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Concentrations of prioritized pharmaceuticals in effluents from 50 large wastewater treatment plants in the US and implications for risk estimation

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ABSTRACT

We measured concentrations of 56 active pharmaceutical ingredients (APIs) in effluent samples from 50 large wastewater treatment plants across the US. Hydrochlorothiazide was found in every sample. Metoprolol, atenolol, and carbamazepine were found in over 90% of the samples. Valsartan had the highest concentration (5300 ng/L), and also had the highest average concentration (1600 ng/L) across all 50 samples. Estimates of potential risks to healthy human adults were greatest for six anti-hypertensive APIs (lisinopril, hydrochlorothiazide, valsartan, atenolol, enalaprilat, and metoprolol), but nevertheless suggest risks of exposure to individual APIs as well as their mixtures are generally very low. Estimates of potential risks to aquatic life were also low for most APIs, but suggest more detailed study of potential ecological impacts from four analytes (sertraline, propranolol, desmethylsertraline, and valsartan).

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1. Introduction

Active pharmaceutical ingredients (APIs) have been frequently detected in surface waters of developed nations (Halling-Sorensen et al., 1998), raising concerns about potential risks to humans and the environment (Daughton and Ternes, 1999). The primary route of APIs into surface waters is believed to be excretion by patients into wastewater collection systems, survival of wastewater treatment, and subsequent introduction into the aquatic environment as a component of the treated wastewater flow (Fent et al., 2006).

Estimating risks from APIs requires characterizing their environmental occurrence, but this is complicated by the number and variety of APIs in common use: over 1000 APIs are approved for use in the US (U. S. Food and Drug Administration, 2009), but most studies examining environmental occurrence only report concentrations of a handful of analytes. Differences in analytical methods and reporting formats have limited the potential of combining individual studies to generate a more complete picture of API occurrence. Furthermore, little or no measured concentration data are available for a number of widely prescribed APIs (Kostich et al., 2010).

In order to efficiently explore potential risks from this broad class of contaminants, our group conducted a preliminary risk assessment of human prescription pharmaceuticals available in the US to identify a manageable subset with the highest estimated potential for environmental impact (Kostich and Lazorchak, 2008). We then developed an analytical method targeting these priority APIs (Batt et al., 2008). Here we report the measured concentrations of 56 APIs and 7 API metabolites in effluent samples from fifty very large (15–660 MGD) wastewater treatment plants (WWTPs) located across the US. We use these results, in combination with a previously described risk assessment approach and summary of published occurrence data (Kostich et al., 2010), to draw tentative conclusions about risks from aquatic exposure for all human prescription pharmaceuticals, including those that have never been surveyed.

2. Materials and methods

2.1. Plant selection

The Clean Watershed Needs Survey (CWNS; U. S. Environmental Protection Agency, 2004) lists the size of the population served and the flow rate for most WWTPs in the US, as reported by plant operators. The survey includes data on 22,795 WWTPs with discharges, including 13,819 WWTPs that discharge into

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surface waters (which does not include ocean discharge). WWTPs listed in CWNS were incorporated into our selection process if they discharged to surface water, served a population greater than 100 people, had at least 75% of their flow originating from municipal (as opposed to industrial or storm water) sources, served a population consisting of at least 75% local residents, and reported per capita wastewater production between 50 and 1000 L per person per day. This process produced a subset of 11,040 WWTPs. The largest (based on daily flow rate) 50 plants meeting the criteria were selected for the present survey. Five of these plants declined to participate. The next five largest plants, ordered by flow-rate, were selected to take their place. In aggregate, the 50 plants we sampled serve over 46 million people and discharge a total of 6.0 billion GPD (22.7 million m^3), or about 17% of all the wastewater produced by WWTPs in the US. These WWTPs are located in 20 out of 50 US States, and 8 out of 10 US Environmental Protection Agency (EPA) Regions (U. S. Environmental Protection Agency, 2013). Regions 1 and 10 did not have WWTPs included in the sample.

2.2. Effluent sample collection

Effluent samples were collected between January 11th and April 5th, 2011. Sample collection containers (1 L, amber glass) were washed in hot water with Alconox, rinsed in hot water, rinsed three times with distilled water, rinsed three times with acetone, and then baked in a heated oven at 250 °C for a minimum of four hours. A 24-h composite sample (500 mL of effluent) was collected by WWTP operators from each WWTP, using their own equipment, and 2 mL of a solution containing 5.0 g/L of Na_2EDTA and 25 mg/L of ascorbic acid was added at the time of collection. The samples were shipped overnight on wet ice, and stored at 4 °C until extraction.

Because of the large number of sampling sites and chemical analytes, it was logistically too difficult and expensive to collect and analyze field blanks as well as duplicates from each location. Field blanks were collected from 20% of the sampling sites, with the field blanks being prepared from laboratory distilled water that was transferred into sampling containers and preserved at the time of collection. Duplicates were collected and analyzed for 10% of the sample sites.

2.3. Sample preparation and analysis

Effluent samples were extracted and analyzed using two previously reported methods (Batt and Aga, 2005; Batt et al., 2008). All samples were extracted within two days of collection and extracts were stored in silanized glass vials at −10 °C until analysis. A laboratory blank consisting of distilled water, a spiked distilled water control sample, and a matrix spike control sample were also included in each extraction batch along with the wastewater effluent samples. Five hundred milliliters of each sample was filtered through a 0.7 μm filter and then spiked with respective isotopically labeled procedural internal standards (at a concentration of 1 μg/L) prior to extraction.

For Method 1 (Batt et al., 2008) analytes (see Supplemental File 1), samples were extracted with 150 mg Oasis HLB MCX cartridges at an unadjusted pH. Acidic and neutral analytes were eluted by acetonitrile and basic analytes were eluted by 95% acetonitrile and 5% ammonium hydroxide into separate silanized glass tubes. The extracts were then concentrated to dryness under a constant flow of nitrogen at 40 °C prior to reconstitution. Reconstituted extracts were transferred to polypropylene vials for immediate liquid chromatography–tandem mass spectrometry (LC–MS/MS) analysis. Extracts were analyzed for 54 APIs using a Waters Aquity ultra performance liquid chromatograph coupled to a Micromass Quattro Micro triple-quadrupole mass spectrometer with an electrospray ionization source operated using multiple reaction monitoring (MRM). Analytes were separated on a BEH C18 column (1.0 × 100 mm 1.7 μm) equipped with 0.2 μm inline filter. Four separate injections were used to cover the range of analytes, in accordance with LC–MS/MS conditions described in Batt et al. (2008).

For Method 2 analytes (Supplemental File 1), a previously reported method (Batt and Aga, 2005) was adapted for the analysis of human and veterinary antibiotics. Sample pH was adjusted to between 2.8 and 3.0 using a dilute solution of hydrochloric acid. Samples were extracted with 200 mg Oasis HLB cartridges and collected in silanized glass vials with a single elution using acetonitrile. The extracts were then concentrated to dryness under a constant flow of nitrogen at 40 °C, and reconstituted in 20% acetonitrile. Reconstituted extracts were then transferred to polypropylene vials for immediate LC–MS/MS analysis. Extracts were analyzed for 14 pharmaceuticals in a single LC–MS/MS analysis with an electrospray ionization source operated in positive ion mode using MRM. Analytes were separated on a BEH Phenyl column (1.0 × 100 mm 1.7 μm) equipped with 0.2 μm inline filter. The LC–MS/MS methodology is described in detail in the supporting information section (Supplemental File 2; see also Batt and Aga, 2005).

Percent recovery for each analyte was calculated in a laboratory fortified distilled water blank and the matrix spike control sample, which were included with each extraction batch for a total of thirteen distilled water and matrix spike samples. Due to the complexity of the sample matrix, the acceptable target recoveries were set between 70% and 130% for compounds with an exact match isotopic standard and 50% and 150% for compounds without an exact match isotopic procedural internal standard. Reported data was not corrected using matrix spike recovery, instead the addition of isotopically labeled procedural internal standards was used to account for sample-to-sample matrix variations. Cimetidine, betamethasone, 2-hydroxyibuprofen, glipizide, and glyburide were excluded from data analysis since, in the vast majority of samples, these analytes failed method quality standards. Any analyte detected in either a field blank or laboratory blank were treated as estimated (flagged with a "B" flag) if the concentration of the analyte in the sample was less than ten times the blank concentration. The average of duplicate concentration measurements from an individual site was used in the reported data analysis.

2.4. Data analysis

Data analysis was performed using R 2.14.2 (R Development Core Team, 2012), using built-in functions and functions from the standard base packages. Effect level parameters of minimum daily dose (DdMin), maximum plasma concentration after a minimum dose (Cmax), fraction bound to plasma proteins (Fb), lowest minimum inhibitory concentration (MIC), and antibiotic breakpoint (BP), as well as the modes of action (MOAs), and predicted environmental concentration (PEC) listed in Supplemental File 1 were adapted from Kostich and Lazorchak (2008); or from Batt et al. (2008).

3. Results and discussion

3.1. Measured concentrations

A summary of occurrence data is presented in Table 1. Detailed plant-by-plant data for each analyte, including quality control flags is provided in Supplemental File 3. Of the 63 analytes measured, 43 were detected at least once. The 20 analytes we did not detect include 14 that were targeted because they appeared in our previous prioritization. That prioritization was driven by marketing data, and did not incorporate estimates of wastewater removal rates since that parameter is uncharacterized for the vast majority of pharmaceuticals. The absence of these analytes in effluent suggests that they are readily degraded within wastewater treatment facilities, diverted into the biosolids waste stream, or their usage rates are overestimated by the marketing data-based model. One API (hydrochlorothiazide, a diuretic used for the treatment of hypertension whose aquatic concentration has rarely been reported) was detected in all 50 effluents examined. In addition, metoprolol (an antihypertensive), atenolol (another antihypertensive), and carbamazepine (an anticonvulsant also used for other neurological and psychiatric conditions) were detected in more than 90% of effluents examined.

Our summaries of concentration data incorporated only data that was not flagged as estimated (see Supplemental File 3). The highest concentration measured for any API was 5300 ng/L (see Table 1) for valsartan (an antihypertensive), which also had the highest average concentration (1600 ng/L) across all 50 samples. The peak concentrations we saw for several analytes (i.e. ibuprofen) were somewhat lower than the highest concentrations reported in some other studies (reviewed in Kostich et al., 2010), but as we describe in the following sections, the conclusions from this study and from our previous summary of literature results (Kostich et al., 2010) are consistent with one another. In part, differences in concentrations reported here and those reported elsewhere in the literature may reflect differences in sampling locations or analytical methodologies. They may also reflect the contrast between our 24-h composites, versus the grab samples used in some other studies. In addition, we only sampled plants once, during the colder months of the year. This may prove advantageous for detecting analytes from pharmaceuticals with higher usage rates during winter months (i.e. antipyretics), and pharmaceuticals which are less efficiently removed during wastewater treatment in winter weather (see, for instance, Nelson et al., 2010). Conversely, it may lead to our study underestimating peak concentrations of pharmaceuticals that are used more in warmer weather (i.e. antihistamines). More detailed studies on the daily and seasonal profiles of effluent concentrations would be helpful for understanding the temporal dynamics of contaminant loading.

Table 1
Concentrations across all 50 effluent samples.

Analyte	CasNumber	Method[a]	RL[b] (ng/L)	Number of measurements	Number of detections	PEC[c] (ng/L)	Mean[d] (ng/L)	Max[d] (ng/L)
10-Hydroxy-amitriptyline	64520-05-4	1	5	50	6	5029	<RL	<RL
Acetaminophen	103-90-2	1	5	50	7	306,955	79 (300)	1500 (4500)
Albuterol	18559-94-9	1	9.7	50	27	471	14	35
Alprazolam	28981-97-7	1	9.1	50	15	103	10	31
Amitriptyline	549-18-8	1	5	50	20	5029	11	110
Amlodipine	111470-99-6	1	5	50	11	94	6.9	18
Amphetamine	51-63-8	1	1.6	50	5	387	3.5	40
Atenolol	29122-68-7	1	6	50	48	4137	940	3000
Atorvastatin	134523-00-5	1	38	48	4	2906	<RL	<RL
Benztropine	86-13-5	1	10	50	0	33	ND	ND
Carbamazepine	298-46-4	1	4.4	50	48	5607	97 (140)	240 (460)
Ciprofloxacin	85721-33-1	2	10	49	30	NA	67 (72)	260 (320)
Clonidine	4205-91-8	1	35	50	0	43	ND	ND
Desmethylsertraline	79902-63-9	1	9.4	50	9	615	9.9 (10)	24
Diltiazem	33286-22-5	1	2.8	49	41	3343	85	340
Diltiazem-desmethyl	130606-60-9	1	1.6	50	34	3343	24	100
Enalapril	76095-16-4	1	1	50	9	369	4.6	38
Enalapril	76095-16-4	2	11	49	13	369	13	32
Enalaprilat	76420-72-9	2	9	49	5	369	14 (18)	150
Florfenicol	73231-34-2	2	60	49	0	NA	ND	ND
Fluocinonide	356-12-7	1	10	50	0	12	ND	ND
Fluoxetine	59333-67-4	1	2.8	48	18	NA	8.7	31
Fluticasone	57-83-0	1	19	50	0	4.2	ND	ND
Furosemide	54-31-9	1	38	50	45	7283	280 (350)	810 (2100)
Gemfibrozil	25812-30-0	1	10	50	38	NA	420 (480)	2300
Hydrochlorothiazide	58-93-5	1	10	50	50	13,947	1100 (1200)	2800
Hydrocodone	143-71-5	1	3.8	50	22	2561	22 (24)	92 (100)
Hydrocortisone	50-23-7	1	25	50	0	2368	ND	ND
Ibuprofen	15687-27-1	1	12	50	23	20,257	460 (690)	4200 (4600)
Lincomycin	859-18-7	2	8	49	0	NA	ND	ND
Lisinopril	83915-83-7	2	45	49	23	814	180 (1700)	3300 (13,000)
Melengestrol acetate	2919-66-6	2	9	49	0	NA	ND	ND
Methylprednisolone	83-43-2	1	25	50	0	250	ND	ND
Metoprolol	56392-17-7	1	14	50	49	1451	410 (450)	660 (1200)
Norethindrone	68-22-4	1	6.9	50	0	111	ND	ND
Norfluoxetine	83891-03-6	1	7.2	46	8	NA	7.7	15
Norverapamil	67814-42-4	1	4.4	48	25	5328	5.8	20
Ofloxacin	82419-36-1	2	10	49	44	NA	160	660
Oxycodone	124-90-3	1	2.5	50	30	NA	53	310
Paroxetine	110429-35-1	1	5	50	0	NA	ND	ND
Prednisolone	50-24-8	1	11	50	0	1421	ND	ND
Prednisone	53-03-2	1	30	50	0	2194	ND	ND
Progesterone	80474-14-2	1	188	50	2	NA	<RL	<RL
Progesterone	80474-14-2	2	9	49	0	NA	ND	ND
Promethazine	58-33-3	1	5	50	0	1668	ND	ND
Propoxyphene	1639-60-7	1	16	48	12	8300	17	34 (46)
Propranolol	318-98-9	1	4.4	50	44	991	33	260
Ranitidine	66357-59-3	1	11	50	19	NA	120	1400
Sertraline	79559-97-0	1	5	50	32	615	21	71
Simvastatin	79902-63-9	1	41	50	12	548	<RL	<RL
Sulfadimethoxine	122-11-2	2	1	49	0	NA	ND	ND
Sulfamethazine	57-68-1	2	10	49	1	NA	12	87
Sulfamethoxazole	723-46-6	1	1.6	50	40	NA	910	2900
Sulfamethoxazole	723-46-6	2	1	49	44	NA	330	1000
Testosterone	58-55-9	1	3.5	50	0	NA	ND	ND
Testosterone	58-55-9	2	1	49	0	NA	ND	ND
Theophylline	58-55-9	1	88	50	4	5696	<RL (88)	<RL (100)
Triamterene	396-01-0	1	1.3	50	35	4504	37	170
Trimethoprim	738-70-5	1	2.5	43	37	NA	170	370
Trimethoprim	738-70-5	2	1	49	40	NA	90	210
Valsartan	396-01-0	1	11	41	40	2628	1600 (1700)	5300 (8200)
Verapamil	137862-53-4	1	2.5	49	39	5328	26	97
Warfarin	81-81-2	1	11	50	0	28	ND	ND

[a] Method employed.
[b] Reporting limit, defined as 3X the EPA MDL (method detection limit) or the lowest calibration point, whichever is greater.
[c] Predicted national average concentration from Kostich and Lazorchak (2008).
[d] Numbers in parentheses include estimated concentrations from samples that failed quantification criteria.

Previously, our group attempted to conservatively estimate annual average concentrations for the entire US (Kostich and Lazorchak, 2008). Subsequent efforts to estimate geographic and seasonal variations in pharmaceutical prescribing practices, together with a review of variations from study to study in peak concentrations reported in the literature (Kostich et al., 2010), led us to suggest a 10-fold 'assessment factor' (uncertainty factor) on predictions of national averages to capture the upper limits of

spatial and temporal variation. Comparing measured concentrations to national average predicted environmental concentrations (PECs) reveals the highest ratio of measured concentrations to predicted concentrations is about five (Table 1), well within our anticipated 10-fold assessment factor.

3.2. Potential toxicity

Although good estimates of no-effect levels of APIs are not typically available for either humans or other taxa, clinical data can offer some guidance on expected potency. Previously (Kostich and Lazorchak, 2008), we used minimum daily therapeutic dose rate (DdMin) as a semi-standardized estimate of effective concentrations for humans (originally proposed in Richardson and Bowron (1985), also suggested by Webb et al. (2003)), and the free plasma concentration after therapeutic dosing (Cmax-free), which was intended as a more conservative semi-standardized estimate of potentially bioactive concentrations intended for estimating potential for effects in non-human taxa. Plasma concentrations have previously been proposed for this purpose in: Lange and Dietrich (2002), Huggett et al. (2003) and Owen et al. (2007). Plasma concentrations have been previously used as a toxicity metric in: Brown et al. (2007), Fick et al. (2010), Mehinto et al. (2010), Cuklev et al. (2011), and Lahti et al. (2011). In this approach, one assumes a very pessimistic pharmacokinetic scenario where APIs readily enter an organism, but the organism lacks the ability to actively rid itself of the API. This conservatively accounts for uncertainties in pharmacokinetic parameters across the many organisms that may be exposed to components from the effluent stream. In this circumstance, responses are entirely determined by pharmacodynamic parameters, which are assumed similar to those in humans. Both DdMin and Cmax-free reference effect levels clearly above a traditional NOEC or LOEC. Instead they correspond to levels inducing a clinically useful (although usually not overtly toxic) physiological effect. This API comparison scheme also has the shortcoming of not discriminating between different endpoints elicited by different APIs (for instance, mixing the often toxic effects of anticancer drugs, with the typically more benign physiological effects of compounds such as antipyretics). In addition, for many APIs, therapeutic dosage rates are not established for pregnant women, small children, those with severe liver or kidney disorders, or those with allergies to the API. Therefore this approach does not extend to these potentially more sensitive subpopulations. Despite these issues, given the absence of traditional NOEC and LOEC estimates for pharmaceuticals, and the fact that DdMin and Cmax-free are typically well established in the course of API regulatory approval for clinical use, we believe these benchmarks may represent the best generally available potency data for prioritizing APIs and estimating the likelihood of eliciting some sort of biological effects. We adapted parameter values for DdMin and Cmax-free (listed in Supplemental File 1) from Kostich and Lazorchak (2008) and Batt et al. (2008). DdMin values were originally derived from prescribing information and represent the minimum daily dose for any approved use in healthy adults.

For all individual APIs we looked at, measured concentrations were consistently well below the DdMin. Lisinopril (an antihypertensive) showed the highest ratio of concentration (3300 ng/L) to DdMin (2.5 mg/day). Assuming someone was drinking two liters per day of water at this concentration, that person would consume slightly less than one minimum daily dose of lisinopril per year. The next highest ratio of concentration to daily dose was seen for hydrochlorothiazide (another antihypertensive), corresponding to one dose every six years. For all other APIs we investigated, the ratio of maximum measured concentration to daily dose equated to a potential dose rate of less than one daily dose equivalent per decade. These results are consistent with an analysis based on data reported elsewhere in the literature (summarized in Kostich et al., 2010), as well as our initial model predictions (Kostich and Lazorchak, 2008). It is worth keeping in mind that all the measurements are of treated wastewater, and people do not drink or typically even come in direct contact with wastewater effluent. Concentrations for most analytes in ambient waters and in finished drinking water are expected to be considerably lower than the effluent concentrations we report here due to in-stream dilution, natural degradation, and drinking water treatment. Therefore, this analysis should be thought of as putting an upper limit on concentrations (and potential risks) that might be encountered in ambient water rather than predicting most likely exposure rates. On the other hand, treatment of wastewater and drinking water can occasionally result in the production of byproducts that are more toxic than the parent. Our analysis does not address this possibility because, for the compounds we measured, there is insufficient information available on what byproducts might be produced and their corresponding toxicity profiles. Generally, our data suggest that, based on comparison between measured concentrations and minimum therapeutic dosage rates, risks to healthy human adults from wastewater derived APIs appearing in drinking water are very low.

For most analytes we looked at, peak concentrations were also well below the Cmax-free, with only 4 analytes having maximum concentrations above one tenth of the Cmax-free. The ratio of maximum measured effluent concentration to Cmax-free was 0.71 for the antidepressant sertraline, 0.65 for the anti-hypertensive ingredient propranolol, 0.24 for the sertraline metabolite desmethyl-sertraline, and 0.18 for the anti-hypertensive valsartan, suggesting the effluent concentrations of these analytes are close to plasma concentrations which are known to cause readily measurable responses in patients and lab animals. Assuming the validity of a concentration addition model (Loewe and Muischnek, 1926) within modes of action for mixtures of analytes, this suggests hazard ratios of about 1 for both anti-hypertensives and for antidepressants, further emphasizing the potential for physiological effects. The connection between this simple mechanistic model for predicting toxicity and actual real-world toxicological responses is not completely established, but the limited available data suggests, for instance, that the plasma concentration of propranolol in fish continuously exposed to propranolol in the water at a variety of concentrations reaches steady state concentrations similar to what is in the water (Owen et al., 2007). Similar results have been obtained for other APIs (Lahti et al., 2011). Other work has demonstrated that at least some APIs induce measurable changes in fish gene expression when present in fish plasma at concentrations similar to the human therapeutic plasma concentration (Cuklev et al., 2011). Together these results corroborate the plausibility of this model for initial conservative screening for potential risks from APIs where more detailed concentration response data are lacking. Combined with the measurement data presented here and elsewhere, these results suggest closer examination of risks to fish and other aquatic life are justified for a handful of APIs.

3.3. Contributions to antibiotic resistance

In addition to direct toxicological risks, concern has been raised about the potential for antibiotic residues in wastewater giving rise to antibiotic resistant human pathogens (Webb et al., 2003). Microbial sensitivity to antibiotics is typically expressed as the minimum inhibitory concentration (MIC) of the antibiotic, which is the lowest concentration of antibiotic, in a standard in vitro test system, causing reliable inhibition of microbial growth. Clinically significant antibiotic resistance is defined in terms of the concentrations of antibiotic that can be safely maintained in a target tissue in a

patient without causing excessive adverse side-effects. This concentration is termed a 'breakpoint' concentration (BP). Microbes whose MIC is greater than the BP for a given antibiotic are considered to have clinically significant resistance to the antibiotic in question. One way to estimate the selective pressure for development of clinically significant antibiotic resistance is comparison of MECs to the MIC and BP (Webb et al., 2003; Kostich and Lazorchak, 2008). The highest MEC to BP ratio we observed was 0.0003, for the antibiotic ofloxacin (maximum MEC = 660 ng/L, BP = 2 μg/mL, or 2 million ng/L), suggesting no real risk of direct selection of clinically significant resistance. On the other hand, the highest MEC to MIC ratio, 0.66 was also for ofloxacin (MIC = 0.001 μg/mL), and the second highest ratio (0.26) was for ciprofloxacin (MIC = 0.001 μg/mL). Because these ratios are close to one, they suggest the possibility for growth inhibition of some naturally occurring (and potentially beneficial) bacteria, and perhaps for initial acquisition of low level antibiotic resistance by exposed pathogens, particularly if assuming a concentration addition model for mixtures of antibiotics with common modes of action. Such low level antibiotic resistance would not be directly clinically relevant, but it may facilitate faster development of clinically significant resistance when further selection with higher concentrations of antibiotics is applied, for instance in a treated patient.

3.4. Implications for risk assessment

In principle, if our prioritization is perfect, our sampling representative, and our measurements exact, this work would allow us to put upper limits on the hazard posed to humans and aquatic life by any API, not just the ones measured in the present study. This follows from the fact that we prioritized the analyte list based on potential risk, so the APIs we did not measure should present lower risks than the ones we did measure. Furthermore, our measurements suggest the maximum locally measured concentrations of APIs do not exceed predicted national average concentrations by more than a 10-fold assessment factor. Assuming the same assessment factor is applicable to the many pharmaceuticals that have never been measured in the aquatic environment, it should be possible to put a ceiling on potential risks for any API. For instance, the highest priority pharmaceutical we did not measure was doxepin. Based on marketing data, we estimated that no more than 4,333,023,418 daily dose equivalents of doxepin are dispensed in the US each year (Kostich and Lazorchak, 2008). After multiplying by our proposed 10-fold assessment factor, this would result in highest possible local concentrations corresponding to a worst-case potential human exposure rate of 4.4 daily doses per decade for this API, and lower daily dose equivalents for the remaining thousand or so lower priority APIs.

Our prioritization was based on market data and wastewater production data which are both incomplete and of uncharacterized accuracy. Nevertheless, measurement data presented here, as well as published by other groups (summarized in Kostich et al., 2010), generally corroborate our model predictions. Although our sampling is not perfectly representative of all US wastewater, it comes close to representing the widest swath of wastewater possible with 50 samples. Nevertheless, it remains possible that concentration profiles at smaller WWTPs (including household septic systems) may be different than the large facilities we sampled.

We expect the greatest weakness of our approach to stem from the sparseness of available dose response data for non-human taxa. Although the use of Cmax-free as a surrogate for non-human dose response has some experimental support, more work will be required to test its broader applicability. In addition, comprehensive risk assessment requires further measurements on biosolids, sediments, and biota, including human food sources. Also, our work only looked at human use of pharmaceuticals as a source of APIs. Additional characterization of agricultural and industrial sources of APIs is needed for a comprehensive risk estimate.

4. Conclusions

Based on the data presented, risks of direct toxicity to humans, particularly healthy adults, from APIs released into the aquatic environment appear low. Residual risks to susceptible human subpopulations are hard to evaluate without effect level data for these groups, which is typically not available. Risks to aquatic life are still a significant concern for a handful of APIs, but further work will be required to explore this possibility. Risks of direct selection for clinically significant antibiotic resistance appear low, but antibiotic concentrations may inhibit the growth of some naturally occurring beneficial microbes, and may facilitate early steps in the acquisition of clinically significant resistance. These conclusions can be tentatively extended to all prescription pharmaceuticals in current use. Our conclusions are limited to potential exposure through the water column. Additional work will be required to evaluate exposure routes involving biosolids, sediments, exposure within food webs, and agricultural as well as industrial sources of pharmaceutical residues.

Author contributions

MK initiated the project, organized the work, designed the sampling regime, analyzed the finished measurement data, and coordinated manuscript preparation. All three authors cooperated in developing the experimental design and writing the manuscript. AB managed sample extraction, and performed chemical analysis, including method adaptation, instrument runs, as well as analysis of LC–MS/MS data. JL negotiated, coordinated, and participated in sample collection with EPA Regional personnel, State environmental managers, and WWTP operators. JL also managed shipping and custody chains of samples until sample extraction.

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Appendix A. Supplementary data

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