface waters (treated SW / SW). Figure 2b shows the ratio of river bank filtrates to surface waters (RBF / SW). Figure 2c shows the ratio of drinking water produced from river bank filtrate to river bank filtrate (DW-RBF / RBF). The '*' indicates that the concentrations in the two water types significantly differ (two tailed t-test, $p < 0.05$). Number of ratios is given between brackets, data are only presented if two or more ratios could be obtained.

The concentrations of 16 out of 20 compounds in surface water exceeded concentrations observed in river bank filtrates (Table 1). Lower (or undetectable) concentrations in river bank filtrates can be explained by degradation and soil sorption during infiltration. However, various factors such as: i) the heterogeneity of the residence time of water in river banks, ii) variable physical properties and chemical conditions in riverbanks, iii) temporal variations of concentrations in the source water (i.e. surface waters), and iv) potential dilution with groundwater, complicate the direct comparison of concentrations in river bank filtrates and surface waters. Remarkably, the concentrations of phenazone and 1-acetyl-1-methyl-2-phenylhydrazide (AMPH), an environmental transformation product of dimethylaminophenazone (Reddersen et al., 2002; Zuehlke et al., 2007) and possibly phenazone, in river bank filtrates significantly exceed the concentrations in surface waters by almost one order of magnitude (Figure 2b). The residence time of water in river banks ranges from less than a year to several decades. Generally river bank filtrates are mixtures of younger (years) and older (decades or longer) water (personal communication with hydrologists from drinking water companies). Phenazone and AMPH in river bank filtrated waters most likely originate from historical surface water contamination. The higher concentrations of phenazone and AMPH in de river bank filtrates might therefore be explained by higher concentrations in surface waters due to higher consumption of phenazone and dimethylaminophenazone in the river Meuse and Rhine catchments some decades ago (Brune, 1997; Reddersen et al., 2002). It is therefore expected that concentrations of these compounds will eventually decrease in river bank filtrates as well. Additionally, propyphenazone and dimethylaminophenazone are solely observed above quantification limits in river bank filtrates (Table 1). Contrastingly, the concentrations of two human metabolites of phenazone-type pharmaceuticals, FAA and AAA, are lower in river bank filtrates than in surface waters. These pharmacologically inactive metabolites are mainly formed from dimethylaminophenazone and its pro-drug metamizole (Levy et al., 1995; Medicines Complete, 2011) of which the latter is currently used in large quantities in German hospitals and also as a veterinary medicine (Rohweder, 2003; Feldmann et al., 2008).

Relatively low concentrations of phenazone, AMPH and hydroxycarbamazepine were observed in three drinking water samples produced from river bank filtrates (Figure 2c). The occurrence of phenazone and AMPH in produced drinking water is likely a result of the relatively high concentrations in the source water (river bank filtrate) and the hydrophilic character of phenazone and AMPH, as their respective LogK_{OW} values are 0.59 and -0.76 (U.S. Environmental Protection Agency, 2000). However, in surface waters, concentrations of hydroxycarbamazepine were similar or even higher in surface waters than in river bank filtrate (Figure 1a). Therefore, the small residues of hydroxycarbamazepine (~1 ng/L) in the produced drinking water from river bank filtrates and their absence in drinking water from surface waters might be explained by the less advanced treatment of river bank filtrate than surface water to produce drinking water (de Moel et al., 2006).

3.3 Ratios of pharmaceuticals and their transformation products

Figure 3 shows the transformation product/parent ratios of tramadol, venlafaxine and carbamazepine. The ratios could be calculated when parent and product were both observed in a sample. The ratios of treated waters and surface waters are pooled.

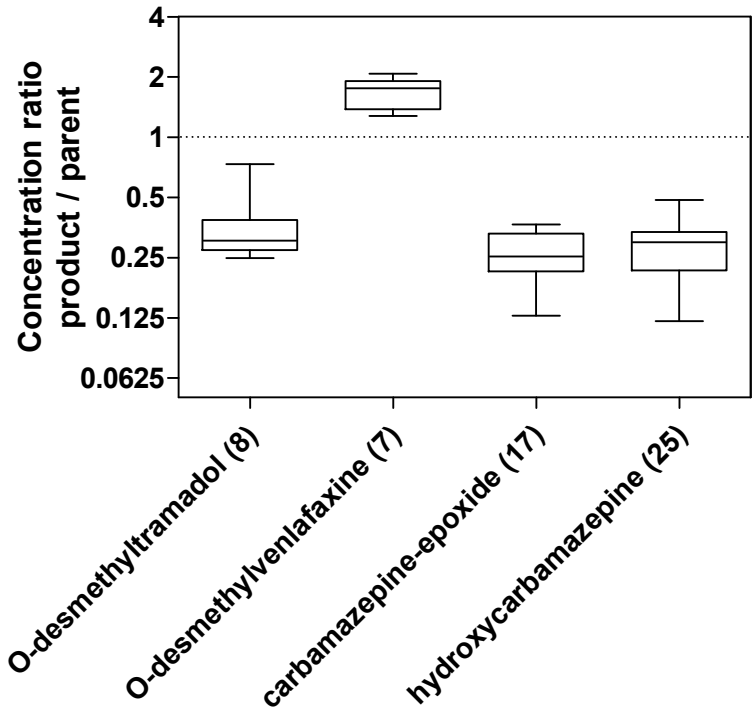

Figure 3: Ratios of concentrations of the transformation products vs. parent compounds in various types of water; O-desmethyltramadol vs. tramadol in SW (n=7), O-desmethylvenlafaxine vs. venlafaxine in SW (n=8), carbamazepine 10, 11-epoxide vs. carbamazepine in SW (n=11) and pre-treated SW (n=6) and hydroxycarbamazepine vs. carbamazepine in SW (n=12), pre-treated SW (n=8) and RBF (n=5). Number of ratios is given between brackets.

Concentrations of O-desmethyltramadol in the surface water samples ranged between 27% to 73% of its parent compound tramadol. O-desmethyltramadol is a human metabolite that is mainly excreted via urine. The ratio observed in the surface water samples corresponds to ratios observed in urine excreted by humans that range from 26% to 57% (Chitil et al., 2009). This implies that ratios present in urine are preserved and indicates that wastewater treatment is approximately equally effective in removing the parent and transformation product. Concentrations of O-desmethylvenlafaxine were between 128% and 208% of its parent venlafaxine in the surface water samples. Similar ratios were observed in effluents of wastewater treatment plants of the Netherlands (154% to 211%, n=7, unpublished data). These ratios, however, do not correspond to excretion ratios by humans. In humans, 92% of the venlafaxine dose is excreted via urine and less than 5% is excreted via feces (Howell et al., 1993). In urine, only 5% of the dose is excreted non metabolized, while 29% is excreted as unconjugated O-desmethylvenlafaxine, 26% as conjugated O-desmethylvenlafaxine and the rest as minor inactive metabolites (Howell et al., 1993). Even if the 5% excreted via feces is solely venlafaxine, only 7% of the total dose is non-metabolized,

while over 50% is excreted as (conjugated) O-desmethylvenlafaxine. Consequently, the O-desmethylvenlafaxine/venlafaxine ratio in human excrements is higher than the ratios observed in wastewater effluents and the aqueous environment.

Concentrations of hydroxycarbamazepine and carbamazepine-10,11-epoxide were both between 12% and 37% of their parent carbamazepine in surface water, treated surface water and river bank filtrates. This does not correspond to human excretion ratio's of carbamazepine-10,11-epoxide as this metabolite is largely transformed to carbamazepine 10-11 diol before excretion (Cunningham et al., 2010). However, a study on wastewater influents and effluents showed product/parent ratios that were 13% & 12% for carbamazepine-10,11-epoxide, 33 & 31% for 2-hydroxycarbamazepine and 26% & 24% for 3-hydroxycarbamazepine, respectively (Miao and Metcalfe, 2003). These data more closely resemble the ratios observed in surface waters, treated surface waters and river bank filtrates in the current study, suggesting that ratios of carbamazepine and metabolites in wastewater are preserved. Finally, the product/parent ratios of phenazone-type pharmaceuticals (phenazone, dimethylaminophenazone, propyphenazone) are not shown in Figure 3 because their human (i.e. AAA and FAA) and environmental (i.e. AMPH) transformation products can be formed from multiple parents that were not all analyzed in this study (Reddersen et al., 2002). Product/parent ratios of tramadol, venlafaxine and carbamazepine appear to be stable over samples taken from different water sources. Whether this is also the case for other pharmaceuticals commonly observed in the environment remains to be studied. Such studies can potentially provide valuable information for the fate of pharmaceuticals, as stable ratios of the parents and products allow predicting concentration of transformation products from those of their parents (and vice versa).

3.4 Toxicological relevance of pharmaceuticals and transformation products

Table 2 summarizes the data and parameters used for the derivation of the pGLVs for the pharmaceuticals and transformation products detected. The pGLVs for the compounds phenazone, carbamazepine, metoprolol and bezafibrate were obtained from literature (Versteegh et al., 2003; Versteegh et al., 2007; Cunningham et al., 2010; Schriks et al., 2010). For the antibiotic clindamycin, the World Health Organization derived an ADI (WHO, 2000), which was used to derive a pGLV. For erythromycin-H_2O, which is the inactive dehydrated form of the macrolide antibiotic erythromycin, the pGLV of the parent compound derived by Versteegh *et al* was used as a conservative approach (Versteegh et al., 2007). If no established ADI or TDI was available in the literature, the lowest therapeutic dose was used to derive a provisional TDI and a pGLV. This was the case for the parent compounds propyphenazone, tramadol, oxcarbazepine, atenolol, sotalol, venlafaxine and the human metabolite O-desmethylvenlafaxine. The latter compound is registered for clinical use as a antidepressant in the United States and Australia, but not in Europe (Medicines Complete, 2011), and its lowest therapeutic dose may be used to derive a provisional TDI.

Table 2. A) Data and parameters used for the derivation of provisional drinking water guideline values (pGLV) and (group) pGLVs for each (group of) pharmaceuticals and B) Comparison of the maximum (sum) concentration levels present in the different water samples (see Table 1) to the pGLV expressed as quotients.

Compound	Point of departure	Ref	UF	TDI or ADI (mg/kg bw/d)	pGLV (µg/L)	Group pGLV (µg/L)	Quotient DW-RBF	Quotient pre-treated SW	Quotient RBF	Quotient SW
phenazone	lowest daily therapeutic dose of 3.6 mg/kg bw/day for phenazone	1,2	100	0.036	125	35	0.002	0.009	0.02	0.01
dimethylaminophenazone	pharmacological NOEL of 10 mg/kg bw/d for metamizole	3	1000	0.010	35					
propyphenazone	lowest daily therapeutic dose of 2.1 mg/kg bw/day for propyphenazone	4	100	0.021	75					
1-acetyl-1-methyl-2-phenylhydrazide (AMPH)	pharmacological NOEL of 10 mg/kg bw/d for metamizole	3	1000	0.010	35					
4-acetylaminoantipyrine (AAA)	pharmacological NOEL of 10 mg/kg bw/d for metamizole	3	1000	0.010	35					
4-formylaminoantipyrine (FAA)	pharmacological NOEL of 10 mg/kg bw/d for metamizole	3	1000	0.010	35					
tramadol	lowest daily therapeutic dose of 0.71 mg/kg bw/d for tramadol	4	100	0.0071	25	6	-	0.009	-	0.03
O-desmethyl-tramadol	lowest daily therapeutic dose of 0.71 mg/kg bw/d for tramadol	4	400	0.0018	6					
erythromycin-H20	microbiological ADI of 4.3 µg/kg bw/day for erythromycin	1,2	na	0.0043	15	na	-	0.001	-	0.002
clindamycine	microbiological NOEL of 3 mg/kg bw/d for clindamycin	5	100	0.030	105	na	-	-	-	0.0002
carbamazepine	lowest daily therapeutic dose and lowest LOAEL of 1.43 mg/kg bw/d for carbamazepine	6	90	0.016	56	56	0.00005	0.001	0.001	0.004
carbamazepine-10,11-epoxide	lowest daily therapeutic dose and lowest LOAEL of 1.43 mg/kg bw/d for carbamazepine	6	90	0.016	56					
hydroxycarbamazepine	lowest daily therapeutic dose and lowest LOAEL of 1.43 mg/kg bw/d for carbamazepine	6	90	0.016	56					
oxcarbazepine	lowest daily therapeutic dose of 8.6 mg/kg bw/d for oxcarbazepine	4	100	0.086	300					
atenolol	lowest daily therapeutic dose of 0.71 mg/kg bw/d for atenolol	4	100	0.0071	25	25	-	0.0006	-	0.009
metoprolol	lowest daily therapeutic dose of 1.4 mg/kg bw/d for metoprolol	2,7	100	0.014	50					
sotalol	lowest daily therapeutic dose of 1.1 mg/kg bw/d for sotalol	4	100	0.011	40					
venlafaxine	lowest daily therapeutic dose of 0.54 mg/kg bw/d for venlafaxine	4	100	0.0054	19	19	-	0.0007	-	0.01
O-desmethylvenlafaxine	lowest daily therapeutic dose of 0.71 mg/kg bw/d for O-desmethylvenlafaxine	4	100	0.0071	25					
bezafibrate	lowest daily therapeutic dose of 1 mg/kg bw/d for bezafibrate	1	100	0.010	35	na	-	-	-	0.0005

UF, uncertainty factor; ref, references; TDI, Tolerable Daily Intake; ADI, Acceptable Daily Intake; pGLV, provisional guideline value; DW-RBF, drinking water produced from river bank filtrate; SW, surface water; RBF, river bank filtrate. References: 1 (Versteegh et al., 2007), 2 (Schriks et al., 2010), 3 (EMEA, 2003), 4 (Medicines Complete, 2011), 5 (WHO, 2000), 6 (Cunningham et al., 2010), 7 (Versteegh et al., 2003)

Insufficient toxicological data is available in the literature to derive compound-specific TDIs for the pharmacologically active dimethylaminophenazone, its environmental transformation product AMPH with unknown activity and the inactive human metabolites FAA and AAA (EMEA, 2003). As these compounds are structurally related to metamizole or their transformation products (Levy et al., 1995; Reddersen et al., 2002), the ADI derived by the European Medicines Agency based on the pharmacological NOAEL of metamizole was used to calculate a pGLV (Table 2) (EMEA, 2003). Here we assumed an equal toxic potency, also for (inactive) the transformation products. We consider this approach rather conservative as in general the transformation products are equal or less toxic than their parent compounds (Escher and Fenner, 2011). An exception is the pharmacologically active O-desmethyltramadol, the major metabolite of tramadol, which is a centrally acting opioid analgesic. Due to bio-activation, O-desmethyltramadol possesses a 2- to 4-fold higher pharmacologic activity than its parent (National Library of Medicine, 2010). Therefore, the TDI for tramadol based on the lowest daily therapeutic dose was used for O-desmethyltramadol with an additional uncertainty factor of four; taking into account that this major metabolite has a higher pharmacological activity than tramadol. For carbamazepine, two TDIs were derived in the literature based on different approaches. A TDI of 0.00034 mg/kg bw/day was derived by Snyder *et al* based on a maximum tolerated dose of 250 mg/kg bw/day obtained from a 2-year study in rats showing evidence of carcinogenicity (Snyder et al., 2008). For risk assessment purposes, a "virtually safe dose" corresponding to a cancer risk of 1 in a million can be estimated (e.g. a TDI) by dividing the maximum tolerated dose of from 90-day studies in rodents by a factor 740,000 (Gaylor and Swirsky Gold, 1998). In the case of carbamazepine, we consider this TDI as rather conservative, as here the maximum tolerated dose was derived from a 2-year study instead of a 90-day study. From this TDI, a pGLV of 1 µg/L may be derived (Schriks et al., 2010). The point of departure for a second TDI for carbamazepine is the lowest therapeutic dose as well as the LOAEL (1.43 mg/kg bw/day) (Cunningham et al., 2010). This TDI of 0.016 mg/kg bw/day was derived by the application of an uncertainty factor of 90 to extrapolate to a NOAEL and to sensitive sub-populations, and for uncertainty regarding (non-genotoxic) carcinogenic effects observed in rodent studies but not in humans. In this study, we selected the latter TDI to derive a pGLV of 56 µg/L for carbamazepine (Table 2). The compound carbamazepine-10,11-epoxide is the major human metabolite of the anticonvulsant carbamazepine, which accounts for about 40% of the absorbed dose. Carbamazepine-10,11-epoxide also has anticonvulsant activity as demonstrated in several *in vivo* animal models of seizures (National Library of Medicine, 2010). Although clinical activity for the epoxide has been postulated, the significance of its activity with respect to the safety and efficacy of carbamazepine has not been established (National Library of Medicine, 2010). One study showed that chronic exposure to both carbamazepine and carbamazepine-10,11-epoxide in pregnant rats resulted in a similar spectrum of fetal malformations including soft tissue defects and skeletal defects (Bennett et al., 1996). When assuming equivalent potency, the TDI derived by Cunningham *et al* for carbamazepine was also applied for the

carbamazepine-10,11-epoxide (Cunningham et al., 2010). In addition, also for the OH-substituted metabolite hydroxycarbamazepine the TDI derived for carbamazepine was applied as no pharmacological or toxicological data for this metabolite were found.

Most human health risk assessments of pharmaceuticals and other anthropogenic compounds in drinking water only focus on the risks of exposure to individual compounds and do not address mixture toxicity (Kumar et al., 2010). In the present case study, we took into account a quantitative consideration for mixture toxicity by deriving so-called group pGLVs for groups of pharmaceuticals with a shared toxicophore or pharmacological mechanism of action. We assumed additive effects of the compounds within each group. In our case study, we distinguished a group of phenazone-type drugs including transformation products based on a common analgesic effect, a group of carbamazepine-type of drugs including oxcarbazepine and transformation products based on a common pharmacological mechanism of action and a group of beta-blockers based on their common β-receptor antagonistic activity (Table 2) (National Library of Medicine, 2010). The other parent compounds in our case study shared no common mechanism of action. For the remaining compounds, we composed groups consisting of a parent compound and its corresponding transformation product under the assumption of an equivalent pharmacological or toxicological potency (Table 2). After the classification, we derived a group pGLV for each group. This group pGLV was set at the level of the lowest pGLV within the group as a conservative approach. A similar methodology was recently applied by other authors (van der Aa et al., 2011).

After derivation of the (group) pGLVs, quotients for each group were calculated by dividing the maximum (sum) concentration levels present in the different water samples (Table 1) by the (group) pGLVs (Table 2). For the compounds and the compiled groups in this study, all quotients were below 1 and also below the thresholds to carry out an additional assessment of 0.2 and 0.1 for sources of drinking water and drinking water, respectively (see methods section). These findings imply that the compounds observed in the water samples present no appreciable concern to human health. The quotients range from 0.00005 for carbamazepine-type compounds in drinking water produced from river bank filtrates to 0.03 for tramadol and O-desmethyltramadol in surface water. The finding that a substantial margin of exposure exists between the maximum concentrations of these compounds present in different water samples and the derived pGLVs are in agreement with other studies which also assessed the toxicological relevance of pharmaceuticals and other organic contaminants in (drinking) water, e.g. (Snyder et al., 2008; Kumar et al., 2010; Schriks et al., 2010) and a recent review on risk assessment of pharmaceuticals in drinking water (WHO, 2011). Due to the grouping applied in our study, the margin becomes slightly smaller than when the risk assessment is performed on a case by case basis, but is still substantial. This study illustrates that when taking into account potential additive effects, current environmental concentrations and concentrations in drinking water of pharmaceuticals and their

transformation products are well below levels where potential effects on human health would be expected.

For carbamazepine two pGLVs could be derived based on two different TDIs. When applying the pGLV of 1 µg/L (Snyder et al., 2008; Schriks et al., 2010) instead of the pGLV of 56 µg/L as used in this study, the quotient for drinking water would be 0.003 instead of 0.0005. So in both cases a large margin of safety exists. In this study, for the parent compounds propyphenazone, tramadol, oxcarbazepine, atenolol, sotalol, venlafaxine and O-desmethylvenlafaxine, we derived a provisional TDI from the lowest therapeutic daily dose using a general uncertainty factor of 100 under the implicit assumption that this dose is equivalent to a lowest observed adverse effect level (LOAEL) (Versteegh et al., 2007). Other authors applied a compound specific uncertainty factor to derive a provisional TDI based on lowest or minimum therapeutic dose. They selected an appropriate uncertainty factor based upon extrapolation uncertainties including LOAEL to NOAEL, duration of exposure, intra-individual susceptibility and quality of the available data (Schwab et al., 2005; Cunningham et al., 2009; Bruce et al., 2010). In these studies, the applied uncertainty factors ranged between e.g. 27 for paracetamol to 1000 for dehydronefidipine. This methodology results in a more precise, compound specific estimation, whereas our methodology gives a more general estimation. However, for this reason we propose to carry out an additional, more extensive assessment for compounds or groups with quotients below the thresholds of 0.2 and 0.1 for sources of drinking water and drinking water, respectively. In contrast to our study, no 10% allocation-factor was applied in the above-mentioned studies, to allocate the proportion of a TDI attributable to drinking-water in the derivation of a predicted no effect concentration (PNEC) or drinking water equivalent levels (DWELs).

Although there is no indication of a human health risk with respect to the pharmaceuticals and transformation products detected in finished drinking water, alertness may be required as presence of these compounds in (sources of) drinking water may change in future. More thorough monitoring yielding information on statistical uncertainty and variability in time and space may be recommended as concentrations of pharmaceuticals can vary in time (ter Laak et al., 2010). Additionally, a potential drawback of this practical approach is that only additive effects within a group are taken into account. Synergistic effects of mixtures of compounds within a pharmacological class are largely unknown (Bull et al., 2011). At low concentrations, these synergistic effects may be more important than additive effects from a toxicological point of view (Kumar et al., 2010). Understanding and implementing such information is important in human health risk assessment, however, due to a current lack of knowledge and data in this field, further research is needed. Furthermore, this study only took into account a selection pharmaceuticals and possible transformation products that (can) occur in drinking water sources. The selection was based on occurrence in the environment, transformation processes, but also on the availability of standards and their detectability in the analysis. For example, the three beta-

blockers, classified as one group based on their common β-receptor antagonistic activity, is not exhaustive as for example bisoprolol and propranolol were not included. Adding these substances to the risk assessment may slightly increase the ratio, but, as the use (Bull et al., 2011) and kinetics will not differ too much from the other beta-blockers, we do not expect that the current ratios of 0.009 for surface waters and 0.0006 for pre-treated surface water will reach a value of 0.2.

4 Conclusion

This study describes the presence and risks of a selection of pharmaceuticals and transformation products in Dutch surface waters and drinking water. The study shows that the largest number and highest concentrations of pharmaceuticals and transformation products were observed in surface waters, while concentrations and number of pharmaceuticals reduced with passage of river banks and water treatment. However, concentrations of phenazone and AMPH largely exceeded surface water concentrations. This is likely a result of historical contamination, as the sampled river bank filtrates originate from surface water of years or decades before. Minor residues of some pharmaceuticals were observed in drinking water produced from river bank filtrates, whereas in drinking water produced from surface water no pharmaceuticals could be quantified. Interestingly, transformation products of some pharmaceuticals were observed in similar concentrations as their parents. These ratios of the concentrations of parents and products were rather stable across the different samples. Stable ratios might enable prediction of concentrations of transformation products from concentrations of parent pharmaceuticals. However, additional studies are necessary to investigate the potential of such predictions.

This study shows a practical approach to assess the human health risk of mixtures of pharmaceuticals and transformation products by deriving a group pGLV for a group of (related) pharmaceuticals and transformation products by assuming additive mechanisms of action. Despite the relatively high abundance of some transformation products compared to their parent compounds, this study showed that still a substantial margin exists between the maximum summed concentrations of these compounds present in different water samples and the derived (group) pGLVs. So earlier drawn conclusions based on parent compounds (Bruce et al., 2010; Schriks et al., 2010; WHO, 2011) do still hold when transformation products are included. Based on the results of this limited screening campaign no adverse health effects of the studied compounds are expected in (sources of) drinking water in the Netherlands. The presence of transformation products, which may have similar pharmacological activities and concentrations as their parents, illustrates the relevance of monitoring transformation products, and including these in future risk assessments. However, more thorough monitoring yielding information on statistical uncertainty and variability in time and space is necessary (ter Laak et al., 2010). Additionally, ongoing research on possible synergistic effects of low concentration mixtures of compounds belonging to similar pharmacological classes requires attention as well.

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7 Supplemental Information

Table S1 gives the recoveries of the tested pharmaceuticals and transformation products and the tentative identification and quantification of hydroxycarbamazepine are described.

Table S1: Pharmaceuticals and transformation products analyzed and their recoveries.

Compound	Selected extraction method	Recovery drinking water	Standard deviation	Recovery surface water	Standard deviation
phenazone	acid	68%	5%	61%	2%
dimethylaminophenazone	neutral	96%	2%	95%	1%
propyphenazone	acid	57%	5%	51%	5%
1-acetyl-1-methyl-2-phenylhydrazide (AMPH) [1]	acid	98%	17%	96%	13%
4-acetylaminoantipyrine (AAA) [1]	acid	34%	7%	39%	1%
4-formylaminoantipyrine (FAA) [1]	acid	30%	6%	27%	17%
tramadol	acid	86%	21%	89%	14%
O-desmethyltramadol [1]	neutral	79%	3%	77%	7%
trimethoprim	acid	80%	13%	59%	2%
erythromycin-H20 [1]	acid	107%	19%	80%	18%
clindamycine	acid	98%	7%	72%	18%
carbamazepine	acid	87%	7%	108%	24%
carbamazepine 10,11-epoxide [1]	neutral	106%	3%	115%	6%
oxcarbazepine	acid	61%	6%	53%	10%
hydroxycarbamazepine [1,2]	neutral	-	-	-	-
atenolol	neutral	59%	3%	51%	13%
metoprolol	acid	90%	9%	115%	5%
O-desmethylmetoprolol [1]	neutral	96%	6%	91%	4%
sotalol	neutral	81%	2%	72%	10%
fluoxetine	acid	76%	20%	34%	14%
norfluoxetine [1]	acid	104%	38%	49%	21%
paroxetine	acid	89%	22%	38%	19%
venlafaxine	acid	81%	71%	87%	0%
O-desmethylvenlafaxine [1]	acid	37%	7%	41%	13%
bezafibrate	acid	55%	8%	54%	10%
cyclophosphamide	acid	73%	7%	61%	11%
iphosphamide	acid	54%	11%	46%	11%

[1] transformation product

[2] there was no standard injected or recovery experiment executed for 2-hydroxycarbamazepine or 3-hydroxycarbamazepine (isomer is unknown) so no recoveries could be not obtained.

Quantifying hydroxycarbamazepine

Hydroxycarbamazepine was not included in the external standard series, and no recovery experiments were performed with this compound. Its response was clearly correlated to the response of carbamazepine. Additionally, its retention time was 20% shorter than carbamazepine which can be expected with the addition of a polar hydroxyl group. Furthermore, hydroxycarbamazepine was not

observed in blanks spiked with all standards, excluding that hydroxycarbamazepine is a contamination of (internal) standards or the extraction and analytical procedure. For quantification, the concentration-response factor was assumed to be identical to carbamazepine. This was not tested, but the similar structure of the molecules likely gives a similar response. This was for example observed for carbamazepine versus oxcarbazepine and carbamazepine-10,11-epoxide of which the response factors deviated only 5% and 20%, respectively. All this circumstantial evidence supports our assumptions on the identity and quantity of hydroxycarbamazepine. Nevertheless, it should be noted that the presented results are not conclusive for this compound.

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