Running title: Aquatic concentrations of pharmaceuticals.

Predicting variability of aquatic concentrations of human pharmaceuticals.

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Abstract

Potential exposure to active pharmaceutical ingredients (APIs) in the aquatic environment is a subject of ongoing concern. We recently published maximum likely exposure rates for several hundred human prescription pharmaceuticals commonly used in the US. These rates were estimated from nationally aggregated marketing data and wastewater production rates. The accuracy of these estimates is unclear, and it is unclear how to use the national-level estimates of exposure to predict local exposure rates. In this study we compare our previous predicted environmental concentrations (PECs), which were based on marketing data, with PECs based on regulatory data. We then use local dispensing rates for 12 APIs along with local wastewater production rates to estimate the distribution of local PECs relative to national averages, in order to identify an 'application factor' suitable for converting national-level PECs into reliable bounds for local concentrations. We compare the national-level PECs and the proposed application factor with measured environmental concentrations (MECs) published in 62 recent peer-reviewed publications. Regulatory data-based national average PECs are uniformly lower than marketing data-based national average PECs, corroborating the intended conservative nature of the marketing data-based PECs. Variability in local API usage and wastewater production rates suggest local PECs may occasionally exceed national averages by about 10-fold. Multiplying national average PECs by an 'application factor' of 10 and comparing the resulting predicted maximum local PECs to published MEC data for 83 APIs corroborates the usefulness of 10-fold adjusted national PECs as a reasonable ceiling for measured environmental concentrations.

Key words: pharmaceutical; antibiotic; wastewater; aquatic; drinking water

List of abreviations:

AIC: Akaike Information Criterion

aPEC: ARCOS-based national average PEC

API: active pharmaceutical ingredient

ARCOS: Automation of Reports and Consolidated Orders System

bMOA: broad mechanism of action

CWNS: Clean Watersheds Needs Survey

DDmin: minimum daily dose

DPD: doses per decade

EE2: ethinyl estradiol

LOEC: lowest observable effect concentration

MEC: measured environmental concentration

MOA: mechanism of action

mPEC: marketing data-based national average PEC

MRL: method reporting limit

nMOA: narrow mechanism of action

PEC: predicted environmental concentration

POCIS: polar organic chemical integrative sampler

WWTP: wastewater treatment plant

ZCTA: zip-code tabulation area

1. Introduction

Active pharmaceutical ingredients (APIs) have been detected at low concentrations (typically below 10 μg/L) in municipal wastewater effluents and surface waters for more than three decades (Hignite and Azaznoff, 1977; Richardson and Bowron, 1984; Kolpin et al., 2002a). The primary route for their introduction into the environment is thought to be excretion from humans into wastewater collection systems, persistence through wastewater treatment, and subsequent discharge into surface or ground water (Fent et al., 2006). Risks posed by these contaminants to humans and aquatic life are of ongoing concern (Daughton and Ternes, 1999). Characterizing aquatic exposure rates is complicated by the large number of APIs in use, which can vary greatly from one another with regard to usage rate, transport, fate, and potency. Although about 1,800 APIs are currently approved for prescription use in the US (US FDA, 2009) individual monitoring efforts have been limited to about 50 analytes each, with most studies looking at fewer than 10 analytes (Gros et al., 2006). This fact suggests exhaustive monitoring of all APIs is impractical and instead indirect means of estimating potential exposure rates are needed in order to prioritize future investigation as well as estimate overall risks.

We recently estimated (Kostich and Lazorchak, 2008) relative maximum likely risks, at the national level, posed by waterborne APIs originating from US municipal wastewater. Marketing data-based predicted environmental concentrations (mPECs) were conservatively estimated from nationally aggregated API sales and wastewater production rates. Lowest observable effect concentrations (LOECs) for humans were assumed proportional to the minimum daily dose (DDmin) recommended for therapeutic use. Relative aquatic risk for each API was expressed as the ratio of each API's mPEC to its DDmin. Because of uncertainties in

fate parameters, such as partitioning, breakdown, and in-stream dilution, we did not attempt to make central estimates of water-column associated exposure. Instead, each mPEC was calculated making the conservative assumption that these dissipative processes were negligible. The resulting mPECs therefore estimate the upper end of possible national average environmental concentrations, rather than the most likely national average concentrations. However, reliance on marketing data of unknown quality, together with uncertainties in factors for converting marketing data from dollars sold or numbers of prescriptions written into mass of API dispensed, introduces uncertainties into the mPECs. In addition, evaluating the approach by comparing mPECs to measured environmental concentrations (MECs) is not straightforward, as MECs reflect local variation. For similar reasons, it is unclear how to use national-level mPECs to estimate local hazards posed by an API.

In this study we assess our previously derived national average mPECs, and estimate the range of local variability in API concentrations relative to the mPEC. We use regulatory data on legal distribution of APIs classified in the US as 'controlled substances' (Doig and Cordy, 2004) to examine the accuracy of national estimates arrived at using marketing data. Then data on local distribution of APIs classified as controlled substances is combined with census data to estimate local per capita rates of API use. Local API usage rates and local wastewater production rates are combined to estimate an upper 99th percentile wastewater concentration, relative to the national average. This upper 99th percentile is proposed as a general 'application factor' suitable for converting predicted national average mPECs into reliable upper bounds for local concentrations. This factor is then applied to mPECs, and the resulting predicted local concentration ceilings are compared to MECs for a range of APIs reported in recent peer-reviewed studies. This comparison serves as an evaluation of the generality of the application factor and the usefulness

of the marketing data-derived national PECs.

2. Materials and methods

2.1 Data analysis

Data analysis was performed using R 2.8.1 (R Development Core Team, 2008). In addition to the base package, functions from the stats (cor, cor.test, lm, summary.lm, plot.lm), boot (boot, boot.ci, plot.boot) and MASS (dropterm) packages were used.

Variables were log-transformed prior to linear regression or calculation of Pearson's r, in order to stabilize variances, moderate the effects of outliers on parameter estimation, and extend the range of variables below zero. Regression was performed by ordinary least-squares fitting. Semi-partial correlations (section 3.2) between variable A and variable B after removing the effects of variable C were calculated as Pearson's *r* between variable A and the residuals from bivariate linear regression with B dependent upon C.

Akaike Information Criterion (AIC) changes were calculated using the MASS::dropterm function (Venables and Ripley, 2002). Hypothesis tests were conducted at $p <= 0.05$. Testing whether a sample value of Pearson's *r* arose from random assortment of unassociated variables assumes a Student's t (df=n-2) sampling distribution of *r*, and was conducted with the function stats::cor.test. The Bias Correction-accelerated algorithm (Davison and Hinckley, 1997) was used to estimate 95% confidence intervals for Pearson's *r*, using boot::boot.ci on 9999 bootstrap samples generated with boot::boot. Permutation analysis in section 3.3 was conducted using 1 million permutations generated with the function base::sample.

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152 2.2. API usage rates

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154 The Automation of Reports and Consolidated Orders System (ARCOS, US DEA, 2004)
155 documents legally regulated distribution within the US of 12 APIs classified as controlled
156 substances, and is organized by state and three-digit zip code. A three-digit zip code identifies a
157 region corresponding to the union of the regions whose postal zip codes share the same first three
158 digits. Geographic coordinates of postal zip codes were estimated using the coordinates of the
159 centroids of zip-code tabulation areas (ZCTA, US Census Bureau, 2000), which approximate the
160 region served by a postal zip code.

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162 2.3. Wastewater production rates

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164 The Clean Watershed Needs Survey (CWNS, US EPA, 2004) lists the size of the population
165 served and the flow rate for most wastewater treatment plants (WWTPs) in the US. WWTPs
166 listed in CWNS were included in our variability predictions if they served a population greater
167 than 100, at least 75% of their flow was of municipal origin, at least 75% of their served
168 population was local residents, and per capita wastewater production was between 50 and 1,000
169 liters per person per day.

170 CWNS contains state identifiers for all listed WWTPs, geographical coordinates of
171 discharge outfalls for many WWTPs, and zip codes (included as part of the WWTP mailing
172 address) for many WWTPs. When the outfall location was available, the facility was assigned
173 the zip code corresponding to the closest (calculated with haversine formula -- Sinott, 1984)

ZCTA centroid within the same state. If outfall location was unavailable, but a mailing address was listed, the mailing zip code was assigned to the facility.

Of the 16,521 discharging facilities listed in CWNS, 7,176 met inclusion criteria listed above and could also be assigned a zip code. These WWTPs, on which our distributional analysis is based, produce 14.6 billion gallons of wastewater per day (out of a CWNS total of 33.7 billion gallons), and serve 114,136,107 people (out of a CWNS total of 229,071,206 people).

2.4. PECs and spatial variation

The likely upper bound for the average US PEC for each API was calculated by dividing the mass of API dispensed nationwide each year by an estimate of annual US wastewater production (6.8×10^{13} L/yr -- adapted from Kostich and Lazorchak, 2008):

PEC for an API in ng/L = (mass of that API dispensed in kg/yr) * (10^{12} ng/kg) / (6.8×10^{13} L/yr)

Degradation of parent drug by patient metabolism or wastewater treatment was not accounted for, so the resulting estimates should be conservative for many APIs. In order to express potential exposure in units with an intuitive relationship to risk, and also adaptable to describing exposure rates to mixtures, PECs were converted into doses per decade (DPD). DPD are the equivalent number of DDmin that would be consumed in one decade, assuming consumption of 2 liters of water per day with API present at the PEC:

$$DPD = (PEC * 2 * 3650) / (DDmin * 10^6)$$

where PEC is in ng/L, 2 is the number of liters consumed per day, DDmin is in mg/day, and the factor 10^6 is used to convert mg to ng.

Each CWNS facility to which a zip code was assigned (section 2.3) was associated with 12 local per capita API usage rates (one for each of the 12 APIs in ARCOS -- section 2.2) by matching three digit zip codes and state identifiers. The local usage rate for each API was divided by the per capita wastewater production rate for that facility (section 2.3), to yield a local PEC for that particular WWTP. Local PECs were normalized by division by the ARCOS-based national average PEC (aPEC) of the corresponding API, resulting in a local PEC expressed as a multiple of the corresponding API's national average aPEC. For each API, the distribution of local PECs was expressed in terms of the proportion of all wastewater produced by WWTPs with PECs lower than a given PEC: WWTPs were sorted by their associated local PECs; for each local PEC, the total volume of wastewater produced by all WWTPs with lower local PECs was divided by the total volume of wastewater produced by all WWTPs, yielding the wastewater volume percentile for that local PEC.

2.5. Comparison to MECs

Peer reviewed publications reporting MECs for any API (controlled substance or not) were identified via literature search. Studies were included if they were conducted in the US, published between January 2001 and January 2009, and reported some mass spectrometry data. Only data on human prescription pharmaceutical active ingredients that are currently used and

220 are not naturally occurring hormones were summarized. Measurements from wastewater, surface
221 water, and ground water were included. MECs from hospital effluents and treated drinking water
222 were excluded. POCIS data were excluded. Non-detections and detections that could not be
223 quantified were recoded as the method reporting limit (MRL). Estimated concentrations reported
224 as a range of possible values were recoded as the lower end of the range.
225 For DPD calculations, metabolites were considered equipotent with the parent on a mass
226 basis. For metabolites with MECs (section 3.3), this simplification results in differences of 7.5%
227 or less relative to DPD calculations performed on a molar basis. Levofloxacin was recoded as
228 ofloxacin, since none of the studies summarized here used methods that distinguish enantiomers.
229 Data reported in Kolpin et al. (2002a) were corrected per Kolpin et al. (2002b). DDmin and
230 MOA are adapted from Kostich and Lazorchak, 2008, if available, or from product prescribing
231 information.
232 An error in our previous mPEC calculations was corrected: the minimum price of
233 erythromycin had been transcribed as $0.0687/mg. The original marketing data source actually
234 listed $0.0006164/mg. As a result, the erythromycin mPEC is underestimated by 111-fold in
235 Kostich and Lazorchak (2008). The corrected mPEC was used in the present analysis and
236 reported in Appendix 2 of the supporting information.
237
238 **3. Results and discussion**
239
240 3.1. National average PECs
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242 Of the 12 APIs in ARCOS, only nine (Table 1) are dispensed frequently enough to be included in

243 the marketing data for 'top drugs' that was previously used (Kostich and Lazorchak, 2008) to
244 estimate mPECs for 371 APIs. These nine APIs span the marketing data-based risk rankings
245 from #8 (codeine) to #158 (methadone). mPECs exceeded the corresponding ARCOS-based
246 national level PECs (aPECs) by 1.2- to 13.5-fold (Table 1), depending on the API, corroborating
247 the intended conservative nature of the mPECs. Within this sample, a modest linear relationship
248 was found between log-transformed mPECs and log-transformed aPECs, with Pearson's
249 correlation *r* for this sample equal to 0.82. The hypothesis that this sample value of *r* arose by
250 chance assortment of variables with no real association was rejected with a one-sided (only
251 positive associations are expected) p-value of 0.003. Assuming that this set of 9 APIs can be
252 considered a simple random sample from the larger population of 371 'top drugs', a 95%
253 confidence interval for Pearson's *r* in the corresponding population was found to be 0.21-0.97. It
254 is not clear how representative these APIs are of all the APIs in use in the US, but consistency of
255 local PECs based on this assumption with MECs for a much broader range of APIs (sections 3.3
256 and 3.4) suggests the assumption is approximately correct.
257
258 3.2. Predicting spatial variation
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260 Combining local per capita wastewater production rates with local per capita API distribution
261 rates for all 12 APIs in ARCOS (Table 2) suggests that 99% of municipal wastewater (on a
262 volume basis) contains API residue concentrations less than ten times the corresponding API's
263 national average aPEC. Given the small sample size on which this estimate is based, perhaps 15
264 or 20 would be a more prudent application factor for converting national average mPECs into
265 reliable upper bounds for local concentrations. Nevertheless, we use ten as an application factor

for comparing national level mPECs to MECs in section 3.3, since this is the factor suggested by our limited data. Log-transformed local wastewater production and API usage rates showed little correlation with one another (sample Pearson's R-squared was consistently <= 0.03), providing a nearly additive partition of local PEC variability between these drivers. Local API usage rates had greater coefficients of variation than local wastewater production rates (0.5-1.2, depending on API, vs. 0.3 for wastewater production). This variability in API usage accounted for most of the variation in local PECs (squared semi-partial correlations of 0.64-0.93, depending on API, after removing effects of wastewater production) compared to variations in wastewater production (squared semi-partial correlations of 0.05-0.32, after removing effects of API usage). This means that for these 12 APIs, most variability between locales in the PEC for any single API is accounted for by variations in local per capita API usage, with substantially less accounted for by variability in local per capita wastewater production.

3.3. Comparing predictions to measurements

A search of peer-reviewed literature identified 62 studies meeting criteria for inclusion (section 2.5). In aggregate, these studies report MECs for 133 API-related analytes corresponding to 111 APIs found in prescription drugs (Appendix 1). Individual studies measured between 1 and 51 (median study=6.5, when ranked by number of analytes) analytes, corresponding to between 1 and 45 (median study=6) APIs. Individual studies reported between 1 and 336 (median study=12.5) independent (with respect to time or site of sample collection) measurements per analyte, on samples collected from between 1 and 115 (median study=6) sites. For each API, the combined set of studies provided between 1 and 1,237 (median API=42) independent

289 measurements from between 1 and 542 (median API=23) distinct sites.

290 MECs and mPECs (adapted from Kostich and Lazorchak, 2008) for each API were
291 potency normalized and expressed as DPD (section 2.4). The highest MEC for each API was
292 compared with the corresponding mPEC (Appendix 2). Of the 111 APIs for which MECs were
293 found, 87 are among the 362 APIs which have mPECs but are not natural hormones. Natural
294 hormones were not considered, as they have substantial sources other than pharmaceutical use
295 which were not accounted for during generation of the mPECs. Of the remaining 87 APIs with
296 both mPECs and MECs, one (digoxin) has never been detected (all reported MECs are less than
297 corresponding MRLs) in the studies considered (section 2.5), but MRLs are more than 10-fold
298 greater than the corresponding mPEC, limiting the utility of comparing MECs to the digoxin
299 mPEC. For an additional three APIs (fluticasone, methotrexate, and norgestrel) with mPECs and
300 reported MECs, MECs have been below the corresponding MRL, but MRLs exceeded the API's
301 mPEC. In addition to these 87 APIs, MECs were found for 24 APIs without corresponding
302 mPECs, and no MECs were found for 275 APIs with mPECs. APIs with MECs span the
303 marketing data-based risk rankings from #3 (hydrochlorothiazide) to #309 (lindane) out of the
304 362 APIs with mPECs.

305 For 14 of 83 APIs that have been detected or have MRLs less than the corresponding
306 mPEC, the highest reported MEC exceeds the mPEC (Table 3). The most prominent among
307 these 14 APIs is ethinyl estradiol (EE2), for which the MEC exceeds the mPEC by a factor of 41
308 (see below). In all other cases, the highest MEC is less than the mPEC or exceeds the mPEC by
309 less than the proposed application factor of 10. By contrast, 30 of 83 APIs have a maximum
310 MEC less than one tenth of their mPEC, 11 have a MEC less than one percent of their mPEC,
311 and three have a MEC less than 0.1 percent of their mPEC. ARCOS-based aPECs agree more

312 closely with MECs (Table 2), with the exception of methamphetamine, whose highest MEC
313 exceeds the aPEC by 190-fold. This discrepancy is not surprising, since nationwide therapeutic
314 use of methamphetamine is only about 12 kg/yr, while illicit supply is probably in excess of 120
315 tons/yr (National Drug Intelligence Center, 2005).

316 APIs were sorted in descending order by maximum MEC (measured in DPD), with the
317 highest ranking APIs listed in Table 4. The only APIs whose MECs correspond to greater than 3
318 doses per decade are EE2 (100 DPD), mestranol (59 DPD), and norethindrone/norethisterone (6
319 DPD). Maximum MECs for these structurally related contraceptive APIs were reported in the
320 same study (Kolpin et al. 2002a) and measured using the same method (Barber et al., 2000).
321 Although the majority of measurements reported in this extensive study appear reasonable,
322 concerns have been raised (Ericson et al, 2002; responses in Kolpin et al., 2002b) that
323 measurements for these three APIs (particularly EE2) are too high to reflect typical human use,
324 and might result from isobaric interfering substances in the samples. The highest EE2 MEC
325 reported in other US studies (N=10 other studies) has been 6 ng/L (compared with 273 ng/L in
326 Kolpin et al., 2002a), while norethindrone and mestranol, whose monitoring has not been as
327 extensive (N=1 other study for each API), have not been detected in the other studies
328 summarized here. Nevertheless, the high MECs might also be explained by unorthodox use of
329 these compounds, for instance in livestock production. Further investigation is strongly
330 warranted to determine if the surprisingly high (and correspondingly worrisome) measurements
331 for these three APIs are correct.

332 Although the level of agreement described above between mPEC-based ceilings and
333 maximum MECs is encouraging, it is not clear how specific the assignment of mPEC-based
334 ceilings to individual APIs is. For example, perhaps all the mPECs are too high to be reached by

any API, in which case the assignment of individual mPECs to APIs for ranking purposes would have little value. In order to test for this sort of trivial agreement between MECs and mPECs, mPECs were randomly re-associated with APIs, after which agreement between MECs and mPECs in the permuted dataset was compared to agreement in the un-permuted data. Including data for all 87 APIs except digoxin (which cannot be informatively compared to ten times its mPEC -- see section 3.1), and expressing concentrations as DPD results in one disagreement between mPEC-based ceilings and MECs in the unpermuted data (EE2), a level of agreement only reached in about 1 in 10,000 random permutations. Expressing concentrations as mass per unit volume (ng/L) results in a more dramatic contrast, with only about one in one million random permutations reaching the level of agreement seen in the unpermuted data. This suggests meaningful, specific association of mPECs with APIs.

Pearson's *r* was calculated between log-transformed MECs and mPECs (expressed as DPD) for the 83 APIs that have either been detected and have mPECs, or have not been detected (despite having been looked for) but have a MRL less than the corresponding mPEC. Pearson's *r* for this sample was very modest (0.47), but the possibility that this value arose from chance assortment of unassociated variables was rejected with a one-sided p-value $< 4 \times 10^{-6}$. A 95% confidence interval for the population value of *r* was estimated as 0.30-0.60.

The highest reported MEC for any given API summarizes results from a varying (by API) number of environmental samples, and will reliably approach the true upper limits of environmental concentrations (what we are trying to estimate by multiplying the mPEC for that API by an application factor of 10) only when the number of samples is large. Including sample number as a predictor variable might therefore improve prediction of maximum MECs from mPECs, even though sample number might not be a good predictor on its own. Modeling log-

transformed maximum MECs across APIs as a linear function of the corresponding log-transformed mPECs and the log of the sample number on which each maximum MEC is based shows a fair fit with well-behaved residuals. Deletion of either explanatory variable (mPEC or sample number) is accompanied by a rise in AIC (signaling a loss of useful information), and estimated coefficients for both explanatory variables are positive and significantly different from zero ($p<7x10^{-8}$ for mPEC, and $p<7x10^{-5}$ for sample number), suggesting both variables contribute significantly to prediction of maximum MECs. Sample values of Pearson's *r* between log-transformed maximum MECs and the fitted values from the linear model rose to 0.60 when both variables are included in the regression. Consistent with expectations, sample number appears to be a poor predictor of MECs on its own (sample R-squared = 0.08), but improves prediction more than this would imply (adjusted sample R-squared improves by about 0.14 with inclusion of sample number, compared to a model with mPECs as the only predictor of MECs).

These data suggest that national average mPECs, when adjusted by a 10-fold application factor to account for spatial variability, provide reasonable upper bounds on MECs. By contrast, mPECs are only marginally useful for predicting maximum MECs, with the highest reported MEC for many APIs falling far below the corresponding mPEC. This can be understood in terms of the conservative nature of the mPEC calculations, in particular the omission of terms for dissipative processes, such as transformation, partitioning and in-stream dilution. It can also be partially explained by the variability in the maximum MEC that is dependent on the number of samples analyzed. These explanations are corroborated by the observation that, within the data sets examined, the mPECs are more strongly associated with aPECs (see section 3.1), which are not affected by these issues, than they are with MECs.

Given that our previously published national mPECs for most APIs were quite low (there

were only 20 APIs with mPECs greater than 1 DPD), the sufficiency of a 10-fold application factor for estimating maximum local concentrations suggests that potential aquatic exposure rates to most APIs are far below levels required to elicit clinical effects. For the 20 APIs with mPECs greater than 1 DPD, MEC data summarized here also suggests potential aquatic exposure rates are quite low, but data are not very abundant for many of these APIs. Even though the 10-fold factor still suggests aquatic exposure rates for these 20 APIs are well below those resulting from clinical API administration, the margins of safety are narrower, potentially raising questions about risks from potential aquatic exposure to particularly sensitive human sub-populations or sensitive non-human species. Therefore, we feel further investigation of these APIs is warranted.

It is worth keeping in mind that the scope of the present exposure study extends only to APIs dissolved in the water column. Greater exposure rates may be possible through contact with other environmental media in which APIs might become concentrated, including fish, plants, and sediments. Less data exist on API distributions in these media, and more research will be required to determine associated risks. In addition, the general approach adopted in this work assumes risks decline monotonically with exposure rates, which has been disputed in some cases. See Kostich and Lazorchak, 2008, for a more in-depth discussion of this issue.

3.4. Exposure rates for mixtures

Potential exposure rates to multiple APIs sharing a common MOA were estimated using a potency-normalized concentration addition model for each of 15 broad MOA (bMOA) and 40 narrow MOA (nMOA) categories (Appendix 2; MOA adapted from Kostich and Lazorchak,

2008) that have associated MEC data. For each MOA, the highest reported MEC for each API in the MOA category was expressed as DPD, and MECs were summed across APIs belonging to the MOA. These MEC-based exposure rate estimates were compared to mixture exposure rates estimated from mPECs (Table 5). Maximum potential cumulative exposure along each bMOA was estimated as less than 6 DPD for all bMOA except for 'reproductive hormone modulator' (165 DPD; but see discussion of EE2, mestranol, and norethindrone in section 3.3). Although MEC-based estimates occasionally exceeded the mPEC-based estimates for bMOA (MECs and mPECs could be compared for 13 bMOA), they do so to a lesser degree than was seen for individual APIs. The ratio was less than three for all 13 bMOA categories except 'reproductive hormone modulator', for which the ratio was 47. The exposure rate for the bMOA 'reproductive hormone modulator' is reduced to 68 DPD, and the MEC/mPEC ratio is reduced to 19 if the highest EE2 MEC is adjusted to 6 ng/L (see section 3.3 for rationale). The exposure rate for this bMOA is reduced to 2.2 DPD, and the MEC/mPEC ratio is reduced to 0.86 when mestranol and norethindrone are also deleted (we could not adjust these to the next highest value, as other reported measurements for these APIs are non-detects) from the analysis.

The ratio of MEC-based estimates to mPEC-based estimates for nMOA (MECs and mPECs could be compared for 38 nMOA; Table 6 shows the 12 nMOA with the highest MEC/mPEC ratio) was more variable, often approaching the ratio seen for individual APIs. This may be explained by the smaller number of APIs being averaged into each nMOA mPEC, compared to the larger bMOA categories. Estimated maximum cumulative exposure along each nMOA was less than four DPD for all 40 nMOA categories with MEC data, except estrogens (159 DPD) and progestins (six DPD). Adjusting EE2 MECs to 6 ng/L and deleting mestranol along with norethindrone results in exposure rates for estrogens of 2.2 DPD, and progestin

exposure rates of 0.022 DPD. MEC/mPEC ratios after these adjustments are 0.90 for estrogens, and 0.16 for progestins.

Pearson's *r* between log-transformed MECs and mPECs (expressed as DPD) for the 13 bMOA which include APIs with both MECs and mPECs was significantly greater than zero (one-sided p-value <0.003), and the central estimate suggested a stronger association (sample *r*=0.73, with a 95% confidence interval for the population value of *r* being 0.41-0.86) than the mPEC:MEC association seen for individual APIs. Pearson's *r* between log-transformed MECs and mPECs for the 38 nMOA which include API with both MECs and mPECs was significantly greater than zero (one-sided p-value <0.0001), with the central estimate (sample r=0.57, with a 95% confidence interval of 0.34-0.70) falling between the mPEC:MEC association seen for bMOA and that seen for individual API.

4. Conclusions.

Examination of the ARCOS database suggests previously published (Kostich and Lazorchak, 2008) marketing data-based national average mPECs exceed regulatory data-based estimates, corroborating the intended conservative nature of the marketing data-based estimates. Analysis of ARCOS spatially explicit usage data for 12 APIs, along with CWNS data on local wastewater production rates, suggests local PECs may on occasion exceed national average PECs by about 10-fold. Multiplying national average marketing data-based PECs by an 'application factor' of 10 and comparing the resulting predicted maximum local PECs to published MEC data for 83 APIs corroborates the usefulness of the adjusted mPECs as a reasonable ceiling for measured environmental concentrations.

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API	Market rank	Market kg/yr	ARCOS kg/yr	Ratio	DDmin mg	Market DPD	ARCOS DPD
Fentanyl	108	463	371	1.2	0.29	0.17	0.14
Methylphenidate	63	34988	14053	2.5	10	0.38	0.15
Hydromorphone	186	1825	655	2.8	4	0.049	0.018
Oxycodone	51	86660	29178	3.0	20	0.47	0.16
Methadone	157	14875	4730	3.1	20	0.080	0.025
Amphetamine	32	32839	6485	5.1	5	0.71	0.14
Hydrocodone	7	177184	24082	7.4	5	3.8	0.52
Morphine	60	108786	14319	7.6	30	0.39	0.051
Codeine	69	274219	20265	13.5	90	0.33	0.024

Table 1. Comparing national average PECs. Marketing data-based mPECs compared to ARCOS-based aPECs. Market rank is based on mPECs expressed as days per dose (DPD). Kg/yr is the nationwide mass of API dispensed annually, estimated from marketing data or ARCOS. Ratio is the ratio of the marketing data-based estimate to the ARCOS-based estimate. DDmin is the minimum daily therapeutic dose.

API	50%	75%	90%	95%	99%	ARCOS ng/L	ARCOS DPD	Market DPD	MEC DPD
Amphetamine	0.94	1.49	2.30	2.82	6.06	95	0.14	0.71	0.00044
Cocaine	1.02	1.94	3.10	4.10	7.24	0.96	0.00014		
Codeine	1.35	2.12	3.39	4.18	4.71	298	0.024	0.33	0.081
Fentanyl	1.11	1.64	2.28	2.85	4.69	5.45	0.14	0.17	
Hydrocodone	1.12	1.73	2.58	3.06	8.28	354	0.52	3.8	0.28
Hydromorphone	1.10	1.78	2.90	3.65	6.41	10	0.018	0.049	
Meperidine	0.85	1.34	2.45	3.37	5.78	71	0.0017		
Methadone	0.95	1.77	3.05	3.82	5.92	70	0.026	0.080	
Methamphetamine	1.09	2.66	5.15	6.11	9.73	0.18	0.00026		0.050
Methylphenidate	0.97	1.48	2.12	2.59	4.33	207	0.15	0.38	
Morphine	1.19	1.80	2.77	3.77	5.83	211	0.051	0.39	
Oxycodone	1.07	1.61	2.50	3.42	5.77	429	0.16	0.47	0.055
Sum(stim) N=4	0.97	1.49	2.19	2.70	5.18		0.29	1.1	
Sum(opiates) N=8	1.17	1.65	2.35	3.02	6.29		0.93	5.3	
Sum(all) N=12	1.14	1.66	2.17	2.86	5.10		1.2	6.4	

Table 2. PEC variability. PEC wastewater volume percentiles, relative to the national average ARCOS-based aPEC. DPD is the national average ARCOS-based aPEC, marketing data-based mPEC, or maximum MEC, expressed as doses per decade. The sums represent sums of DPD across stimulants (stim), opiates, or all 12 APIs.

API	MEC ng/L	mPEC ng/L	MEC/ mPEC	MEC DPD	mPEC DPD	Sample count
ethinyl estradiol	273	6.7	41	100	2.4	495
ofloxacin	23500	2505	9.4	1.4	0.15	124
azithromycin	14900	1631	9.1	0.44	0.048	101
norethindrone	872	124	7.0	6.4	0.91	78
trimethoprim	37000	8934	4.1	1.7	0.41	995
atenolol	14200	4343	3.3	2.1	0.63	386
ciprofloxacin	5600	1908	2.9	0.082	0.028	538
warfarin	330	162	2.0	1.2	0.59	381
citalopram	600	327	1.8	0.22	0.12	22
naproxen	24600	16212	1.5	0.36	0.24	293
ibuprofen	68700	48001	1.4	2.5	1.8	1027
metformin	47253	36331	1.3	1.4	1.1	144
gemfibrozil	4770	4264	1.1	0.029	0.026	527
propranolol	1900	2075	0.9	0.46	0.50	117

Table 3. Top MEC/mPEC ratios. Highest reported MECs compared to mPECs. DPD is the concentration expressed as doses per decade. Sample count is the number of samples on which the maximum MEC is based.

API	MEC ng/L	mPEC ng/L	MEC/ mPEC	MEC DPD	mPEC DPD	Sample count
ethinyl estradiol	273	6.7	41	100	2.4	495
mestranol	407	NA	NA	59	NA	72
norethindrone	872	124	7.0	6.4	0.91	78
ibuprofen	68700	48001	1.4	2.5	1.8	1027
atenolol	14200	4343	3.3	2.1	0.63	386
hydrochlorothiazide	2950	13947	0.2	1.7	8.1	8
trimethoprim	37000	8934	4.1	1.7	0.41	995
metformin	47253	36331	1.3	1.4	1.1	144
ofloxacin	23500	2505	9.4	1.4	0.15	124
metoprolol	2269	7536	0.3	1.3	4.4	88
warfarin	330	162	2.0	1.2	0.59	381
betamethasone	25	93	0.3	0.73	2.7	8

Table 4. Top MEC by DPD. DPD is the concentration expressed as doses per decade. Sample count is the number of samples on which the maximum MEC is based.

Broad MOA	MEC DPD	PEC1 DPD	MEC #API	mPEC1 #API	mPEC2 #API	MEC/ mPEC1	PEC1/ mPEC2
anti-arthropod	0.0027	0.0034	1	1	1	0.79	1
anti-bacterial	3.8	1.6	26	14	32	2.3	0.84
anti-coagulant	1.2	0.59	1	1	5	2.0	0.8
anti-fungal	0.00066	NA	1	0	8	NA	NA
anti-helminthic	0.0013	NA	1	0	0	NA	NA
anti-hyperglycemic	2.2	3.3	3	3	6	0.66	0.85
anti-hypertensive	2.9	18	10	10	36	0.16	0.79
anti-inflammatory	4.4	13	14	10	30	0.33	0.91
bronchodilator	0.00044	0.63	1	1	1	0.001	1
decreases blood viscosity	0.000026	0.049	1	1	1	0.001	1
gastric antacid	0.042	0.35	2	2	9	0.12	0.3
h1 anti-histamine	0.034	0.75	2	2	11	0.046	0.39
lipid modifier	0.45	5.0	6	5	9	0.09	0.92
neurotransmitter modulator	5.8	21	34	30	105	0.28	0.74
reproductive hormone mod.	165	3.5	4	3	21	47	0.72

Table 5. Broad MOA. MECs and mPECs were expressed as doses per decade (DPD) and summed within broadly defined MOA. mPEC1 represents the sum of DPD across API belonging to the MOA that have both MECs and mPECs. mPEC2 represents the sum of DPD across API belonging to the MOA that have mPECs, but may or may not have MECs. 'MEC #API' is the number of API within the MOA that have MECs. 'mPEC1 #API' is the number of API represented by mPEC1. 'mPEC2 #API' is the number of APIs on which mPEC2 is based. All broad MOA with MECs are shown.

Narrow MOA	MEC DPD	mPEC1 DPD	MEC #API	mPEC1 #API	mPEC2 #API	MEC/ mPEC1	PEC1/ mPEC2
estrogen	159	2.4	2	1	1	65	1
quinolone	1.5	0.17	4	2	4	8.4	0.95
progestin	6.4	1.0	2	2	10	6.1	0.5
macrolide	0.47	0.087	3	3	3	5.4	1
folate synthesis inhibitor	1.7	0.74	4	2	2	2.3	1
anti-clotting factor	1.2	0.59	1	1	2	2.0	0.98
pkaa activator	1.4	1.1	1	1	1	1.3	1
tetracycline	0.090	0.094	5	3	3	0.96	1
nsaid	3.6	4.1	9	5	10	0.88	0.97
beta-blocker (adrenergic)	0.54	0.63	3	3	5	0.85	0.66
anti-arthropod	0.0027	0.0034	1	1	1	0.79	1
beta-1-blocker (adrenergic)	3.4	5.0	2	2	3	0.68	0.95

Table 6. Top narrow MOA ratios. MECs and mPECs were expressed as doses per decade (DPD) and summed within narrowly defined MOA. mPEC1 represents the sum of DPD across API that belong to the MOA and that have both MECs and mPECs. mPEC2 represents the sum of DPD across API belonging to the MOA that have mPECs. MEC #API is the number of API within the MOA that have MECs. mPEC1 #API is the number of API on which mPEC1 is based. mPEC2 #API is the number of APIs on which mPEC2 is based. The top 12 of 38 narrow MOA (by MEC/mPEC1) are shown.

Supporting Information Available.

Appendix 1. Maximum measured concentrations of API from 62 peer-reviewed studies.

Appendix 2. Comparing maximum measured concentrations with marketing data-based predicted concentrations.

Appendix 3. Literature references cited in Appendix 1.