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Environmental toxicology and risk assessment of pharmaceuticals from hospital wastewater

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ABSTRACT

In this paper, we evaluated the ecotoxicological potential of the 100 pharmaceuticals expected to occur in highest quantities in the wastewater of a general hospital and a psychiatric center in Switzerland. We related the toxicity data to predicted concentrations in different wastewater streams to assess the overall risk potential for different scenarios, including conventional biological pretreatment in the hospital and urine source separation. The concentrations in wastewater were estimated with pharmaceutical usage information provided by the hospitals and literature data on human excretion into feces and urine. Environmental concentrations in the effluents of the exposure scenarios were predicted by estimating dilution in sewers and with literature data on elimination during wastewater treatment. Effect assessment was performed using quantitative structure activity relationships because experimental ecotoxicity data were only available for less than 20% of the 100 pharmaceuticals with expected highest loads. As many pharmaceuticals are acids or bases, a correction for the speciation was implemented in the toxicity prediction model.

The lists of Top-100 pharmaceuticals were distinctly different between the two hospital types with only 37 pharmaceuticals overlapping in both datasets. 31 Pharmaceuticals in the general hospital and 42 pharmaceuticals in the psychiatric center had a risk quotient above 0.01 and thus contributed to the mixture risk quotient. However, together they constituted only 14% (hospital) and 30% (psychiatry) of the load of pharmaceuticals. Hence, medical consumption data alone are insufficient predictors of environmental risk. The risk quotients were dominated by amiodarone, ritonavir, clotrimazole, and diclofenac. Only diclofenac is well researched in ecotoxicology, while amiodarone, ritonavir, and clotrimazole have no or very limited experimental fate or toxicity data available. The presented computational analysis thus helps setting priorities for further testing.

Separate treatment of hospital wastewater would reduce the pharmaceutical load of wastewater treatment plants, and the risk from the newly identified priority pharmaceuticals. However, because high-risk pharmaceuticals are excreted mainly with feces, urine source separation is not a viable option for reducing the risk potential from hospital wastewater, while a sorption step could be beneficial.

KEYWORDS

Pharmaceuticals

Quantitative structure-activity relationship

Predicted no-effect concentration

Risk quotient

Elimination

Source Separation

Wastewater

Hospital

1. INTRODUCTION

1.1. Environmental effects of pharmaceuticals

Pharmaceuticals are increasingly detected in surface waters, ground waters, and drinking water (Kolpin et al., 2002, Benotti et al., 2009, Watkinson et al., 2009) as not all are removed in conventional wastewater treatment plants (Joss et al., 2008). Often, it is difficult to establish cause-effect relationships of negative consequences for aquatic ecosystems (Ankley et al., 2007). Assessed as single compounds, most pharmaceuticals apparently pose no or moderate environmental risk. A notable exception is the negative effects on fish reproduction after exposure to estrogenic compounds (Routledge et al., 1998, Kidd et al., 2007). Likewise, the adverse effect of diclofenac on vulture populations in Pakistan (Oaks et al., 2004) demonstrates that under specific exposure conditions pharmaceuticals can cause problems. Increasingly, also negative effects of pharmaceuticals that are not related to the pharmacological effect (Owen et al., 2007) or its side effect (Oaks et al., 2004) are found (Tarazona et al., 2010), e.g. specific inhibition of photosynthesis in algae caused by ß-blockers (Escher et al., 2006) and fluoxetine (Neuwoehner et al., 2009). Furthermore, in reality, rather than single compounds we find complex mixtures of pharmaceuticals and metabolites that may interact or show concentration additivity (e.g. Altenburger et al., 2004, Brian et al., 2007). Wastewater experts and policy makers are currently discussing whether micropollutants give sufficient rise to concern to justify removal measures from wastewater streams (FOEN, 2009).

1.2. Removal of pharmaceuticals from wastewater

There are four approaches to remove micropollutants: optimize existing technology at wastewater treatment plants (WWTP), upgrade WWTP with new technology, source control, and source separation (Larsen et al., 2004). The main focus usually lies on end-of-pipe measures, and ozonation of the WWTP effluent or addition of powdered activated carbon were evaluated as promising tertiary treatment step. There was satisfactory removal of most pharmaceuticals by ozonation in a full-scale pilot plant (Hollender et al., 2009, Reungoat et al., 2010). However, removal of iodinated X-ray contrast agents is often not satisfactory. Dosages of 10 – 20 mg L^{-1} powdered activated carbon also result in a good removal of a broad spectrum of micropollutants (Nowotny et al., 2007, Snyder et al., 2007).

1.3. Source separation: the example of hospitals

Source control measures include strict prohibition (as for phosphate in detergents), emission standards (as for nutrients from WWTP), or designing pharmaceuticals with improved bio-degradability in cooperation with the industry. Urine source separation with NoMix toilets can contribute to reducing pharmaceuticals from diffuse household sources (Larsen et al., 2009, Lienert and Larsen, 2010, www.novaquatis.eawag.ch), which would on average reduce 60–70% of the mass (Lienert et al., 2007a) and approximately 50% of the ecotoxicological risk of human pharmaceuticals from wastewater (Lienert et al., 2007b). The type and quantity of pharmaceuticals used in hospitals differs from what is used in the general population (Kummerer, 2001). Therefore, hospitals or homes for the elderly can be considered as point sources, and separate treatment of this wastewater is being discussed (Moser et al., 2007, Heinzmann et al., 2008).

To date the contribution of hospitals to the pharmaceutical load in wastewater is unclear, since e.g. contraceptives or painkillers are widely used in the population. Various projects, including a large EU-consortium called “PILLS” (www.pills-project.eu) are currently determining the significance of hospitals as point sources for pharmaceuticals and pathogens, including multi-antibiotic resistant bacteria. In Switzerland, 18% of the total volume of the “most-sold top 100 active compounds list of pharmaceuticals” (IMS, 2004) is being administered in hospitals (Weissbrodt et al., 2009). In mass flow studies in a Swiss hospital, 50% of all X-ray contrast media, but only a few percent of the investigated cytostatics were recovered in the hospital sewer (Weissbrodt et al., 2009). The low recovery is mainly explained by pharmaceuticals consumed in the hospital but excreted at home by out-patients (50% out-patients for X-ray

contrast media and 70% for cytostatics in this example). Cytostatics are considered to be especially harmful to the environment and are mainly administered in hospital settings (Lenz et al., 2007).

Ort et al. recently determined the fractions of pharmaceuticals stemming from hospitals using a clever sampling design and chemical analytical quantification of 59 pharmaceuticals (Ort et al., 2010). For most pharmaceuticals the contribution of hospitals to overall wastewater was lower than 15%, with exception of two antibiotics (contrast media were not included in this study). These Australian results were consistent with a Norwegian analysis (Langford and Thomas, 2009). Similarly, the load of endocrine-disrupting chemicals did not differ between hospital and general wastewater (Pauwels et al., 2008).

1.4. The dose makes the poison

Mass fluxes alone are insufficient to evaluate the risk stemming from pharmaceuticals; their ecotoxic potential needs to be considered, what to our knowledge has not been done for hospital wastewater so far. The risk quotient (RQ) is defined as predicted environmental concentration (PEC) divided by the predicted no effect concentration (PNEC), which is extrapolated preferentially from chronic toxicity data, or, if no chronic data are available, from acute toxicity data (EMEA, 2006, European Parliament and European Council, 2006a). Despite recent large efforts to increase the database on ecotoxicological effects of pharmaceuticals (PhACT Database, 2006), there remain significant data gaps, especially when it comes to chronic effect data (Crane et al., 2006). Data gaps can be closed with predictive models using Quantitative Structure Activity Relationships (QSAR) but again chronic QSARs are less readily available (European Chemicals Agency, 2008, Escher et al., 2009). Therefore, the following analysis is based on acute toxicity data and uses an assessment factor of 1000 to extrapolate the PNEC, which is 100 times higher than the assessment factor of 10 recommended in the EMEA guideline to be used in association with chronic toxicity data. This implicitly accounts for an acute-to-chronic ratio of 100, which should be protective for most modes of toxic action, apart from endocrine effects as is discussed in section 3.3.

Previous attempts to predict the risk of large lists of pharmaceuticals (Sanderson et al., 2004) were of limited use because they did not account for the speciation of pharmaceuticals. However, over 60% of pharmaceuticals are acids or bases that are fully or partially dissociated at ambient pH (Avdeef, 2003). Therefore, classical QSAR models cannot be applied without adaption and consideration of speciation of pharmaceuticals (Tarazona et al., 2010).

Additionally to the risk from individual pharmaceuticals, also the risk from different mixtures should be estimated. We recently developed a toxicity model for mixtures consisting of an individual pharmaceutical and its metabolic transformation products (Escher et al., 2006, Lienert et al., 2007b). It can also be applied to mixtures of groups of different pharmaceuticals with a common (therapeutic) mode of action using the assumption of concentration addition of mixture toxicity – or for concentration addition of the underlying baseline toxicity for all groups of pharmaceuticals as discussed below.

1.5. Mixture toxicity of pharmaceuticals

Pharmaceuticals are designed to be bioactive (with exception of contrast agents, which are rather diagnostics than pharmaceuticals). In non-target aquatic life many act as baseline toxicants. However, some exhibit the therapeutic effect also in aquatic life as the unwanted estrogenic effects on fish (Kidd et al., 2007). Others act via a different specific mode of toxic action, as evidenced for fluoxetine effects on algae (Neuwoehner et al., 2009). It is generally accepted that mixtures with components exhibiting the same mode of action act according to the model of concentration addition. If all components act according to a strictly different mode of action they cannot be modeled with concentration addition but act according to the model of independent action (Altenburger et al., 2003). For practical purposes, the concept of concentration addition is usually a realistic worst-case scenario because its prediction is often within an order of magnitude of the experimental findings (Altenburger et al., 2004). The majority of mixture studies with pharmaceuticals was on estrogenic chemicals (Brian et al., 2005, Thorpe et al., 2006, Brian et al., 2007, Kortenkamp, 2002, 2008); with few exceptions on other classes of pharmaceuticals (Escher et al., 2002, Cleuvers, 2004, Escher et al., 2006) and they generally confirmed concentration addition for pharmaceuticals from the same therapeutic class. Also analysis of a large number of pesticide mixtures confirmed that their aquatic mixture toxicity could be predicted by concentration addition in 90% of over 200 mixtures (Deneer et al, 2000). Furthermore, Hermens and Leeuwangh (1982) put forward the hypothesis that for mixtures of large numbers of chemicals with diverse specific modes of action, where the individual concentrations are well below the threshold of individual effect, the underlying baseline toxicity may add up to a significant mixture effect.

All chemicals, regardless of whether they have a specific mode of toxic action, also exert a baseline toxic effect (van Wezel and Opperhuizen, 1995). There is typically a threshold concentration below which the specific mode of toxic action is not observed and above which

it is. At the concentrations at which acute toxicity usually occurs, the toxicity of a single pharmaceutical will be predominantly due to the specific mode of toxic action. However, in mixtures the concentration of each single component decreases, while the number of components with various different specific modes of toxic action increases. Therefore, the contribution to the total toxicity by the specific mode of toxic action decreases while that for the non-specific baseline toxicity increases (ECETOC, 2001). Warne and Hawker used this concept to develop the Funnel Hypothesis (Warne and Hawker, 1995). The Funnel Hypothesis argues that the more components an equitoxic mixture (a mixture where each chemical contributes the same to toxicity) contains, the larger the likelihood is that the compounds with specific modes of toxic action will not dominate the mixture toxicity. Thus the components will increasingly act only by their baseline mechanism of action and should be concentration additive.

In wastewater, we have a large number of components of varying modes of toxic action. Thus we can assume that the toxicity of a very complex mixture is governed by the underlying baseline toxicity, not the specific mode of toxic action of single components. For risk assessment, if concentration addition can be assumed, the risk quotients of the individual pharmaceuticals can be added up to yield a sum risk quotient (RQ_{mix}).

1.6. Ecotoxicological risk potential in four scenarios

The aim of this study was to estimate the risk potential of wastewater containing pharmaceutical mixtures from two point sources. The 100 active ingredients excreted in the highest amounts in 2007 from two different hospitals, one general hospital and one psychiatric center were compared.

To evaluate the elimination of pharmaceuticals in conventional wastewater treatment plants (WWTP) and the effect of dilution of the hospitals' wastewater in the sewer, we compared the following four scenarios for both hospitals:

Scenario 1 HWW: Risk potential of the wastewater of the hospital main wing, before discharge to the sewer (i.e. full risk potential without any degradation or dilution).

Scenario 2 WWTP influent: Risk potential at inlet of the WWTP (i.e. reduction of risk potential through dilution in sewers).

Scenario 3 WWTP effluent: Risk potential at discharge of the WWTP (i.e. reduction of risk potential through degradation and sorption process during conventional biological treatment; including dilution in sewers).

Scenario 4 HWWTP effluent: Risk potential at the hospital main wing after hypothetical conventional biological treatment (i.e. reduction of risk potential through degradation and sorption process in conventional biological treatment without dilution). This scenario thus assumes that some sort of biological treatment would be installed in the main wing of the hospital to deal with the wastewater; in an ideal case, the wastewater might then be directly discharged to surface waters or infiltrated).

2. MATERIALS AND METHODS

2.1. General hospital

The first case is a typical, regionally important general hospital in Switzerland with 338 used beds serving more than 250000 inhabitants. In 2007, there were 122814 "days of care" and 16013 patients leaving the hospital. The whole range of medical services is offered, e.g. internal medicine, oncology, surgery, maternity clinic, nuclear medicine, and radiology, including computer tomography (CT) and magnetic resonance imaging (MRI). In 2008, 11767 CTs were carried out, of which 7490 were with X-ray contrast media; and 5154 MRIs (2691 with X-ray contrast media). Around two thirds of these X-rays were carried out with out-patients.

In 2008, 209251 m^3 water was used in total, and 115690 m^3 in the main hospital wing that hosts patients and where pharmaceuticals are excreted. Wastewater is discharged to a WWTP with conventional biological treatment, which serves 54133 inhabitants. In 2007, the WWTP treated 8641486 m^3 wastewater, and discharged 564993 m^3 without treatment in combined sewer overflows during rain events. Pharmaceutical concentrations in the hospital wastewater were calculated for the main hospital wing. For the dilution to the WWTP influent, the combined sewer overflow was not considered, resulting in a dilution factor df of 0.013,

The hospital kindly provided data of the pharmaceuticals administered in 2007. We additionally purchased Swiss pharmaceuticals sales data for 2004 from IMS, Hergiswil, Switzerland. Website: http://www.ihaims.ch (accessed 7.10.2009); info@ch.imshealth.com.

The amount of active ingredient in the pharmaceuticals was evaluated from Swiss drug documentations (Documed, 2009) and the sum of each ingredient calculated. Amounts of active compounds excreted unchanged in urine and feces were calculated using excretion rates from literature (Lienert et al., 2007a, Documed, 2009). If excretion was not clearly given, worst case scenarios with highest suggested excretion were taken. For active ingredients been

used as cremes, an excretion of 75–100% was assumed, since wash off from the skin is also a source of water contamination without undergoing metabolism in human body. We assumed that all pharmaceuticals were excreted in the hospital, i.e. we neglected pharmaceuticals thrown away, and excretion by out-patients. The 100 active ingredients excreted in the highest amounts (Top-100 pharmaceuticals) were analyzed further in this study.

In 2007, 1154 kg of pharmaceuticals were consumed in the hospital, of which 779 kg were excreted. The Top-100 list accounts for 1137 kg consumed pharmaceuticals (777 kg excreted). "Natural" ingredients such as metals, carbohydrates, sugars, enzymes, paraffin oil, herbal medicines etc. were omitted from the analysis. However, we included synthetic laxatives and synthetic sugars.

In Swiss households, approximately 74.8 g pharmaceuticals per inhabitant per year were consumed, of which 23.4 g were excreted; based on data from IMS health of the Top-40 pharmaceuticals sold in pharmacies, drug stores, and doctor's practices. Out of the Top-100 data received, 60 substances belong to the natural ingredients excluded for this study. The amount of pharmaceuticals discharged into this WWTP from households totals 1267 kg per year or 62% of the total pharmaceutical load in the WWTP (2044 kg per year). Thus, around 38% of the pharmaceuticals at the WWTP in this case study stem from the hospital.

2.2. Psychiatric center

The psychiatric case study is a regionally important Swiss psychiatric center with 211 used beds, providing stationary and ambulatory services. In 2007, 2008 patients received stationary treatment, with 76855 "days of care". Besides acute adult psychiatry, there are e.g. wards for psychotherapy, addictive disorders, and geriatric psychiatry. There is also a housing group and working place for long-term psychiatric patients. According to interviews with head physicians and nurses (Lienert and Mosler, in prep.), many patients have acute psychiatric disorders. These are often in an extreme state at admission requiring strong medication. Therefore, there is a focus on pharmaceutical treatment.

In 2007, 23250 m^3 water was used in the psychiatric hospital. It is discharged to a WWTP, which treats 1742000 m^3 raw wastewater with conventional biological treatment and serves 14603 inhabitants, yielding a dilution factor of the wastewater df of 0.013.

In 2007, 52 kg of pharmaceuticals were consumed in the psychiatric hospital, of which 17 kg were excreted. As above, these numbers were calculated from the amounts of pharmaceuticals administered, which were kindly provided by the hospital. The Top-100 list, which consists of

the 100 active ingredients excreted in the highest amounts, accounts for 50 kg of consumed pharmaceuticals, of which 17 kg were excreted. Again, "natural" ingredients such as metals, carbohydrates, sugars, enzymes, paraffin oil, herbal medicines etc. were discarded, but synthetic laxatives, such as synthetic sugars, included. These 17 kg excreted in the psychiatric center represent approximately 5% of the pharmaceuticals reaching the WWTP (359 kg per year in total) assuming a general excretion of 23.4 g per year per Swiss inhabitant as explained above.

2.3. Exposure Assessment

In the following, the calculation of the predicted environmental concentration PEC for the four scenarios is described. Only parent compounds were regarded and concentrations were corrected for metabolism in the human body. Metabolites were neglected because previous analysis showed that the contribution of metabolites to the overall risk is typically not very high. Moreover, exposure to metabolites is very difficult to assess due to highly variable literature reports on excreted metabolite fractions (Lienert et al., 2007b).

In scenario 1, PEC_{HWW} was defined as the concentration of active ingredient expected in hospital wastewater. PEC_{HWW} was calculated from the amount of each active ingredient consumed in the hospital, M (g), the fraction excreted $f_{excreted}$ of unchanged active ingredient in urine and feces and the volume of the hospital wastewater in the main wing where pharmaceuticals are consumed V_{HWW} (L).

$$PEC_{HWW} = \frac{M \cdot f_{excreted}}{V_{HWW}} \quad (1)$$

M was summed up from all amounts m_i (g) of active ingredient consumed in the different drug preparations. We derived m_i from the units consumed for each drug preparation, U_i, and the amount of active ingredient contained in each unit, m_{Ui} (g).

$$M = \sum_{i=1}^{n} m_i = \sum_{i=1}^{n} U_i m_{Ui} \quad (2)$$

In scenario 2, $PEC_{WWTPinfluent}$ was defined to be equivalent to the PEC_{HWW} multiplied with the dilution factor df in the sewer and corresponds to the concentration of pharmaceuticals at the inlet of the WWTP. The df was 0.013 for both, the the general hospital and the the psychiatric center.

$$PEC_{WWTPinfluent} = df \cdot PEC_{HWW} \quad (3)$$

In scenario 3, $PEC_{WWTPeffluent}$ refers to the discharge of the WWTP, where the $PEC_{WWTPinfluent}$ was reduced by conventional biological secondary treatment with sludge age > 3 days in municipal wastewater treatment, including removal of organic material and denitrification/nitrification. Data on biodegradation were compiled from the literature (Supporting Information, Tables SI-1 and SI-2). The fraction eliminated in the treatment plant $f_{elimination\ in\ WWTP}$ was assumed to be 0% if no literature data were available.

$$PEC_{WWTPeffluent} = f_{eliminationin\ WWTP} \cdot PEC_{WWTPinfluent} \quad (4)$$

For scenario 4, the same elimination rates were assumed for the wastewater treatment directly in the hospital (without dilution in the sewer), which yields the $PEC_{HWWTPeffluent}$.

$$PEC_{HWWTPeffluent} = f_{eliminationin\ WWTP} \cdot PEC_{HWW} \quad (5)$$

2.4. Effect Assessment

2.4.1. Experimental ecotoxicity data

Literature was screened for ecotoxicity data for all 100 quantitatively most important compounds in each case study. For screening, a straightforward search approach was defined:

1. Screen database on ecotoxicity data PhRMA PhACT(R) (PhACT Database, 2006). PhACT database is currently limited to members of PhRMA (US trade association) and was used with permission.
2. Screen the ECOTOX database of the U.S. EPA (http://cfpub.epa.gov/ecotox/)
3. Screen selected reports, books, and papers which compiled ecotoxicity data for pharmaceuticals (Hanisch et al., 2002, BLAC, 2003, Kümmerer, 2004, Besse and Garric, 2007, SRU, 2007).
4. Search for data with google scholar (http://scholar.google.com.au/) using search terms "compound name", "EC50", and "algae"/"daphnia"/"fish".

Whenever possible, toxicity data that are consistent with the species of the selected QSAR were chosen to calculate baseline toxicity (see below). If such data were not available, the lowest acute EC/LC50 of another closely related biological species was chosen. If no acute value was available, also chronic toxicity data were used. However, as the discussion below demonstrates, ecotoxicological literature data on pharmaceuticals remains scarce and there is not enough chronic toxicity data available to base the analysis upon. Therefore toxicity was estimated with QSARs exclusively to avoid inconsistencies between data-rich and data-poor compounds.

2.4.2. QSAR model to predict baseline toxicity

To calculate baseline toxicity of the 100 quantitatively most important compounds in each case study, established QSARs for algae-, daphnia-, and fish toxicity were used. The QSARs were selected from the Technical Guidance Document of the EU (European Commission, 2003) because they constitute a well-validated and often applied set.

Most published baseline QSAR models were set up for neutral organic molecules and use the octanol-water partition coefficient K_{ow} as hydrophobicity descriptor. However, many pharmaceuticals are acids or bases (Tarazona et al., 2010). For these, K_{ow} is an unsuitable measure of bioaccumulation and surrogate for biomembranes, the target site for baseline toxicants. In pharmaceutical science, the liposome water distribution coefficient at a defined pH value, e.g. pH 7, D_{lipw}(pH 7) has replaced the K_{ow} as a descriptor for uptake into biological membranes. More recently, this model was also adapted in environmental science. For a historic overview refer to (Escher and Sigg, 2004). The logarithm of D_{lipw}(pH 7) was therefore used in the QSARs for baseline toxicity (Table 1) to calculate the toxicity of the compound towards the three aquatic organisms, algae, daphnia, and fish.

2.4.3. Estimating the hydrophobicity descriptor logD_{lipw}(pH 7)

D_{lipw}(pH 7) is the lipid-water distribution coefficient that corrects for speciation at pH 7 in the case of organic acids and bases, since partitioning into membranes not only depends on the hydrophobicity of a compound but also on its charge and specific interactions with the membrane (Escher et al., 2000). Ideally (but rarely), the experimental D_{lipw}(pH 7) is available in the literature. If not, the liposome-water partition coefficient of the neutral species K_{lipw} can be used together with an estimate of the speciation derived from the acidity constant pK_a. If K_{lipw} is not available it can be estimated from the K_{ow} (Escher and Schwarzenbach, 2002). For consistency and fair treatment of data-rich and data-poor compounds, we consistently used estimated values of D_{lipw}(pH7) derived from the K_{ow} with the algorithms below.

To calculate D_{lipw}(pH7) from the K_{ow}, following steps were undertaken for each compound:

A. **logK_{ow}-search:** The databases of Kowwin v. 1.67 (U.S.EPA, 2008), ChemPlusID (http://chem.sis.nlm.nih.gov/chemidplus/chemidlite.jsp), and PhysProp (http://www.epa.gov/oppt/exposure/pubs/episuitedl.htm, also accessible via http://www.syrres.com) were checked for an experimentally derived octanol-water

distribution coefficient K_{ow}. If no experimental value was found, the value estimated by a program of U.S. EPA (Kowwin v. 1.67, U.S.EPA, 2008) was used. As comparison, the K_{ow} was also calculated using the online prediction program SPARC (Hilal et al., 2005). Contrary to Kowwin, which is based on a database of compounds with known K_{ow}, SPARC calculates K_{ow} values *ab-initio* from quantum mechanics.

B. **Selecting K_{ow}** and sorting out compounds without baseline toxicity: If the experimental or estimated value by Kowwin was $\log K_{ow} > 0$ and less than 10 times greater or smaller than the value estimated by SPARC ($\log K_{ow} \pm 1$), the former was used for all further calculations. If both $\log K_{ow}$ (experimental/Kowwin and SPARC) were negative (i.e. $\log K_{ow} < 0$, no accumulation in an organism), the compound was considered to show no baseline toxicity due to its low tendency to partition into biomembranes and insignificant contribution to the mixture toxicity. These compounds were excluded from all further calculations. If the two K_{ow} differed more than an order of magnitude, several more estimation programs were used and the K_{ow} from either Kowwin or SPARC closest to the mean values reported by vcc labs (Virtual Computational Chemistry Laboratory, 2009) was used.

C. **$\log K_{lipw}$ calculation:** $\log K_{lipw}$ was calculated from the selected $\log K_{ow}$ using a QSAR for polar compounds (Vaes et al., 1997).

$$\log K_{lipw} = 0.905 \cdot \log K_{ow} + 0.515 \quad (6)$$

D. **Speciation at pH7:** SPARC (Hilal et al., 2005) was used to calculate the fraction that is neutral at pH 7 $f_{neutral}$. The acidity constants pK_a of single functional groups of a compound were also extracted from SPARC and where possible, experimental values from PhysProp database were collected as comparison.

E. **$\log D_{lipw}$(pH 7) calculation:** Calculation of $\log D_{lipw}$(pH 7) based on K_{lipw} of the neutral species and speciation uses the rough assumption that charged species (fraction $1 - f_{neutral}$), independently whether they are positively or negatively charged, partition one order of magnitude less into organic phases than the corresponding neutral species (fraction $f_{neutral}$) (equation 7):

$$\Delta mw = \log K_{lipw}(\text{neutral species}) - \log K_{lipw}(\text{charged species}) = 1 \quad (7)$$

$$\log D_{lipw}(pH7) = \log(f_{neutral} \cdot 10^{\log K_{lipw}} + (1 - f_{neutral}) \cdot 10^{(\log K_{lipw})-1}) \quad (8)$$

We have discussed the limitations of using Δmw of 1 on numerous occasions (Escher and Sigg, 2004, Neuwoehner et al., 2009). Since the database is too limited to generate more

precise estimates for Δmw, we kept the generic value of 1. Zwitterionic compounds were treated with a Δmw of 1, too, despite their overall net neutral charge because often the opposite charges are spatially isolated.

2.4.4. Calculating the predicted no-effect concentration (PNEC)

To estimate the predicted no-effect concentration (PNEC), the lowest QSAR-based EC50 value (i.e. for the most sensitive species; either fish, daphnia, or algae) of each compound was divided by 1000. The Technical Guidance Document of the European Commission (2003) suggests an assessment factor of 1000 if acute toxicity data (for example $EC50_i$, effect concentration of pharmaceutical i) are available in at least three test systems on three trophic levels: algae, daphnia, fish.

$$PNEC_i = \frac{EC50_i}{1000} \quad (9)$$

2.5. Risk analysis

2.5.1. Calculating the risk quotient (RQ) of single compounds

For each pharmaceutical i, the risk quotient RQ was calculated as an indicator for ecotoxicological risk. The RQ is the ratio between the predicted concentration in the environment PEC and the concentration at which no effect is expected PNEC (EMEA 2006).

$$RQ_i = \frac{PEC_i}{PNEC_i} \quad (10)$$

RQ > 1 indicates an ecotoxicological risk for the aquatic environment

RQ < 1 indicates no ecotoxicological risk for the aquatic environment

Note, while for individual chemicals, the PNEC is derived from the most sensitive species, calculations for mixtures must be based on a common species. Therefore, we assessed the risk for algae, daphnia, and fish individually and then selected the species with the highest resulting RQ_{mix} for further analysis. We also point out that for hospital wastewater, cytostatic and antibiotic effects are of particular concern. However, there are only limited and non-standard ecotoxicological data available for these mechanisms.

2.5.2. Mixture toxicity model and risk quotient of mixtures

The sum of the risk quotients of the Top-100 pharmaceuticals in each hospital was computed to allow comparing drug cocktails of variable compositions. According to the concept of concentration addition, the combined effect of the components is equal to the sum of the concentrations of each chemical expressed as a fraction of its own individual toxicity (Brown, 1968, Sprague, 1970). Concentration addition holds if the components of a mixture exhibit the same mode of toxic action. Since toxicity was estimated using a baseline toxicity QSAR for all compounds, this condition is fulfilled for the QSAR estimates but not necessarily for the experimental toxicity. If individual pharmaceuticals exhibit a specific mode of toxic action (which would be underestimated by the baseline toxicity QSAR), this specific effect would contribute to the mixture toxicity according to independent action, which is a generally lower contribution than one from concentration addition. Thus it is likely that the underestimation of specific toxicity is cancelled out because the contribution of this component is given a higher weight by using the mixture toxicity model with concentration addition instead of the model with independent action for specific toxicity.

Hence, to calculate the mixture toxicity RQ_{mix} of all 100 quantitatively most important pharmaceuticals using the model of concentration addition, their risk quotients were summed up with eqn. 13.

$$RQ_{mix} = \sum_{i=1}^{n} RQ_i = \sum_{i=1}^{n} \frac{PEC_i}{PNEC_i} \quad (13)$$

By comparing RQ_i of single compounds to the total risk of the mixture RQ_{mix}, the pharmaceuticals or groups of pharmaceuticals of greatest concern can be identified and further assessed.

3. RESULTS AND DISCUSSION

3.1. Mass fluxes in hospital wastewater

The general and psychiatric hospitals showed very different pharmaceutical usage patterns in 2007 (Tables 2 and 3). First, the total amount of pharmaceuticals differed substantially. In the general hospital, 779 kg were excreted, from which we can predict a load excreted from each "bed" of 2.3 kg per year. In the psychiatric hospital only 17 kg were excreted, which gives an excreted load of 0.08 kg per bed. Second, also the types of pharmaceuticals differed

significantly. In the general hospital, 58% of the excreted load stemmed from X-ray contrast media, 19% from laxatives, 16% from antibiotics, and 8% from others. In the psychiatric hospital, the main fraction came from laxatives with 36%, followed by analgesics/antiphlogistics to 17%, antidiabetics to 15%, psychotropic pharmaceuticals to 11%, and others to 21%.

Even though all these pharmaceuticals were administered in the hospital, it is unclear, which fraction was excreted in the hospital and which fraction was taken home by out-patients. A mass flow study in another hospital showed that only 50% of all X-ray contrast media were excreted there (Weissbrodt et al., 2009). In our case study hospital, two thirds of the patients typically go home after receiving an X-ray, thus a significant fraction of pharmaceuticals will also be excreted at home. Likewise, since many older patients are in hospital, they take a number of pharmaceuticals regularly that they bring into the hospital. Since it is impossible to make an exact mass balance of which pharmaceuticals are excreted where, we assumed the worst case that all pharmaceuticals administered in the hospital would also be excreted there. Likewise, we did not account for the pharmaceuticals brought in by patients.

Currently, a mass flow analysis study is performed at the general hospital. The wastewater from the hospital is analyzed and compared to the wastewater of the receiving treatment plant with the aim to elucidate the load fraction of the hospital (Kovalova et al., 2010).

3.2. High consumption does not always translate to high risk

The ten highest ranked PEC_{HWW}, i.e. the concentration of different active ingredients in the hospital wastewater, constituted 5970 µg/L in the general hospital. This equaled 89% of the sum of all Top-100 PEC_{HWW} (Table 2 and Table SI-1 in the Supporting Information). However, the mixture toxicity RQ_{mix}, i.e. the sum of the risk quotients of these Top-10 pharmaceuticals amounted only to 1.0, equaling 0.4% of the RQ_{mix} of the Top-100 pharmaceuticals. The reason is that among the Top-10 pharmaceuticals only two (4-methylaminoantipyrine and amoxillin) showed significant ecotoxicity ($logD_{lipw}$(pH 7) > 0; Table SI-3). The remainder comprises the polymeric macrogol, which is the laxative polyethylene glycol, and contrast agents such as iodinized and gadalenium compounds of very low hydrophobicity.

A similar result on the exposure side was obtained for the psychiatric hospital, where the Top-10 PEC_{HWW} summed up to 603 µg/L, which is 81% of the sum of all Top-100 PEC_{HWW} (Table 3 and Table SI-2 in the Supporting Information). However, the effect analysis came to a

different conclusion than for the general hospital. There were only four pharmaceuticals in the Top-10 list that were not ecotoxic ($logD_{lipw}$(pH 7) < 0; Table SI-4), namely the laxative macrogol, the antidiabetic metformin, magaldrate, a drug for acid related disorder, and the antiepileptic gabapentin. All others showed substantial ecotoxicity potential (diclofenac, ibuprofen, venlafaxine, amoxicillin, amisulpride, paracetamol). Consequently, the Top-10 pharmaceuticals with respect to their exposure amounted to 23% of RQ_{mix} (Table 3).

Figures 1A and 1B compare the PEC_{HWW} with the risk quotients of the different scenarios investigated. The data are ranked with decreasing PEC_{HWW} and all data are included, while Tables 2 and 3 only include the results with RQ_{HWW} < 0.01). Obviously, there is no correlation between PEC and RQ (Pearson's R < 0.1). There were only few pharmaceuticals with a RQ > 1 in the hospital wastewater and these mostly had a PEC_{HWW} < 10 µg/L. A notable exception is diclofenac, whose risk was equally driven by exposure and effect.

For most other compounds the main driver determining the RQ was the PNEC (Figure 2). This observation is substantiated by the fact that the PEC_{HWW} varied in our selected dataset by less than four (general hospital, Table 2) and three (psychiatric center, Table 3) orders of magnitude, while the PNEC values covered almost eight orders of magnitude, resulting in an overall range of the RQ of more than seven orders of magnitude (Figure 2).

This analysis is relevant to prioritize pharmaceuticals for risk assessment. Generally, those pharmaceuticals with a high consumption are selected for further investigation and risk assessment, which is reflected by many studies on these compounds. However, those pharmaceuticals are not necessarily the most relevant ones with respect to their environmental risk as our present analysis indicates.

3.3. How good is the model for effect assessment?

Ideally, chronic toxicity data should be used for the risk assessment of pharmaceuticals (EMEA, 2006). However, data on the chronic toxicity of pharmaceuticals remain scarce (Crane et al. 2006) and the database is not sufficient for the risk analysis attempted here. The use of acute toxicity data is justified in those cases, where the acute-to chronic ratio (ACR) is in the typical range of 10 to 100 (Roex et al. 2000, Raimondo et al. 2007). However, for pharmaceuticals, the ACR can be much higher, especially for endocrine disruptors such as ethinylestradiol or methyltestosterone, when the ACR may exceed 10^6 because adverse effects on the endocrine system require very low concentrations (Crane et al. 2006) In other aquatic species the ACR is typically much lower, even for endocrine disruptors (Sanderson and

Thomson, 2009). The top-100 list of pharmaceuticals used in hospitals contains only one sex hormone (progesterone) and three corticosteroids (prednisolon, betamethason, dexamethason). Progesterone has not been tested in fish but its synthetic analogue levonogestrel exhibited chronic effects at the low ng/L range in adult fathead minnows (Zeilinger et al., 2009) and the resulting ACR is $>10^6$ (Berninger and Brooks, 2010). However, unlike the synthetic progestins, the natural substrate progesterone is rapidly degraded in wastewater treatment plant and is even not stable in a wastewater sample (Labadie et al., 2005; Esperanza et al, 2007). Due to its instability no toxicity data exist for progesterone and it is justified to neglect the specific progestagen activity in the risk analysis.

As fish have corticosteroid receptors (Prunet et al. 2006), this might translate into a specific effect, but there are no experimental data available for corticosteroids apart from an ACR of 10 for algae (Crane et al. 2006).

In addition, it cannot be fully excluded that none of the other pharmaceuticals exhibits a different and more sensitive mode of toxic action in a chronic toxicity study. This would cause an underestimation of the RQ of the individual components, and if these particular components had a large contribution to the mixture toxicity, the assumptions regarding the mixture toxicity model could be flawed. Nevertheless, based on currently available data, we regard our screening approach as a valuable contribution to risk assessment of hospital pharmaceuticals.

Even experimental acute toxicity data were only available for a very limited set of compounds (Tables SI-5 and SI-6 in the Supporting Information). 16/15 (general/psychiatric hospital) acute EC50 values were found for algae (Tables SI-5A and SI-6A), 19/21 acute EC50 for *Daphnia* (Tables SI-5B and SI-6B), and 16/18 acute LC50 for fish (Tables SI-5C and SI-6C). Thus even if one resigns to acute toxicity data, less than 20% of the pharmaceuticals under investigation actually have experimental toxicity data. This percentage would not be sufficient for the envisaged analysis. Therefore, we had to use the QSAR models for the prediction of toxicity.

To evaluate if the experimental toxicity data point to a specific mode of toxic action or if it can be explained by baseline toxicity, we performed a toxic ratio analysis. This analysis helps to decide if the use of baseline toxicity QSARs is justified or if there is a high probability that QSAR predictions lead to underestimation of toxicity as the pharmaceutical analyzed exhibits a specific mode of toxic action to the organism under evaluation.

The toxic ratio TR (eqn 14) is a measure of the specificity of effect (Maeder et al. 2004). If TR > 10, i.e., the experimental toxicity is at least ten times higher than the one predicted from the baseline toxicity QSAR, then the compound is likely to have a specific mode of action (Verhaar et al. 1992). If TR ≤ 10, the given compound exhibits merely baseline toxicity.

$$TR = \frac{EC_{50,baselinetoxicity}}{EC_{50,experimental}} \quad (14)$$

The majority of pharmaceuticals with experimental toxicity data could be classified as baseline toxicants with a toxic ratio analysis. Of the 15/16 experimental algae toxicity data, only three antibiotics had a TR exceeding 10 (Tables SI-5 and SI-6). Clarythromycin had a TR of 61165, sulfamethoxazole of 2867, and erythromycin of 6585. Metoprolol had a TR of 71, but another algal species was tested than *Pseudokirchneriella subcapitata*, which we use for QSAR calculations. For trimethoprim, a TR of 24 was derived from a NOEC value, so no quantitative comparison should be made due to mismatch of endpoints. Out of the 19 experimental *Daphnia magna* data, two analgesics, tramadol (TR = 814) and paracetamol (TR = 59) indicated specific toxicity. The TR of sulfamethoxazole of 16 was slightly increased but it is uncertain whether it exhibits a specific mode of toxic action as in algae. In fish, only one out of 16/18 experimental data points yielded a TR >10 but this value for tramadol is not reliable, since the fish species tested was not indicated.

If we extrapolate the results of the TR analysis of this fraction of pharmaceuticals for which experimental data were available to all pharmaceuticals evaluated in this study, we can safely assume that > 90% of the top-100 pharmaceuticals act as baseline toxicants to the non-target aquatic organisms and that the remainder (< 10%) will not dominate the toxicity of the mixture (see section 1.5). We conclude that the QSAR model for baseline toxicity is valid to predict the toxicity of our mixtures of hospital wastewater. For 54 (general hospital) and 72 (psychiatric hospital) of the Top-100 pharmaceuticals, it was possible to derive a toxicity estimate. For the remainder, the predicted lipophilicity was so small ($logD_{lipw}$(pH 7) < 0) that independent of the PEC no contribution to the toxicity was expected.

3.4. On which biological species to base risk evaluation?

In principle, PNEC must be derived from the biological species with the lowest EC_{50} by extrapolation with an uncertainty factor of 1000 (TGD, European Commission, 2003). This can be a different biological species for each pharmaceutical. However, for the mixture risk quotient, we have to work with a single species and cannot sum up risk quotients from

different species. To choose the species for the final risk evaluation, PNECs were defined for each species separately, and risk quotients for all single pharmaceuticals calculated and summed up for each species. In the general hospital, RQ_{mix} was 239 for algae, 145 for *Daphnia*, and 48 for fish. In the psychiatric hospital, RQ_{mix} was 114 for algae, 77 for *Daphnia*, and 31 for fish. Hence, for both hospitals, algae produced the highest RQ_{mix} and fish the lowest, with a factor of five between the highest and the lowest RQ_{mix} for the general hospital and a factor of 4 for the psychiatric hospital. Thus in all further evaluations, the effect data for algae were used. The PNEC values reported in Tables 2 and 3 are those for green algae but the results for all biological species are given in the Supporting Information (Tables SI-5 and SI-6).

3.5. Mixture risk quotients in undiluted hospital wastewater

The risk from the mixture of pharmaceuticals RQ_{mix} for scenario 1, i.e., hospital wastewater of the main wing without any dilution in the sewer, was 239 for the general hospital and 114 for the psychiatric hospital (Tables 2 and 3). In the general hospital for 10, 18 and 31 pharmaceuticals, the RQ_{HWW} was above 1, 0.1 and 0.01, respectively (Figure 3A and Table 2), while for the psychiatric center, 9, 26 and 42 pharmaceuticals exceeded an RQ_{HWW} of 1, 0.1 and 0.01, respectively (Figure 3B and Table 3). 31 pharmaceuticals in the general hospital and 42 pharmaceuticals in the psychiatric center made up more than 99 % of the RQ_{mix} (i.e. had a $RQ > 0.01$) but together they constituted only 14% (general hospital) and 30% (psychiatric center) of the PEC_{HWW}. All of those with $RQ_{HWW} > 0.01$ are depicted in Figure 3 and are further discussed below.

3.6. Unexpected "high-risk" pharmaceuticals

Amiodarone, which had the highest ranked risk quotient RQ_{HWW} of 86 in the wastewater of the general hospital (Table 2) is an antiarrhythmic agent with numerous severe side effects. It is used in hospitals for cardiac arrest, serious disrhythmias, and other life-threatening situations (see http://www.drugs.com/amiodarone.html, accessed on 30 Nov 2009). It has been demonstrated that amiodarone disrupts the bacterial cell membrane and decreases bacterial growth (Rosa et al., 2000). Amiodarone, whose reported human side effect is cytotoxicity on thyroid follicular cells, also decreased T4 levels in zebra fish larvae (Raldua and Babin, 2009). No classical experimental ecotoxicity data were available for this pharmaceutical. However, the high experimental $\log K_{ow}$ of 7.8 (Table SI-3) yields a high toxicity prediction despite the

fact that the tertiary amine amiodarone is almost completed protonated and thus positively charged at pH 7.

Ritonavir dominated the RQ_{mix} of the psychiatric hospital with a RQ_{HWW} of 31 (Table 3) despite being only ranked 50th with respect to exposure (Table SI-2). In the general hospital, ritonavir was 3rd (RQ_{HWW} = 53, Table 2) and 60th (PEC_{HWW}; Table SI-1). Ritonavir is an antiretroviral drug to treat HIV infections (see http://www.aidsinfonet.org/fact_sheets/view/442, accessed 30 Nov 2009), which is often administered in a hospital setting. Ritonavir is a very large molecule and its hydrophobicity and ecotoxicity had to be estimated due to lack of experimental data. The high $logK_{ow}$ of 6.27 (Table SI-3 and SI-4) together with its neutral speciation at pH 7 yields an exceptionally low PNEC of 28 ng/L and consequently a high risk quotient (Table 3). Ritonavir is definitively a pharmaceutical warranting further attention and experimental investigations into its environmental risk. A search in ISI Web of Knowledge (http://apps.isiknowledge.com, accessed 21 June 2010) revealed not a single entry for the keywords "ritonavir and (ecotox* or environment*)". This knowledge gap needs to be closed urgently given the high potential environmental risk of ritonavir.

Clotrimazole ranked second for the risk quotient in, both, the general (RQ_{HWW} = 65) and psychiatric hospital (RQ_{HWW} = 28; Tables 2 and 3) despite being ranked only 75th and 67th with respect to exposure (Tables SI-1 and SI-2). Clotrimazole is a widely used over-the-counter antifungal agent. It is very hydrophobic with an experimental $logK_{ow}$ of 6.26 (Table SI-3). As imidazole derivative it has a basic function but the acidity constant pK_a is low enough that at pH 7, the molecule is predominantly neutral. Both physicochemical properties point to very high ecotoxicity, although few experimental data are available. Porsbring et al., (2009) recently demonstrated that clotrimazole has sublethal effects on natural marine microalgal communities (periphyton), altering the chlorophyll content and the cycling of photoprotective xanthophyll pigments already at environmentally relevant concentrations of 500 pM (0.17 µg/L), which is lower than our PEC_{HWW}. Clotrimazole has been found in concentrations of 10 to 100 ng/L in effluents of Swiss wastewater treatment plants (Kahle et al., 2008) and was also detected in UK surface waters (Roberts and Thomas, 2006).

Not unexpectedly, diclofenac ranked third in the psychiatric hospital with a RQ_{HWW} of 22 (Table 3) and also third with respect to exposure (PEC_{HWW} = 73 µg/L, Table SI-2). This reflects that its risk is equally driven by exposure and effect. However, in the general hospital diclofenac ranked much lower (RQ_{HWW} = 0.71, Table 2; exposure: 45th rank, PEC_{HWW} = 2.35

µg/L, Table SI-1). Because of the high exposure, diclofenac is well researched in ecotoxicology (Ferrari et al., 2004, Hallare et al., 2004).

3.7. Comparison of two hospital types

33 Pharmaceuticals were overlapping in the Top-100 set of the general and the psychiatric hospital and 12 of them had a $RQ_{HWW} > 0.01$ in both hospital types. Together they made up 54% (general hospital) and 76% (psychiatric center) of the sum risk quotients. In this overlapping group there were the four commonly in wastewater detected compounds carbamazepine, diclofenac, ibuprofen, and paracetamol. These were also among the highest risk pharmaceuticals for the overall Swiss population including general and hospital use (Lienert et al., 2007b).

Four of these overlapping pharmaceuticals, namely clopidrogrel, clotrimazole, meclozine, and ritonavir were in the lower field of exposure ranking (ranked 50^{th} and higher) but exhibit a high ecotoxicity potential. Ritonavir and clotrimazole stick out with their high log K_{ow} and have risk quotient $RQ_{HWW} > 1$ in both hospitals as described in section 3.6. Meclozine and clopidogrel exhibit $RQ_{HWW} > 1$ in the general hospital. The other common four pharmaceuticals, amoxicillin, oxazepam, tramadol, and pravastatin, have $0.01 < RQ_{HWW} < 1$.

3.8. Effect of biological treatment on risk quotient

The data on elimination of pharmaceuticals during wastewater treatment were collected from various literature sources (Ternes, 2000, Golet et al., 2003, Loffler and Ternes, 2003, Strenn et al., 2003, Joss et al., 2005, Bernhard et al., 2006, Buerge et al., 2006, , Zuehlke et al., 2006, Gobel et al., 2007, Kimura et al., 2007, Mahnik et al., 2007, Maurer et al., 2007, Nakada et al., 2007, Gulkowska et al., 2008, Kahle et al., 2008, Kasprzyk-Hordern et al., 2009, Radjenovic et al., 2009, Watkinson et al., 2007, Wick et al., 2009) and are listed in Tables SI-1 and SI-2 (Supporting Information). This compilation included values from municipal wastewater treatment with activated sludge of a sludge age >3 days where denitrification/nitrification occurs. It does not differentiate between actual degradation and sorption to sludge.

In Figure 3, the risk quotients are plotted for all scenarios including those with elimination during wastewater treatment and dilution in the sewer for all pharmaceuticals with $RQ_{HWW} > 0.01$ and are ranked according to RQ_{HWW}. This analysis is somewhat biased as for 55 of the Top-100 pharmaceuticals in the general hospital and for 66 of the Top-100 pharmaceuticals in

the psychiatric center no literature data for biological elimination in wastewater treatment were available and therefore no elimination was assumed (Tables SI-1 and SI-2). As is evident from Figure 3, dilution in the sewer generally had a larger effect on the decrease of the risk quotient than the actual elimination for most pharmaceuticals.

For the pharmaceuticals with RQ_{HWW} >1, dilution in the sewer decreased the RQ to around or below 1 ($RQ_{WWTPinfluent} \leq 1$). The $RQ_{WWTPeffluent}$ decreased even further for clotrimazole and ritonavir, the Top-2 and Top-3 risk pharmaceuticals for the general hospital, due to high elimination rates in the WWTP. Ibuprofen was the only pharmaceutical in the group of $RQ_{HWW} > 1$ whose risk was reduced due to biological wastewater treatment, yielding a $RQ_{HWWTPeffluent} < 1$. However, for many pharmaceuticals in this group no elimination rates are available.

Dilution in the sewer was more effective than removal by biological treatment. This is also evidenced in the psychiatric center when the fourth highest ranked risk pharmaceuticals (ritonavir, clotrimazole, diclofenac, mefenamic acid) all fell below RQ 1 due to dilution, while biological treatment was beneficial but could not fully compensate for the high ecotoxicity potential (Figure 3B).

A shortcoming of this analysis is that sorption to sewage sludge was not differentiated from actual degradation. Hydrophobic chemicals sorb better to sewage sludge than hydrophilic chemicals. The pharmaceuticals that dominate the RQ_{mix} are all very hydrophobic and can therefore be expected to be eliminated through sorption to sewage sludge. Clotrimazole and Ritonavir are eliminated to > 80% during wastewater treatment (Table SI-1). Unfortunately, for other compounds with a high RQ_{HWW} (e.g. amiodarone) no literature data are available on the elimination during wastewater treatment.

3.10. Effect of urine source separation

The potential effect of urine source separation was also evaluated. Urine source separation is considered beneficial because it reduces the nutrient and micropollutant load of wastewater (Larsen et al., 2009, Lienert and Larsen, 2010). The overall pharmaceutical load is mainly expected in the fraction excreted with urine (e.g. Lienert et al., 2007 a, b). Indeed, our survey confirmed that the pharmaceutical load entering wastewater via feces was much lower than that stemming from urine (Table 4). Exceptions were the laxatives, which are not taken up into the circulation. Additionally, the more hydrophobic compounds tend to be rather eliminated

through feces than urine. In sum, 74% and 47% of the PEC_{HWW} was coming from urine for the general and psychiatric hospital, respectively (Table 4).

However, when the RQ is analyzed, the picture looks different: The contribution of the individual pharmaceuticals to the risk quotient is illustrated in Figure 4, where the RQ in urine and feces, RQ_{HWW}(urine) and RQ_{HWW}(feces) are plotted against the RQ_{HWW}. The dotted line corresponds to one matrix (either urine or feces) dominating the overall risk quotient, all points between the 1:1 line and the bottom points (which indicate no contribution to the RQ) indicate that urine and feces carry a share of the RQ. Despite the higher load of pharmaceuticals in urine in the general hospital, the RQ_{HWW} of the top-risk chemicals was generally dominated by the fraction excreted with feces, while for the low-risk pharmaceuticals urine was also a dominant excretory route (Tables SI-1 and SI-2 and dotted line in Figure 4). The Top-3 pharmaceuticals, amiodarone/diclofenac, clotrimazole, and ritonavir constitute 85% and 71% of RQ_{mix} for the general and psychiatric hospital, respectively, and all are excreted predominantly via feces. For ritonavir, urine also plays a minor role, while for the two others urine is negligible as excretory route. As Figure 4 demonstrates for the example of the general hospital, there is no relationship between the magnitude of RQ_{HWW} and its source of excretion from the human body. The three compounds with the highest risk, which dominate the overall RQ_{HWW}, all show very high excretion via feces. The fourth ranked pharmaceutical progesterone, in contrast, is predominantly excreted via urine.

This analysis clearly demonstrates that urine source separation is a good mean to reduce the overall load of micropollutants, but it does not reduce the high-risk compounds and the risk potential of hospital wastewater. The high-risk compounds are all very hydrophobic, which makes them intrinsically toxic but also causes excretion via feces because hydrophobicity and water (urine) solubility are inversely correlated (Schwarzenbach et al., 2003). Thus, a sorption step as pretreatment of hospital wastewater would potentially be appropriate before release of hospital wastewater into the communal sewer.

4. CONCLUSIONS

Despite limitations of the toxicity estimation model, the results of the present study give a comprehensive picture on the risk posed by hospital wastewater. It allows setting priorities for

1 further experimental testing. Interestingly (but disturbingly), the pharmaceuticals likely to
2 pose the highest environmental risk have rarely been investigated previously. No or very few
3 experimental data are available for the physicochemical properties and/or ecotoxicity of
4 amiodarone, ritonavir, and clotrimazole, the three top-risk compounds in the general hospital.
5 In the psychiatric center, diclofenac was among the three top-risk compounds, together with
6 ritonavir and clotrimazole. Diclofenac is the only one of these pharmaceuticals that is well
7 researched in ecotoxicology and risk assessment.

8 As this analysis has demonstrated, the PNEC is generally the more important driver for the
9 RQ. The reason is that the variability in the PNEC among all pharmaceuticals investigated is
10 more than seven orders of magnitude while the PEC values cover only three to four orders of
11 magnitude among the group of 100 most used pharmaceuticals. This means that if
12 pharmaceuticals are selected only according to their usage pattern and occurrence, one might
13 miss relevant ones that could pose an environmental risk. Therefore, consumption data are less
14 suited to guide prioritization, but often the only available source for compound identification.

15 Thus hazard identification should precede risk assessment to prioritize according to intrinsic
16 hazard properties such as potential for persistence, bioaccumulation, and toxicity (PBT). The
17 regulation for industrial chemicals in Europe, REACH, has exactly taken this step by using a
18 PBT assessment to identify chemicals to be prioritized for further testing and risk assessment
19 (European Parliament and European Council, 2006b). Following this recommendation, the
20 European Medicines Agency's guideline also advises to include PBT assessment in the
21 prescreening phase of risk assessment of pharmaceuticals for pharmaceuticals exceeding a log
22 K_{ow} of 4.5 complementing the exposure estimate as trigger for refined risk assessment
23 (EMEA, 2006).

24

25 **APPENDIX. Supporting information**.

Supporting information related to this article can be found at doi:10.1016/j.watres.2010 xxx.

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TABLES AND FIGURES- CAPTIONS

Fig. 1 – Risk Quotients RQ of the Top-100 pharmaceuticals ranked with decreasing Predicted Environmental Concentration PEC for A. the general hospital and B. the psychiatric center.

Fig. 2 – Range of values in Hospital Wastewater for Predicted Concentration PEC_{HWW}, Predicted No Effect Concentration PNEC, and Risk Quotient RQ_{HWW} in A. the general hospital and B. the psychiatric center.

Fig. 3 – Risk Quotients of the Top-100 pharmaceuticals ranked with decreasing Risk Quotient in Hospital Wastewater RQ_{HWW} for all pharmaceuticals with a RQ >0.01 for A. the general hospital and B. the psychiatric center. The dotted line represents the pharmaceuticals for which one excretion route was dominant.

Fig. 4 – Contribution of urine and feces to the Risk Quotient in Hospital Wastewater, RQ_{HWW} for the general hospital.

Table 1: Rescaled QSARs used to calculate baseline toxicity (Escher et al., 2009). The original QSAR (based on $\log K_{ow}$) were taken from the Technical Guidance Document of the EU (European Commission, 2003).

Table 2: **General Hospital:** Predicted Environmental Concentration in hospital wastewater PEC_{HWW}, Predicted No Effect Concentration PNEC for green algae, and Risk Quotients RQ for all four investigated scenarios[a]. Ranking according to decreasing $\mathbf{RQ_{HWW}}$. Only pharmaceuticals with $RQ_{HWW} > 0.01$ are listed because the contribution of the remainders to the RQ is negligible. In the last row, the summed up risk quotients of the whole mixture of pharmaceuticals $\mathbf{RQ_{mix}}$ are given for all scenarios.

Table 3: **Psychiatric Center:** Predicted Environmental Concentration in hospital wastewater PEC_{HWW}, Predicted No Effect Concentration PNEC for green algae, and risk quotients for all four investigated scenarios. Ranking according to decreasing $\mathbf{RQ_{HWW}}$. Only pharmaceuticals with $RQ_{HWW} > 0.01$ are listed because the contribution of the remainders to the RQ is negligible. In the last row, the summed up risk quotients of the whole mixture of pharmaceuticals $\mathbf{RQ_{mix}}$ are given for all scenarios.

Table 4: Influence of source of Top-100 pharmaceuticals from urine or feces on PEC_{HWW} and RQ_{HWW}.

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Table 1: Rescaled QSARs used to calculate baseline toxicity (Escher et al., 2009). The original QSAR (based on $\log K_{ow}$) were taken from the Technical Guidance Document of the EU (European Commission, 2003).

Baseline toxicity QSAR			
Biological species	**Scientific Name**	**Toxicity endpoint**	**Rescaled QSAR**
Green algae	*Pseudokirchneriella subcapitata*	72-96h EC50	$\log(1/EC50(M)) = 0.95 \cdot \log D_{lipw}(pH\ 7) + 1.53$
Water flea	*Daphnia magna*	48h EC50	$\log(1/EC50(M)) = 0.90 \cdot \log D_{lipw}(pH\ 7) + 1.61$
Fish	*Pimephales promelas*	96h LC50	$\log(1/LC50(M)) = 0.81 \cdot \log D_{lipw}(pH\ 7) + 1.65$

Table 1: **General Hospital:** Predicted Environmental Concentration in hospital wastewater PEC_{HWW}, Predicted No Effect Concentration PNEC for green algae, and Risk Quotients RQ for all four investigated scenarios[a]. Ranking according to decreasing $\mathbf{RQ_{HWW}}$. Only pharmaceuticals with $RQ_{HWW} > 0.01$ are listed because the contribution of the remainders to the RQ is negligible. In the last row, the summed up risk quotients of the whole mixture of pharmaceuticals $\mathbf{RQ_{mix}}$ are given for all scenarios.

	PEC_{HWW} (µg/L)	PNEC (µg/L)	Scenario 1 RQ_{HWW}	Scenario 2 $RQ_{WWTPinfluent}$	Scenario 3 $RQ_{WWTPeffluent}$	Scenario 4 $RQ_{HWWTPeffluent}$
Amiodarone	0.80	0.009	85.7	1.15	1.148	85.7
Clotrimazole	0.90	0.014	64.9	0.87	0.17	13.0
Ritonavir	1	0.028	52.6	0.70	0.106	7.89
Progesterone	15.85	1.4	11.2	0.15	0	0
Meclozine	0.77	0.12	6.29	0.084	0.084	6.29
Atorvastatin	0.99	0.16	6.13	0.082	0.082	6.13
Isoflurane	94	29.8	3.15	0.042	0.042	3.15
Tribenoside	0.79	0.26	3.06	0.041	0.041	3.06
Ibuprofen	11.4	6.6	1.73	0.023	0.001	0.06
Clopidogrel	1.74	1.6	1.09	0.015	0.015	1.09
Amoxicillin	499	625	0.80	0.011	0.001	0.06
Diclofenac	2.35	3.3	0.71	0.0095	0.0063	0.47
4-Methylamino-antipyrine	161.9	961	0.17	0.0023	0.0005	0.04

Flucloxacillin/ Floxacillin	38.9	233	0.17	0.0022	0.0002	0.01
Salicylic acid	17.2	134	0.13	0.0017	0	0
Paracetamol	64	583	0.11	0.0015	0	0
Azithromycin	2.08	19	0.11	0.0014	0.0010	0.07
Thiopental	21.0	201	0.10	0.0014	0.0014	0.10
Oxazepam	1.84	32	0.057	0.0008	0.0007	0.053
Valsartan	1.30	27	0.048	0.0006	0.0001	0.011
Clarithromycin	5.41	122	0.044	0.0006	0.0005	0.035
Rifampicin	0.59	16	0.037	0.0005	0.0005	0.037
Tramadol	1.92	57	0.034	0.0005	0.0004	0.027
Carbamazepine	0.50	18	0.028	0.0004	0.0004	0.028
Tetracaine	0.48	18	0.026	0.0003	0.0003	0.026
Sevelamer	13.7	561	0.024	0.0003	0.0003	0.024
Metoclopramide	3.27	136	0.024	0.0003	0.0003	0.024
Dipyridamole	0.47	21	0.022	0.0003	0.0003	0.022
Pravastatin	1.6	77	0.021	0.0003	0.0001	0.009
Prednisolone	2.1	139	0.015	0.0002	0.0002	0.015
Erythromycin	1.4	132	0.011	0.0001	0.0001	0.008
RQ_{mix}			**239**	**3.2**	**2.4**	**179**

[a]The scenarios are: **1** = Risk potential (RQ) of the wastewater from the hospital main wing before discharge to the sewer (i.e., full RQ of hospital wastewater (HWW) without any degradation or dilution); **2** = reduced RQ of scenario 1 by dilution in sewer (i.e., at influent of WWTP); **3** = reduced RQ of scenario 2 by degradation and sorption process during conventional biological treatment (i.e., at discharge of WWTP); **4** = reduced RQ of scenario 1 by conventional biological treatment in hospital main wing (i.e., in effluent of HWW after on-site treatment).

Table 3: **Psychiatric hospital:** Predicted Environmental Concentration in hospital wastewater PEC_{HWW}, Predicted No Effect Concentration PNEC for green algae, and risk quotients for all four investigated scenarios (see footnote Table 2). Ranking according to decreasing $\mathbf{RQ_{HWW}}$. Only pharmaceuticals with $RQ_{HWW} > 0.01$ are listed because the contribution of the remainders to the RQ is negligible. In the last row, the summed up risk quotients of the whole mixture of pharmaceuticals $\mathbf{RQ_{mix}}$ are given for all scenarios.

	$\mathbf{PEC_{HWW}}$ (µg/L)	PNEC (µg/L)	Scenario 1 $\mathbf{RQ_{HWW}}$	Scenario 2 $\mathbf{RQ_{WWTPinfluent}}$	Scenario 3$\mathbf{RQ_{WWTPeffluent}}$	Scenario 4 $\mathbf{RQ_{HWWTPeffluent}}$
Ritonavir	0.86	0.03	30.8	0.41	0.06	4.62
Clotrimazole	0.39	0.01	28.0	0.37	0.07	5.60
Diclofenac	73.0	3.31	22.1	0.29	0.19	14.6
Mefenamic acid	5.38	0.79	6.77	0.09	0.06	4.33
Lopinavir	0.26	0.05	5.60	0.07	0.07	5.60
Nelfinavir	0.71	0.16	4.47	0.06	0.06	4.47
Ibuprofen	26.3	6.62	3.97	0.05	0.00	0.15
Chlorprothixen	2.53	0.91	2.78	0.04	0.04	2.78
Trimipramine	0.63	0.49	1.28	0.02	0.02	1.28
Quetiapine	7.31	7.98	0.92	0.012	0.012	0.92
Meclozin	0.11	0.12	0.88	0.012	0.012	0.88
Nevirapine	0.98	1.3	0.75	0.010	0.010	0.75
Venlafaxine	24.6	35.5	0.69	0.009	0.009	0.69
Promazine	1.67	2.7	0.62	0.008	0.008	0.62
Efavirenz	0.16	0.3	0.58	0.008	0.008	0.58

Olanzapine	8.41	14.9	0.56	0.008	0.008	0.56
Levomepromazine	1.15	2.4	0.480	0.006	0.006	0.480
Clopidogrel	0.72	1.6	0.452	0.006	0.006	0.452
Methadone	3.75	10.5	0.357	0.005	0.004	0.286
Carbamazepine	5.00	17.7	0.283	0.004	0.004	0.283
Atazanavir	0.14	0.6	0.251	0.003	0.003	0.251
Oxazepam	7.24	32.5	0.223	0.003	0.003	0.207
Hexetidine	0.21	1.0	0.205	0.003	0.003	0.205
Candesartan	0.51	2.9	0.177	0.002	0.002	0.177
Duloxetine	0.38	2.3	0.166	0.002	0.002	0.166
Aripiprazole	0.11	0.7	0.157	0.002	0.002	0.157
Buprenorphine	0.13	1.5	0.089	0.001	0.001	0.089
Benzoylperoxide	0.22	2.5	0.088	0.001	0.001	0.088
Valproate	4.05	51	0.080	0.001	0.001	0.080
Fluoxetine	0.54	6.9	0.078	0.001	0.001	0.052
Lamotrigine	0.65	8.7	0.0750	0.0010	0.0010	0.0750
Clozapine	0.97	16	0.0590	0.0008	0.0008	0.0590
Diazepam	0.48	10	0.0472	0.0006	0.0006	0.0472
Tramadol	2.60	57	0.0456	0.0006	0.0005	0.0362
Pravastatin	3.39	77	0.0441	0.0006	0.0002	0.0181

Trichlorethanol	3.50	86	0.0407	0.0005	0.0005	0.0407
Amoxicillin	22.8	625	0.0366	0.0005	0.0000	0.0026
Doxepin	0.17	4.8	0.0361	0.0005	0.0005	0.0361
Citalopram	0.51	17	0.0310	0.0004	0.0004	0.0310
Paracetamol	9.61	583	0.0165	0.0002	0.0000	0.0000
Pantoprazole	0.72	45	0.0158	0.0002	0.0002	0.0158
Clomethiazole	0.28	23	0.0122	0.0002	0.0002	0.0122
RQ_{mix}			**114**	**1.5**	**0.7**	**52**

Table 4: Influence of source of Top-100 pharmaceuticals from urine or feces on PEC_{HWW} and RQ_{HWW}.

	Sum PEC_{HWW} (µg/L)	**Sum PEC_{HWW} (µg/L) urine**	**Sum PEC_{HWW} (µg/L) feces**	**Sum RQ_{HWW}**	**Sum RQ_{HWW} urine**	**Sum RQ_{HWW} feces**
General hospital	6720	4950	1770	239	28	210
Psychiatric center	364	238	126	114	28	86

Figure 1A

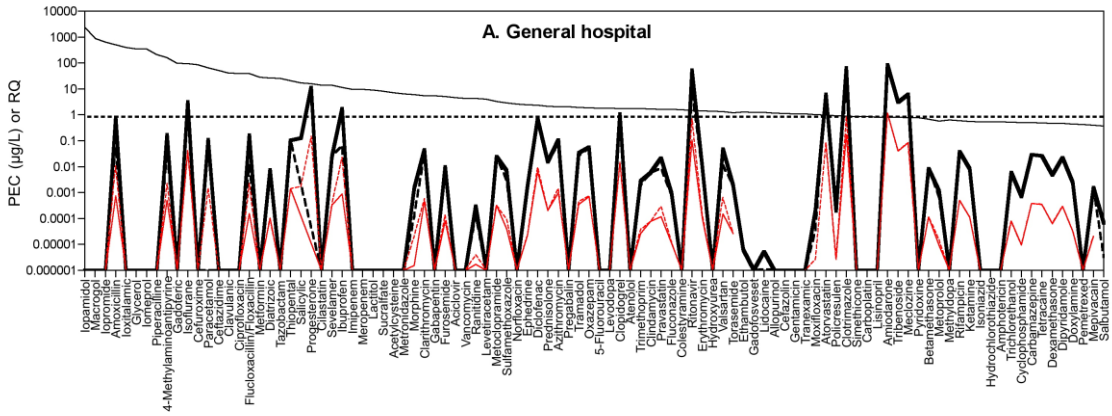


Figure 1B

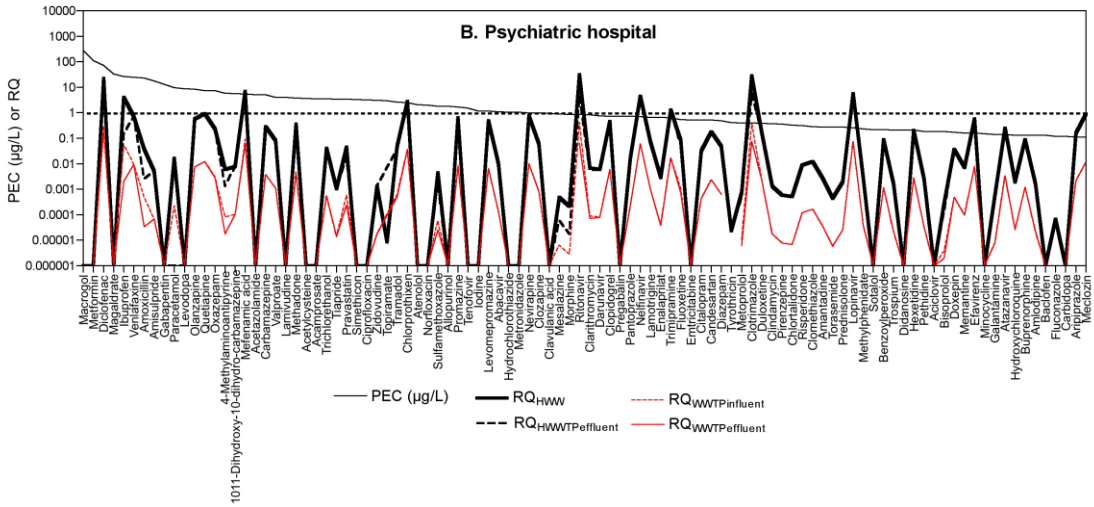
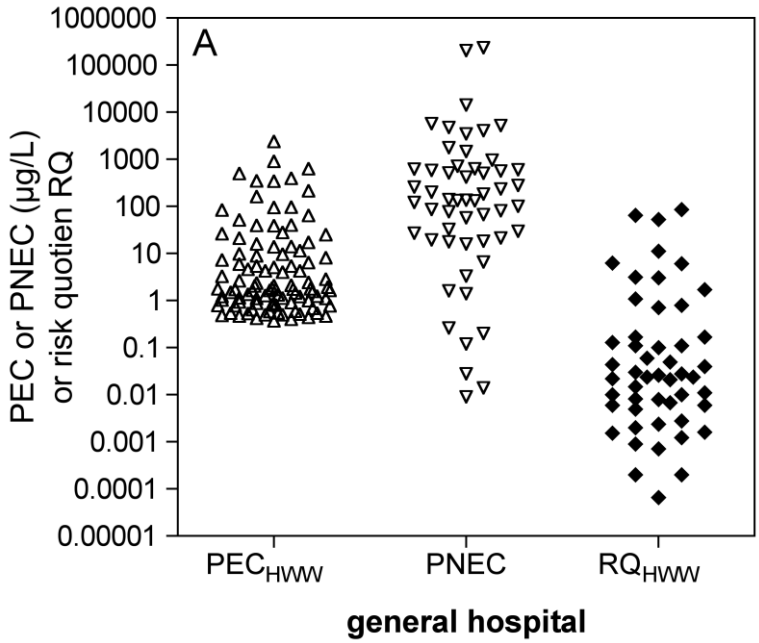


Figure 2A and 2B



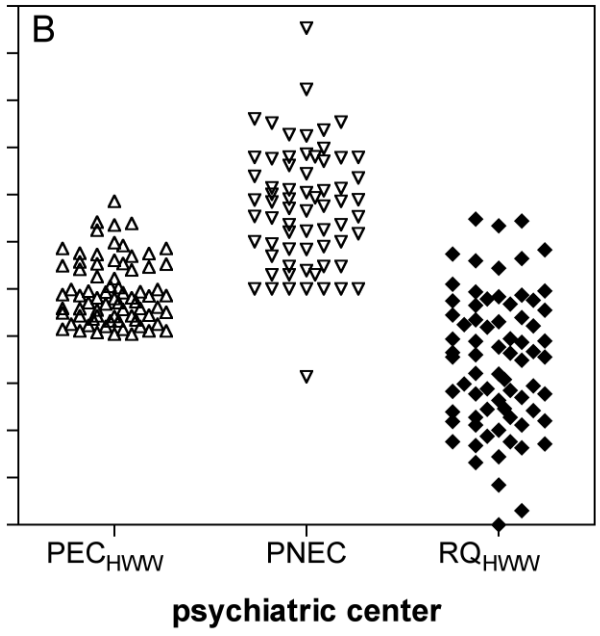


Figure 3A and 3B

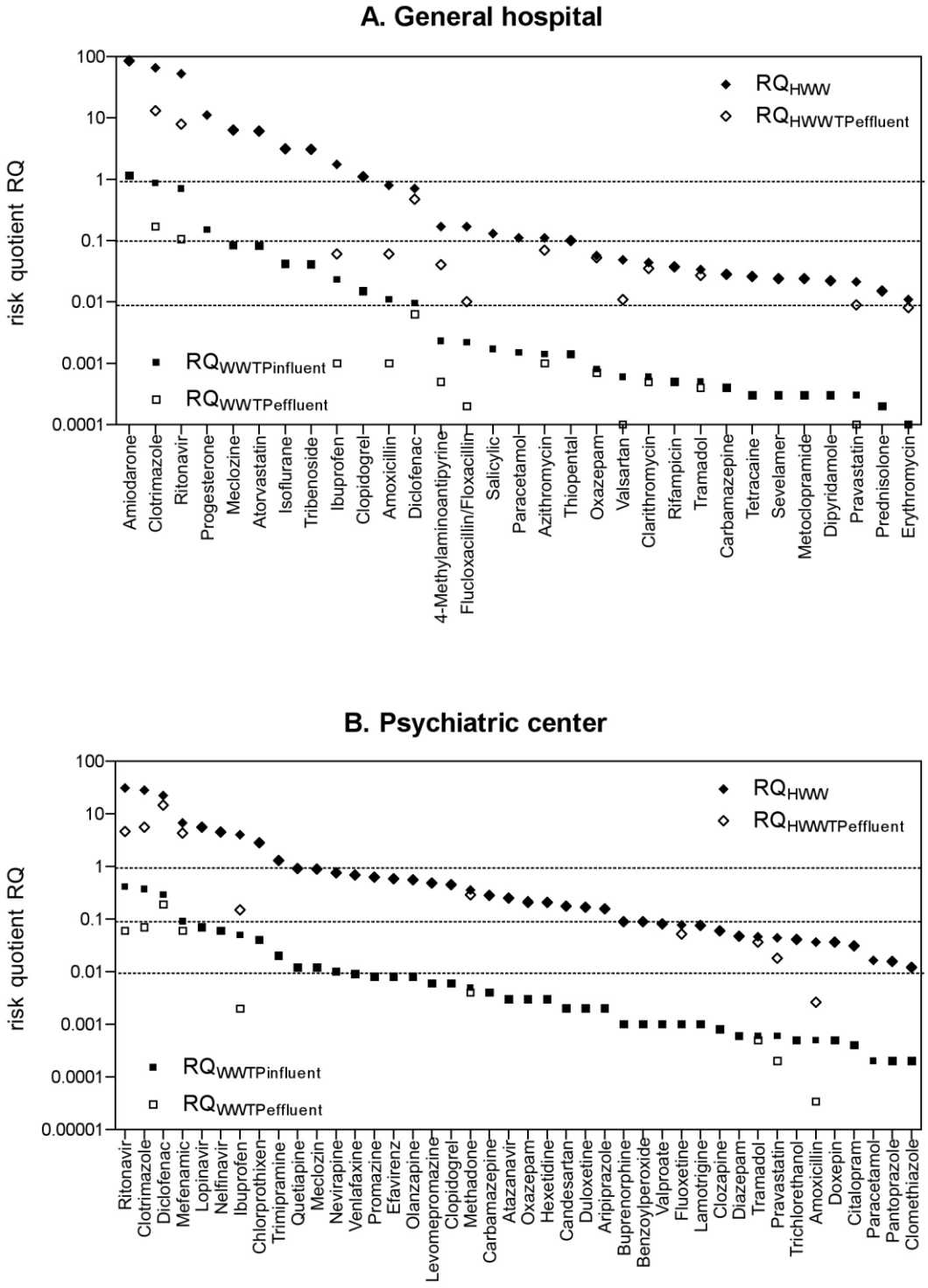
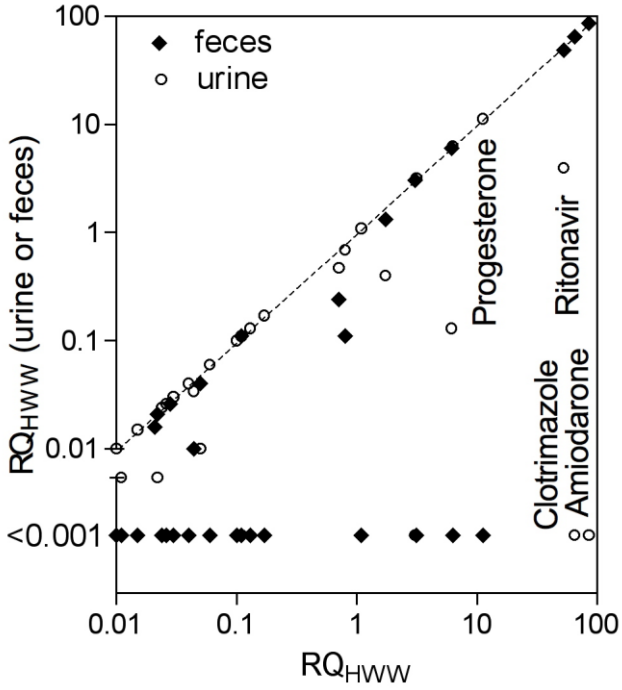


Figure 4


feces
urine
100
10
1
0.1
0.01
<0.001
RQHWW (urine or feces)
0.01
0.1
1
10
100
RQHWW
Progesterone
Ritonavir
Clotrimazole
Amiodarone