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Risks to aquatic organisms posed by human pharmaceutical use

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ABSTRACT

In order to help prioritize future research efforts within the US, risks associated with exposure to human prescription pharmaceutical residues in wastewater were estimated from marketing and pharmacological data. Masses of 371 active pharmaceutical ingredients (APIs) dispensed in the US in 2004 were estimated from marketing data, and then divided by therapeutic dose rate to normalize for potency. Metabolic inactivation of the 50 most dispensed APIs was estimated from published data, and active metabolites were tabulated. Comparing maximum likely average wastewater concentrations of API-associated activity to exposure rates that produce therapeutic effects in humans suggests that the threat to healthy human adults from aquatic exposure is low, even when likely mixture effects are considered. Comparing predicted wastewater concentrations to human therapeutic plasma concentrations suggests that some APIs may be present at sufficient concentrations to affect organisms which eliminate them inefficiently. Comparing predicted antimicrobial concentrations to published minimum inhibitory concentrations suggests that antibacterial APIs in wastewater, but probably not antifungal APIs, may select for low-level antimicrobial resistance. The taxonomic distribution of molecular targets of the 50 most dispensed APIs suggests that potential effects of some APIs are likely restricted to vertebrates, while other APIs can probably affect many eukaryotic and prokaryotic clades.

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1. Introduction

Dozens of active pharmaceutical ingredients (APIs) have been detected at low concentrations in a variety of environmental media across the globe (Richardson and Bowron, 1985; Fent et al., 2006). Human pharmaceutical APIs enter the environment primarily after excretion from patients into wastewater (Fent et al., 2006). Lesser routes include disposal of unused medicine, and release from the manufacturing process. In addition, animal excretion may account for much of the environmental introduction of drugs used in agriculture.

For a few APIs, laboratory exposures to environmentally-relevant concentrations have produced effects in non-human species resembling pathology observed in wild populations of similar species. For instance, laboratory exposure to the contraceptive ethinyl estradiol can impair sexual development and reproduction in fish at concentrations frequently detected in wastewater (Mills and Chichester, 2005). These effects resemble unexplained feminization of fish noted downstream of some wastewater treatment facilities (Jobling et al., 2006), leading to questions about human and non-human health effects resulting from aquatic exposure to residual levels of other APIs (Daughton and Ternes, 1999).

Unfortunately, analysis of occurrence and toxicity of all APIs is impractical. Occurrence studies are complicated by the large number of APIs, many of which are biologically degraded

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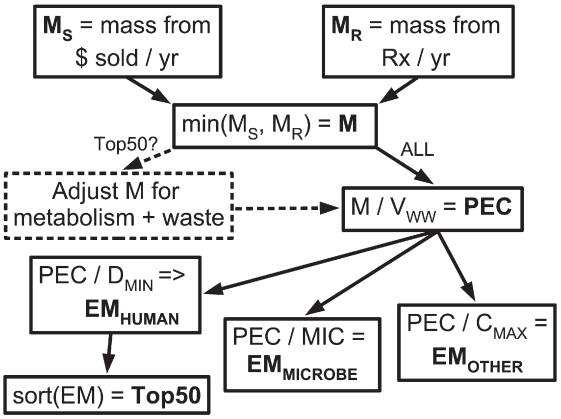

Fig. 1 – Exposure multiples for one API. M_S is the annually dispensed mass of API conservatively estimated from US sales data. M_R is the mass of API conservatively estimated from prescription data. The minimum (M) of M_S and M_R is used in subsequent steps. The aquatic PEC is estimated by dividing M by the volume of US annual wastewater production (V_{WW}). The human exposure multiple (EM_{HUMAN}) is proportional to the PEC divided by the minimum daily therapeutic dose (D_{MIN}). The microbial exposure multiple ($EM_{MICROBE}$) is expressed as the PEC divided by the minimum inhibitory concentration (MIC). The exposure multiple for other organisms (EM_{OTHER}) is expressed as the ratio of the PEC to the freely dissolved plasma concentration (C_{MAX}) in humans after therapeutic dosing. Dotted lines and boxes represent a second calculation for the top 50 APIs (selected by sorting human exposure multiples) that accounts for metabolic inactivation and disposal. The second calculation is only used for exposure multiples represented in Tables 1 and 4.

to active metabolites that should also be accounted for. Current analytical tools can only quantify several dozen analytes in a single run, and availability of suitable standards, especially drug metabolites, is limited. Evaluating API ecotoxicology is even more challenging due to uncertainties about appropriate dosages, durations of exposure, range of sensitive taxa, sensitivity of developmental stages, and toxicological endpoints.

Fortunately, APIs are well studied in several useful respects. Some marketing data are publicly available, allowing estimation of the mass of APIs dispensed (Richardson and Bowron, 1985; Stuer-Lauridsen et al., 2000). Metabolic API transformation into active or inactive metabolites is well studied in humans, facilitating more logical predictions of wastewater API levels and lists of analytes for monitoring (Richardson and Bowron, 1985; Stuer-Lauridsen et al., 2000). Human therapeutic dosage rates (Richardson and Bowron, 1985; Webb et al., 2003) and microbial growth inhibitory concentrations (Webb et al., 2003) have been used to estimate 'no effect' concentrations for risk assessments. Comparing human therapeutic plasma concentrations to aquatic concentrations may be useful for estimating 'no effect' concentrations for non-human exposures (Lange and Dietrich, 2002; Huggett et al., 2003). In addition, the human mode of action of APIs may provide information to guide toxicity endpoint selection, to help determine the range of species potentially vulnerable to API exposure, and to allow estimation of mixture effects (Lange and Dietrich, 2002; Huggett et al., 2003; Cleuvers, 2005; Fent et al., 2006). Although marketing and pharmacological data may not support definitive risk assessment, they may suggest narrowing the scope of research by identifying likely problems and significant data gaps.

In order to prioritize research in support of a definitive risk assessment, aquatic risks posed by human prescription pharmaceutical use in the US were conservatively estimated from available marketing and pharmacological data. Annual use of the 371 most dispensed APIs was estimated from sales data. API amounts were normalized to reflect human therapeutic potency, and then ranked to identify APIs of greatest concern. Identities and physiological activities of known metabolites of the top 50 APIs were identified from published studies. Pharmacological information was compared to predicted wastewater concentrations in order to express exposure multiples for human health, for non-human effects, and for microbial growth inhibition. All 371 APIs were classified based on mode of action, and exposure multiples were estimated for each API class using a potency-normalized concentration addition model. Conservation of human molecular targets across the taxa was examined in order to estimate the range of potentially sensitive species.

2. Materials and methods

2.1. *Amount dispensed per year*

US human prescription drug sales in 2004 were used to estimate the amount of individual APIs dispensed annually (see Fig. 1). Available data sets listed either total dollar value of product sold per year or number of prescriptions written per year for top selling products.

The following dollar sales data were downloaded from the world wide web October 27, 2005:

"Top 200 generic drugs by retail dollars in 2004": <http://www.drugtopics.com/drugtopics/data/articlestandard/drugtopics/112005/150656/article.pdf>
"Top 200 brand-name drugs by retail dollars in 2004": <http://www.drugtopics.com/drugtopics/data/articlestandard/drugtopics/112005/150644/article.pdf>
"The top 200 prescriptions for 2004 by U.S. sales": <http://www.rxlist.com/top200_sales_2004.htm>
"Leading 20 products by U.S. sales, 2004": <http://www.imshealth.com/ims/portal/front/articleC/0,2777,6599_49695983_69890133,00.html>

The following prescription volume data were downloaded from the world wide web October 11, 2005:

"Top 200 generic drugs by units in 2004": <http://www.drugtopics.com/drugtopics/data/articlestandard/drugtopics/102005/150069/article.pdf>
"Top 200 brand-name drugs by units in 2004": <http://www.drugtopics.com/drugtopics/data/articlestandard/drugtopics/102005/150068/article.pdf>

"The top 300 prescriptions for 2004 by number of US prescriptions dispensed": <http://www.rxlist.com/top200.htm>
"Leading 20 products by total U.S. dispensed prescriptions, 2004": <http://www.imshealth.com/ims/portal/front/articleC/0,2777,6599_49695974_68913594,00.html>

Annotation of the most comprehensive dollar value data set (drugtopics.com) indicated this data encompassed about 84% ($144,851,131,000) of the value of the US human prescription drug market in 2004, including brand-name products with $126,427,000 or more in annual sales, and generic drugs with $26,646,000 or more in sales. The most comprehensive prescription count data set (drugtopics.com) encompassed about 85% (2,651,398,000) of US prescriptions, including brand-name products prescribed 1,355,000 or more times per year, and generic products prescribed 1,124,000 or more times per year. The highest encountered figure found for a product was used in subsequent calculations. Vitamins, mineral supplements, minerals, and electrolyte replacements were excluded from consideration.

The dollar value of each product was conservatively converted into amount of API by dividing dollars sold by the lowest available price per unit (milligrams or international units) of API for that product:

(units per year) = (dollars sold per year)/(minimum dollars per unit API).

Lowest prices were identified from the 'Red Book, 2004' (Thompson and Micromedix, 2004). Because published prices may overestimate prices paid (US GAO, 2005) minimum prices were often adjusted downward. If a Federal Upper Limit (FUL) price or Health Care Financing Administration (HCFA) price was listed, then the lowest of FUL, HCFA, Direct Price (DP), and Average Wholesale Price (AWP) was used. Otherwise, if the same combination of APIs was available in a competing product, then the lower of DP or 75% of AWP price was used. Otherwise the lower of 88% of AWP or 108% of DP price was used.

The number of prescriptions was conservatively converted to amount of API by multiplying the number of prescriptions by the maximum suggested daily dose of API, then multiplying by the maximum number of days of therapy typically covered by a prescription for that type of product:

(units per year) = (scripts per year)*(max units per day)*(max days per script).

Daily dose was determined from manufacturer prescribing information. Maximum days of therapy per prescription were assumed to be: analgesic—30, antimicrobial—14, cytotoxic—30, general anesthetic—1, opioid—30, sedative—30, vaccine—1, and other—90.

Several APIs represented by a product listed in the available marketing data were also contained in products not dispensed sufficiently to be listed. Unlisted products were identified in the FDA list of approved drug products (downloaded September 16, 2006 from <http://www.fda.gov/cder/ob/docs/preface/eclink.htm>). For unlisted brand-name products, it was assumed that 1,355,000 prescriptions were written per year, and $126 million worth was sold. For unlisted generic products, it was assumed that 1,124,000 prescriptions were written per year, and $26 million worth was sold. These conservative figures correspond to figures for the least dispensed products listed in the market data.

The lesser estimate of maximum amount of API dispensed per year based either on dollars sold or prescriptions dispensed, was used in subsequent calculations. This amount was normalized for potency by dividing by the API's minimum daily therapeutic dose for a healthy adult. A ranking of the number of minimum daily dose equivalents dispensed per year was used to select the 50 top APIs for analysis accounting for metabolic inactivation.

2.2. *Predicted concentrations*

Metabolic inactivation of the top 50 APIs was estimated from published human studies. It was assumed that conjugation to glucuronide or sulfate was reversible and did not constitute inactivation. Wastewater introduction was assumed to consist of excretion of some portion of administered doses (the portion not metabolically inactivated), and disposal of unused medicine. For medicines prescribed for short-term therapy, 15% of the dispensed amount was assumed wasted. For medicines prescribed for long-term therapy, 5% was assumed wasted. For topical medicines, 33% was assumed wasted. Although knowledge about disposal rates is inexact (Bound and Voulvoulis, 2005), our estimates are relatively conservative (Daughton, 2003). We assumed a higher rate for topical medications because often a substantial portion is not absorbed (Guy et al., 1989), but can instead be washed off, or remains in a patch after use and disposal. Accounting for disposal is particularly important where metabolic inactivation is nearly complete, since considering inactivation, but not wash-off and disposal, can underestimate the amount of API reaching the environment. Metabolic inactivation and disposal rates were not used for mixture effect calculations or for microbial exposure multiple calculations.

The amount of API activity reaching the environment was estimated by:

$$(\text{API activity}) = (M^{*}(1 - Fi)^{*}(1 - Fw)) + (M^{*}Fw), \text{where}$$

M	mass dispensed
Fi	fraction inactivated
Fw	fraction wasted

Figures for US public wastewater treatment in 1996 (Clean Water Needs Survey, downloaded December 10, 2006 from: <http://www.epa.gov/owm/mtb/cwns/1996rtc/toc.htm>), biosolids production (US EPA, 1999), and population estimates for the intervening period (Campbell, 1996) were used to estimate wastewater and biosolids production in 2004. It was assumed that per capita waste production remained constant and that statistics for private wastewater treatment are identical to those for public facilities. This resulted in US annual wastewater production of 6.8×10^{13} L/year and biosolids production of 1.0×10^{10} kg/year.

Predicted environmental concentrations (PEC) in wastewater were estimated by:

(API activity introduced annually)/(annual wastewater volume).

PECs for biosolids were estimated by:

(API activity introduced annually)/(annual biosolids volume).

Wastewater PEC calculations not taking account of inactivation were estimated for all 371 APIs:

PEC = (minimum daily dose equivalents per year)/(annual wastewater volume).

2.3. *Exposure multiples*

Exposure multiples for non-human exposure to each of the top 50 APIs and their metabolites were expressed as the ratio between the wastewater PEC and human peak freely dissolved plasma concentration after administration of a minimum therapeutic dose (Cmaxf):

$$Cmaxf = Cmax/(1 - Fb)$$

Cmax peak plasma concentration after dosing (see Appendix B).
Fb fraction of API bound to plasma proteins (see Appendix B).

Hazard quotients for microbial exposure were expressed as the ratio of the wastewater PEC to the lowest minimum inhibitory concentration (MIC) found for that API. MICs were identified from published ranges (Andrews, 2001), prescribing information, and literature searches.

2.4. *Mixture effects*

Net effects of mixtures of all 371 APIs were estimated using a potency-normalized concentration addition model of interaction between APIs with common modes of action (MOA). APIs were placed into narrowly and broadly defined MOA classes, based on MOA descriptions in prescribing information. The classes were based on the World Health Organization's Anatomical Therapeutic Chemical (ATC) classification system (<http://www.whocc.no/atcddd/indexdatabase/>), but modified to emphasize physiological MOA. Cumulative exposure to all APIs belonging to a MOA category was expressed as the number of days of water consumption required to ingest the equivalent of a single minimum daily therapeutic dose. Exposure was calculated by dividing annual US wastewater volume by the sum of the minimum daily dose equivalents dispensed per year for all APIs in that MOA category, then dividing by 2 L per day (assumed water consumption rate). Effects of antimicrobial mixtures were estimated by adding PEC/MIC ratios for all antimicrobial APIs in that class (either antibacterial or antifungal).

2.5. *Taxa of concern*

Molecular drug targets for the top 50 APIs were identified from published studies. Human sequences of the target proteins were retrieved from NCBI's RefSeq sequence database, release 7 (Pruitt et al., 2005) and used as 'bait' for BLAST (blastall version 2.2.14) (Altschul et al., 1990) sequence similarity searches of GenBank Release 153.0 (Benson et al., 2006). Sequence complexity filtering was turned off, the number of returned alignments was set at 100,000, and minimum expectancy value was set at 1e−5. Hits were re-scored by identifying the 100 residues with the greatest identity in the BLAST amino acid alignment between the hit and the bait. Percent identity in this window (win100 score) was used to rank hit quality.

Source species was identified from each BLAST hit's GenBank record. Based on NCBI's taxonomic hierarchy (Wheeler et al., 2000), 34 taxonomic tree branch points were used as bins to simplify representation of taxonomic distribution.

3. Results

3.1. *Predicted wastewater concentrations*

Available API marketing data included 419 high-volume products, containing 371 distinct APIs. Another 1009 lower-volume human prescription products containing these APIs were identified from a list of FDA approved products. Adding estimates of API amounts dispensed in low-volume products yielded final upper bound estimates (Appendix A) ranging from 143,845,200 kg/year for lactulose to 0.07 kg/year for paricalcitol.

The fraction of activity remaining in human excreta after dosing was estimated from published data for the top 50 APIs, and ranged from 0% for drugs such as insulin, to 100% for drugs such as lisinopril. Degree of inactivation was typically not reported in the literature precisely, so we listed the fraction as a range (Appendix B), and used the lower-end estimate in subsequent calculations. After adding an estimate of unused medicine disposal to the predicted contribution from human excretion, wastewater PECs were calculated assuming complete partitioning of raw wastewater API residues into the aqueous phase. As a supplemental calculation, biosolid PECs were estimated assuming complete partitioning of raw wastewater API residues into biosolids. This led to a 6800-fold higher estimate of API activity in biosolids than in wastewater, reflecting the ratio of wastewater volume to biosolids volume. For the top 50 APIs, the upper bound of wastewater PECs (Appendix C) ranged from 0.15 ng/L for liothyronine, to 306,955 ng/L for acetaminophen.

3.2. *Human exposure rates*

Human exposure rates to API-derived activity potentially in wastewater were expressed as the number of days of water consumption (assuming the upper bound wastewater PEC) required to ingest pharmacological activity equivalent to one minimum therapeutic human daily dose (Table 1 and Appendix C). More than 250 days would be needed for levothyroxine, the API with the greatest potential concentration of activity. More than 500 days would be required for all but two of the rest of the top 50 APIs.

Similar exposure multiples were used to express potential aquatic exposure to mixtures of APIs with common physiological modes of action (MOA). All 371 APIs were placed into narrowly and broadly defined MOA classes (Appendix A). A potency-normalized concentration addition model suggests

Table 1 – Human exposure rates to single agents

Active ingredient	PEC (ppt)	Days/dose
Levothyroxine	19	260
Estradiol	617	406
Hydrochlorothiazide	13,947	448
Hydrocodone	2561	976
Prednisone	2194	1140
Betamethasone	93	1349
Furosemide	7283	1373
Fluticasone	4.2	1471
Lisinopril	814	1536
Atorvastatin	2906	1721

Maximum likely average wastewater concentrations of activity (PECs, in parts per trillion equivalents of parent API) and human aquatic exposure rates estimated for the top 50 APIs, taking into account metabolic inactivation. Exposure rates are expressed as the number of days of water consumption required to ingest the equivalent of one minimum daily therapeutic dose of API. The list was sorted by days per daily dose. Results for the top 10 APIs are shown.

more than 100 days of water consumption would be required to ingest one daily dose equivalent of the narrow MOA class with the greatest aggregate activity (Table 2), and more than 500 days would be needed for all but three other narrow MOA classes. More than 100 days would be required for the broad MOA class with greatest activity (Table 3), and more than 500 days would needed for all but four other broad MOA classes.

3.3. *Non-human exposure rates*

Exposure multiples for non-human organisms were expressed as the ratio of maximum likely average wastewater concentration, and the maximal freely dissolved concentration of API in human plasma after administration of a minimum therapeutic dose (an estimate of cellular potency). This ratio was above one for eleven out of the top 50 APIs (Table 4 and Appendix C).

An exposure multiple for microbial growth inhibition was expressed as the ratio of the wastewater PEC to the lowest minimum inhibitory concentration (MIC) found for an API. This ratio was below 0.04 for each of the eight antifungal drugs in the list of 371 APIs (Table 5). The multiple for the mixture of all eight antifungals was below 0.08. The ratio was above one for four of 30 individual antibacterial drugs (Table 6) in the list of 371 APIs. The multiple for the mixture of all 30 antibacterial APIs was about 21.

3.4. *Potentially sensitive taxa*

The distribution of taxa potentially sensitive to the top 50 APIs was estimated based on sequence conservation of API target

Table 2 – Human exposure to narrow mode of action (MOA) classes

API sub-class	API count	Days/dose
Thyroid hormone	2	119
Corticosteroid	13	168
Estrogen	3	191
Diuretic	9	285
Opioid	11	603
Beta-1-blocker (adrenergic)	3	690
Statin	6	719
Ace inhibitor	11	729
Nsaid	10	876
Benzodiazepine	10	1354

All 371 APIs were assigned to one of 126 narrowly defined classes describing therapeutic MOA. PECs were estimated for all the APIs, without taking account of metabolic inactivation. A potency-normalized concentration addition model was used to estimate effects of a mixture composed of all APIs belonging to a particular MOA class. The number of APIs in each class, and the number of days of water consumption (assuming the wastewater PEC) required to ingest the equivalent of one minimum therapeutic daily dose, for each of the top 10 MOA classes are shown.

Table 3 – Human exposure to broad mode of action (MOA) classes

API super-class	API count	Days/dose
Thyroid hormone modulator	3	119
Neurotransmitter modulator	105	130
Anti-inflammatory	32	138
Anti-hypertensive	36	163
Reproductive hormone modulator	26	166
Anti-hyperglycemic	7	556
Lipid modifier	9	672
H1 anti-histamine	11	1893
Antibacterial	32	1914
Gastric antacid	9	3073

All 371 APIs were assigned to one of 35 broadly defined MOA classes. PECs were estimated for all the APIs, without taking account of metabolic inactivation. A potency-normalized concentration addition model was used to estimate effects of a mixture composed of all APIs belonging to a MOA class. The number of APIs in each class, and the number of days of water consumption (assuming the wastewater PEC) required to ingest the equivalent of one minimum therapeutic daily dose are shown for each of the top 10 classes.

Table 4 – Non-human exposure to single agents

Active ingredient	PEC (ppt)	PEC/Cmax-free
Estradiol	617	2056
Atorvastatin	2906	45
Promethazine	1668	6
Simvastatin	548	6
Ethinyl estradiol	5.4	5
Sertraline	615	4
Hydrocortisone	2368	3
Propranolol	991	3
Amitriptyline	5029	2
Norethindrone	111	1

PECs for the top 50 APIs, taking into account metabolic inactivation, were compared to dissolved plasma concentrations after human administration of a minimum therapeutically effective dose. Maximum likely wastewater concentration (PEC, in parts per trillion of parent API equivalents) of pharmacological activity associated with each API, and the ratio of that concentration to the therapeutic free plasma concentration (Cmax-free) are shown for the top 10 APIs.

Table 5 – Potential fungal growth inhibition

API	PEC ng/L	MIC μg/mL	PEC/MIC	PEC/0.25 μg/mL
Ketoconazole	220	0.007	0.031437	0.00088
Terbinafine	136	0.007	0.019423	0.00054
Clotrimazole	90	0.007	0.012865	0.00036
Fluconazole	106	0.016	0.006638	0.00042
Econazole	26	0.007	0.003646	0.0001
Nystatin	555	0.2	0.002775	0.00221
Caspofungin	1	0.004	0.000158	0.000002
Butoconazole	7	1	0.000007	0.000028
			Sum (PEC/MIC): 0.076949	Sum: 0.0045621

PECs of the eight antifungal APIs among the 371 APIs with market data are compared to lowest minimum inhibitory concentrations (MICs). The PEC/MIC ratio was summed across all eight APIs to estimate potential mixture effects. Individual PECs and potential mixture concentrations are also compared to a presumptive breakpoint of 0.25 μg/mL.

proteins. Published studies revealed the likely molecular mechanism of therapeutic action for all but four of the top 50 APIs (Appendix D). The mechanism of action of acetaminophen is unknown, but may involve cyclooxygenase II. Metformin acts indirectly through mitochondrial protein kinases (PRKAA1 and PRKAA2), but the protein receptor is unknown. Multiple molecular effects have been noted for carbamazepine and theophylline, but relations to therapeutic effects are unclear.

BLAST was used to search protein and nucleotide data for non-mammalian sequences similar to putative human drug targets (Appendix D). Hits similar to (greater than 60% amino acid identity) four targets are widely distributed across eukaryotic and prokaryotic clades, including the P-type sodium pump, histone deacetylase, HMG-CoA reductase, and xanthine oxidase. Mitochondrial protein kinase A-like genes, and insulin receptor-like genes (including daf-2-like genes) could be found broadly distributed across eukaryotic clades, but were not found in prokaryotes.

Several target families were only found in vertebrates. These include some biogenic amine receptor subtypes such as beta-adrenergic receptors, dopamine receptors, histamine receptors, oxyglutarate receptors, and opioid receptors. Invertebrate sequences similar to other biogenic amine receptor subtypes were found, including alpha-adrenergic receptors, muscarinic acetylcholine receptors, receptors for gamma-amino butyric acid, and receptors for adenosine. Hormone receptors for angiotensin, androgens, progesterone, and glucocorticoids were restricted to vertebrates. Invertebrate sequences similar to the estrogen receptor could be found, but may represent constitutively active transcriptional regulators that do not bind estradiol (Thornton et al., 2003).

4. Discussion

4.1. *Overview*

Conservative assumptions were applied to a simple mechanistic model to identify and prioritize human prescription APIs whose residues are possibly present in US wastewater at concentrations sufficient to induce significant effects in humans, microbes, or other organisms. The sole route of exposure considered was consumption or contact with water. The critical exposure rate for inducing significant human effects was assumed to be similar to the minimum therapeutic dose rate. Critical concentrations for inducing microbial effects were assumed to be similar to minimum inhibitory concentrations (MICs) measured for pathogenic microbes. Critical concentrations for inducing effects in other organisms were assumed to be similar to free plasma concentrations of API after human therapeutic dosing. Consequential physiological changes due to low-level API exposure were assumed to be restricted to organisms having receptors similar to the human API receptor. Results suggest that wastewater exposure to residues of the vast majority of individual APIs and their mixtures are unlikely to cause significant biological effects. Subsets of APIs were identified whose effects in this scenario cannot be discounted, as were ranges of organisms most likely to be affected. We suggest that most future US research should focus on these subsets of APIs and their

Table 6 – Potential bacterial growth inhibition

API	PEC ng/L	MIC μg/mL	PEC/MIC	Breakpoint μg/mL	PEC/BP
Penicillin v	4840	0.0005	9.6800	0.25	0.0193600
Amoxicillin	28,465	0.008	3.5582	0.5	0.0569307
Levofloxacin	2505	0.001	2.5047	2	0.0012524
Ciprofloxacin	1908	0.001	1.9079	1	0.0019079
Trimethoprim	8934	0.015	0.5956	4	0.0022335
Piperacillin	1606	0.004	0.4016	2	0.0008032
Ceftriaxone	396	0.001	0.3964	0.25	0.0015856
Moxifloxacin	245	0.001	0.2446	1	0.0002446
Clindamycin	3554	0.015	0.2369	10	0.0003554
Sulfamethoxazole	36,859	0.160	0.2304	76	0.0004850
Metronidazole	10,886	0.060	0.1814	16	0.0006804
Gatifloxacin	138	0.001	0.1380	0.5	0.0002759
Cefuroxime	660	0.008	0.0826	32	0.0000206
Cephalexin	9482	0.120	0.0790	32	0.0002963
Clarithromycin	1108	0.015	0.0739	1	0.0011079
Neomycin	8791	0.130	0.0676	10	0.0008791
Tetracycline	3911	0.060	0.0652	12	0.0003259
Azithromycin	1631	0.030	0.0544	2	0.0008155
Gentamicin	315	0.008	0.0394	1	0.0003150
Doxycycline	715	0.030	0.0238	16	0.0000447
Cefdinir	427	0.030	0.0142	1	0.0004273
Minocycline	367	0.030	0.0122	16	0.0000229
Linezolid	58	0.007	0.0083	2	0.0000292
Cefaclor	883	0.130	0.0068	8	0.0001104
Cefprozil	375	0.250	0.0015	32	0.0000117
Cefadroxil	151	0.120	0.0013	32	0.0000047
Mupirocin	64	0.060	0.0011	4	0.0000161
Erythromycin	15	0.016	0.0009	8	0.0000018
Tobramycin	5	0.008	0.0006	16	0.0000003
Nitrofurantoin	454	1.000	0.0005	128	0.0000035
			Sum: 20.6090		Sum: 0.0905479

Wastewater concentrations (PECs) for the 30 antibacterial APIs among the 371 APIs with market data compared to lowest typical minimum inhibitory concentrations (MIC) and breakpoints (BP) for those APIs. The PEC/MIC ratio was summed across APIs to estimate potential mixture effects.

effects on the taxa of concern, as well as on potential exposure scenarios and effect mechanisms not considered by the model.

Several countries require environmental assessments as part of the drug approval process. In the US, the Food and Drug Administration (FDA) develops guidelines (US FDA, 1998) for these assessments, and the European Medicines Agency (EMEA) issues guidelines (EMEA, 2006) for the EU. Both agencies use a screening step to select APIs for in-depth assessment. In this step, aquatic PECs are estimated by the equivalent of dividing mass of API dispensed nationally by volume of wastewater produced nationally. The FDA and EMEA approaches differ from each other, and from this work, in the way the mass of API is estimated. The FDA uses a manufacturer's five year forward looking estimate of API produced annually for international consumption, and only considers API contributions from one manufacturer at a time. These production estimates are typically not publicly available. Otherwise the FDA PEC calculation is virtually identical to our own (wastewater volumes differ slightly). EMEA assumes 1% of the population takes the maximum daily dose of the API, and assumes a ten-fold dilution of wastewater into receiving waters, which would significantly overestimate dilutions in at least one-quarter of US receiving waters (Brooks et al., 2006). The resulting PECs are usually above measured concentrations, but occasionally underestimate measured concentrations (Liebig et al., 2006; Ferrari et al., 2004). In order to avoid underestimating potential API concentrations, we assume no dilution of effluents in receiving waters. Both the EMEA and FDA procedures compare PECs to a fixed 'action limit' (0.01 ppb, or 1 ppb, respectively) when deciding on further assessment. We avoid using a homogeneous figure for market penetration or 'action limits', because doing so ignores real variations in use and potency that can help prioritize APIs for research.

4.2. *Wastewater concentrations*

Although available data were insufficient to calculate exact API concentrations, they allowed estimates of likely upper bounds of the national average raw wastewater concentration. These upper limits will often vastly overestimate API-associated activity reaching the aquatic environment, because of the conservative assumptions used for converting market data into mass of API dispensed, and for estimating metabolic inactivation of APIs from documented formation of inactive metabolites. Most unaccounted for processes, such as API degradation during wastewater treatment, partitioning out of the aqueous phase and environmental transformation can only decrease aqueous concentrations from those predicted here.

Although our approach is general, parameterization with US marketing and wastewater emission figures restricts the scope of PECs and exposure multiples to the US. Furthermore, PECs calculated from US average prescription and wastewater production rates fail to capture geographic variations in these values, raising questions about the scope of our conclusions within the US. Although detailed data on variations in prescribing patterns were not available to us, a state-by-state analysis of prescription rates for major therapeutic classes of drugs (Motheral et al., 2001) suggests that per capita rates within a therapeutic class (for instance anti-depressants) in one state can exceed the US average by up to 53%. The Clean-Watershed Needs Survey Database (US EPA, 2007) contains both wastewater production volumes and served population sizes for wastewater treatment plants serving 207 million US residents. More than 95% of these residents are served by plants that have effluent production rates of at least 242 L per resident served per day (data not shown). Assuming this lower effluent production rate together with prescription rates 53% higher than average would result in PECs about four times higher than those based on US averages. Other sources suggest that wastewater facility effluent production can be as low as 100 L per person per day (Versteeg et al., 2005). Assuming 53% increased pharmaceutical use along with this minimal flow rate would result in PECs about nine times higher than PECs based on US averages. Applying a nine-fold 'application factor' to account for potential PEC variance would increase API mixture exposure multiples to 0.08 and 0.69 for humans and fungi, respectively. Applying this factor would also increase the number of APIs with exposure multiples greater than one from 11 to 26 for non-humans, and from four to twelve for bacteria. There are also some unusual circumstances (i.e. hospital effluents and campgrounds) where even this application factor may not be conservative enough. Additional research will be required to characterize these exposure scenarios.

4.3. *Human exposure rates*

Worst case human exposure was assumed to be consumption of water with the same concentration of API activity predicted to occur in raw wastewater. Although this scenario likely vastly overestimates human exposure from drinking water, it ignores another plausible route of exposure: consumption of organisms, such as fish (Schwaiger et al., 2004) and plants (Delepee et al., 2004), that bioconcentrate APIs from their environment.

Expressing API exposure rates as the days of water consumption needed to ingest the equivalent of a single minimum therapeutic dose provides a general way of expressing exposure rates relative to those required to produce documented, although usually well tolerated, physiological effects in humans. Results suggest that, under most circumstances, aquatic exposure rates to single APIs and combinations of APIs are at least 100 fold lower than those required to produce minimal therapeutic effects. Since most APIs do not cause frank toxicity except at dosages well above minimum therapeutic dosages, and due to the threshold-like dose responses expected for APIs acting through a receptor (Connolly, 1995), which includes all of the top 50 APIs, the predicted exposure multiples are not expected to be toxic to healthy adults. By contrast, mutagenic cytotoxic agents display cumulative toxicity via introduction of irreversible DNA damage, and long-term effects appear linearly related to dose (Zito, 2001). For these agents, any level of exposure may result in some increase in health risk. However, projected mixture concentrations indicate over 45,000 days (123 years) of wastewater consumption would be needed to ingest a daily dose equivalent of combined cytotoxic API activity, suggesting that associated risks are very low.

The human exposure multiples estimated in this study, were based on daily therapeutic dosages for relatively healthy adult humans. Often, however, reduced dosages are indicated in some subpopulations, particularly the young, the elderly, and those with impairments in liver or kidney function. For the top 50 APIs, dose rates for sensitive subpopulations were uniformly equal to or greater than 1/4 the minimum daily dose listed (data not shown). Given the low predicted exposure multiples, this suggests that toxicity from water exposure is not expected in these subpopulations. On the other hand, some of the top 50 APIs are contraindicated in young children or in patients with very severe kidney or liver dysfunction. APIs are also contraindicated in patients who are known to be allergic to them. For these subpopulations, dose response relationships are typically not known, and it is impossible to calculate exposure multiples using our approach.

For some chemicals, non-monotonic dose responses, such as hormesis have been suggested, challenging linear extrapolation of effects to lower doses. However, hormetic responses are typically only seen at doses 10% or more, and almost always at doses 1% or more, of doses producing traditional endpoints (Calabrese and Blain, 2005). Therefore, hormesis is unlikely at the exposure multiples predicted in this study. Furthermore, the size of hormetic effects typically falls within the range of natural variation, and is more likely to be viewed as adaptive rather than toxic (Calabrese and Baldwin, 2003), further reducing concerns about extrapolation to very low-level API exposure.

Aggregate effects of pharmaceutical mixtures were estimated assuming a potency-normalized concentration addition model for APIs sharing a physiological mode of action (MOA). Although synergistic interactions have been noted for some chemicals, interactions are usually additive, sub-additive, or antagonistic (Hayes, 2001; Carpenter et al., 2002; Altenburger et al., 2003). Also, many of the mechanisms for producing drug interactions (Rodrigues, 2001), such as inhibition of metabolic inactivation, or competition for saturable plasma protein binding sites, are threshold limited and likely negligible at the low exposure levels predicted in this study. Additionally, synergy is typically seen only at doses of interacting chemicals similar to or above their individual threshold effect doses (Seed et al., 1995), suggesting that significant synergy is unlikely at the low exposure multiples predicted here. Therefore a concentration addition model is probably sufficiently conservative for estimating API mixture effects.

Estimates of mixture effects are limited to the 371 APIs with some marketing data, and exclude APIs that share a MOA with some of the 371 APIs, but are not represented in the marketing data. Assuming an even distribution of MOAs among listed and unlisted products, dividing mixture exposure multiples by the fraction of the pharmaceutical market included in the marketing data (0.85) should correct for this missing data. Given the low predicted exposure multiples, reasonable adjustments are unlikely to substantially alter this study's conclusions.

4.4. *Exposure multiples for non-humans*

Little pharmacological data exists in non-mammals, suggesting that more conservative risk estimates should be made. Many inter-species sensitivity differences arise from differences in xenobiotic clearance efficiency (Baggot, 1992). A conservative theoretical model of this case is a freely permeable organism that has cellular sensitivity to APIs similar to humans, but cannot decompose APIs nor excrete them against a concentration gradient. Assuming exposure via passive equilibration with wastewater, the concentration of API dissolved in the modeled organism's extracellular fluid would approach the concentration dissolved in wastewater. Comparing this concentration with the concentration dissolved in human plasma after therapeutic dosing (our surrogate measure of cellular sensitivity) suggests whether a significant effect from aquatic exposure in the modeled organism is possible. Using dissolved human plasma concentration, rather than total plasma concentration, which includes components bound to plasma protein and blood cells, reflects that for most APIs only unbound API is available to cross target cell membranes or interact with molecular receptors (Wright et al., 1996). Therefore the freely dissolved plasma concentration after therapeutic dosing will usually be a more accurate and conservative estimate of API concentrations able to affect exposed cells.

Although the model depicted here is extreme, it may still underestimate effects due to contact with other media, such as sediments or biota, which may concentrate APIs. This model may also underestimate effects seen in organisms which actively concentrate API from the environment, using xenobiotic active transporters such as members of the solute-linked carrier family or the ATP-binding cassette family (Girardin, 2006). The model may also underestimate effects seen in organisms with substantially higher cellular sensitivity to API than humans.

4.5. *Potential antimicrobial effects*

Antimicrobial APIs are selected for high toxicity to pathogenic microbes and for minimal effects on metazoan physiology. Antimicrobial residues are therefore expected to affect microbes more readily than multi-cellular species. Effects may include selection for antimicrobial resistance in pathogens, and growth inhibition of beneficial microbes.

Antimicrobial sensitivity is usually expressed as a minimum inhibitory concentration (MICs). MIC thresholds, called 'breakpoints' (typically between 0.25 and 16 μg/mL), representing antimicrobial concentrations achievable in patients, are standard cutoffs for defining clinically resistant strains (Smaill, 2000). The MICs of 'sensitive' organisms usually fall between 1 ng/mL and the breakpoint (Andrews, 2001). In this study, PECs of antibacterial APIs were at least 20 fold below breakpoints, and antifungal PECs were predicted to be at least 400 fold below presumptive breakpoints of 0.25 μg/mL. Assuming a potency-normalized concentration addition model for mixture effects, antibacterial mixture concentrations are at least 10 fold below breakpoints, and antifungal mixture concentrations are at least 200 fold below presumptive breakpoints. Therefore wastewater concentrations of antimicrobials resulting from human prescription pharmaceutical use are unlikely to select for clinically resistant microbes, in the absence of strong synergies between APIs.

Synergy between antimicrobial APIs is usually expressed as fractional inhibitory concentration index (FICI) (Mukherjee

et al., 2005). FICI for synergistic pair-wise combinations of antimicrobials generally lies between 0.25 and 0.5, and in rare cases reaches 0.02 (Mukherjee et al., 2005). Triple and higher-order combination FICIs are in the same range as pair-wise combinations (Pavicic et al., 1991; Rochon-Edouard et al., 2000; Mukherjee et al., 2005). Multiplying model projections of mixture effects by 50, to conservatively correct for a FICI of 0.02, suggests that synergistic interactions between APIs present in wastewater selecting for clinically significant resistance cannot be ruled out for bacteria, but probably can be for fungi.

Some antibacterial PECs are similar to MICs for the most sensitive known bacterial strains. Also, partial growth inhibition is often seen at concentrations 5 to 20 fold below the MIC (Dessus-Babus et al., 1998; Barrett et al., 1998), and other effects are occasionally discernible at less than 0.1% of the MIC (Kahn et al., 2006), suggesting that wastewater concentrations of antibacterial APIs may select for weak resistance in highly sensitive strains of bacteria. Although unlikely to be clinically significant, weak resistance may serve to initiate step-wise development of clinically significant resistance (Baquero, 2001). The importance of this process relative to shedding of highly resistant microbes from treated patients is unknown.

Much less data is available on the sensitivity of naturally occurring microbes to antimicrobial agents, or the correlation between natural conditions and conditions used to measure MICs (Smaill, 2000). Available evidence suggests a similar range of sensitivity in non-pathogenic microbes as is seen in pathogens (Harrass et al., 1985; Lutzhoft et al., 1999; Halling-Sorensen, 2000), suggesting that antibacterial wastewater PECs may be sufficient to alter the growth of particularly sensitive naturally occurring prokaryotes, with unknown effects on higher trophic levels.

4.6. *Range of potentially sensitive taxa*

Public biological sequence data was mined to identify taxa with proteins similar to human molecular drug targets of the top 50 APIs, suggesting that these taxa might be sensitive to the corresponding APIs. Although only a few clades contain fully sequenced organisms, these clades are broadly dispersed across the tree of life, providing strong hints about taxonomic dispersal of potential API sensitivity. This analysis, along with information about the physiological roles of these molecular pathways in different organisms, provides guidance on the range of species and types of endpoints that should be considered in chronic toxicity studies.

Conserved molecular mechanisms can serve different purposes in non-human species than in humans. For instance, thyroid signaling controls timing of metamorphosis in amphibians (Tata, 1998), and serotonin signaling regulates egg-release in mollusks (Matsutani and Nomura, 1987), phenomena without obvious counterparts in humans. Nevertheless, the presence in a species of homologs of the human molecular receptor should still indicate whether some sort of response to low-level API exposure is expected. In other cases, pathways other than those responsible for therapeutic effects can be activated in humans but may show no overt effects or be considered side-effects. Although typically too little is known about these pathways to construct exposure multiples or identify sensitive taxa, similar ones may occasionally mediate significant ecotoxicological effects (Seiler, 2002).

5. Conclusion

Under conditions typical of the US, significant human, non-human, and microbial impacts resulting from aquatic exposure to most APIs and their mixtures are likely only possible through contact with concentrating sources, through unexpected dose responses, or in extremely sensitive subpopulations. For a small subset of APIs, effects of direct aquatic exposure to single agents cannot be ruled out for non-humans, microbes, or very sensitive humans. Hazard quotients for non-human exposure were over one for a few APIs, suggesting possible effects, and predicted wastewater concentrations of several antibiotics are sufficient to impair growth or select for low-level resistance in microorganisms. The conservative approach used here for estimating aquatic effects of API exposure seems useful for prioritizing future research, since it substantially narrows the number of APIs of concern, and suggests which taxa should be used for initial ecotoxicity testing.

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Supplementary data

Supplementary data associated with this article (Appendices A–E) can be found, in the online version, at doi:10.1016/j.scitotenv.2007.09.008.

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