Abstract: A framework is presented that is intended to facilitate the evaluation of potential aquatic ecological risks resulting from discharges of down-the-drain chemicals. A scenario is presented using representatives of many of the types of chemicals that are treated domestically. Predicted environmental chemical concentrations are based on reported loading rates and routine removal rates for 3 types of treatment: trickling filter, activated sludge secondary treatment, and activated sludge plus advanced oxidation process as well as instream effluent dilution. In tier I, predicted effluent concentrations were compared with the lowest predicted-no-effect concentration (PNEC) obtained from the literature using safety factors as needed. A cumulative risk characterization ratio (cumRCR) < 1.0 indicates that risk is unlikely and no further action is needed. Otherwise, a tier 2 assessment is used, in which PNECs are based on trophic level. If tier 2 indicates a possible risk, then a retrospective assessment is recommended. In tier I, the cumRCR was > 1.0 for all 3 treatment types in our scenario, even though no chemical exceeded a hazard quotient of 1.0 in activated sludge or advanced oxidation process. In tier 2, activated sludge yielded a lower cumRCR than trickling filter because of higher removal rates, and the cumRCR in the advanced oxidation process was << 1.0. Based on the maximum cumulative risk ratio (MCR), more than one-third of the predicted risk was accounted for by one chemical, and at least 90% was accounted for by 3 chemicals, indicating that few chemicals influenced the mixture risk in our scenario. We show how a retrospective assessment can test whether certain chemicals hypothesized as potential drivers in the prospective assessment could have, or are having, deleterious effects on aquatic life.

Keywords: Prospective risk; Down-the-drain; Contaminants of emerging concern; Retrospective risk assessment; Wastewater treatment

INTRODUCTION

Regulatory bodies around the world have typically evaluated ecological risks on a chemical-by-chemical basis, in which it is assumed that the environment is protected if each chemical is regulated at or below its safe concentration. More recent developments in the European Union and elsewhere, however, have raised concerns about this approach, citing instances in
which the mixture or combined exposure may pose potential risks even though each of the component chemicals is at or below its safe concentration. A number of researchers and organizations have developed approaches for evaluating ecological risks of chemical mixtures, many of which require knowledge of the chemicals comprising the mixture, their exposure concentrations, and their effect threshold levels (Meek et al. 2011; European Centre for Ecotoxicology and Toxicology of Chemicals 2011; Kienzler et al. 2014; Umweltbundesamt 2014; Altenburger et al. 2015). The complexity of mixtures being released into the environment presents formidable challenges both for environmental risk assessors and for regulatory frameworks that are seeking a national or multinational approach for situations where the chemical composition of the wastewater mixture is often unknown, difficult to define, or varied.

Concerns about underestimating chemical mixture risks to aquatic life have been especially directed toward municipal wastewater treatment plant (WWTP) discharges, which are known to be complex mixtures (Anderson 2008; Diamond et al. 2010; Ohlinger et al. 2013). Municipal WWTPs are often a source of various types of down-the-drain chemicals from diverse products, but the particular mixture present in a given discharge will depend on the type of wastewater treatment used and its operation efficiency, population size, associated wastewater flow, and perhaps the geographic region and/or climatic regime (Diamond et al. 2011). Prospective risk assessments of WWTP discharges have not typically used mixture exposure information in a meaningful way, and incorporating such information is challenging. Prospective assessments of down-the-drain chemicals in WWTP discharges ideally require knowledge of the various chemicals that could be present in the effluent after treatment, as well as knowledge of their potential effects and mode of action. In addition, information pertaining to potential exposure in the receiving waterbody is desirable, which will be influenced at least in part by available effluent dilution and fate properties of the chemicals in the receiving waterbody.

Despite the challenges noted above, the evaluation of potential ecological risks from WWTP discharges is believed to be tractable for down-the-drain chemicals. Over the past 15 yr or more, considerable progress has been made in characterizing chemicals in municipal WWTP effluents, including contaminants of emerging concern, in relation to different types of wastewater treatment (Miege et al. 2008; Martinez et al. 2012; Saweson et al. 2010). This research has shown that certain types of treatment are more efficient than others at removing a wide variety of down-the-drain chemicals. For WWTPs with greater removal efficiencies, the potential mixture discharged to receiving waters should contain fewer chemicals that could cause adverse effects. In addition, extensive research on consumer usage of household products and pharmaceuticals has shown that the concentration of chemicals from domestic use of consumer products may be relatively constant over time, given a certain population size and geographic region (Drewes et al. 2009). This knowledge can then be used to select representative chemicals with different physicochemical properties for an evaluation of those with the greatest potential for causing adverse effects. This prospective approach allows one to assess chemicals individually and also within the context of a mixture.

The present study presents a framework for conducting a mixture assessment of down-the-drain chemicals in WWTP discharges.
effluents using an effluent scenario that has a representative but simplified mixture composition. This framework is intended to facilitate the evaluation of potential aquatic ecological risks resulting from WWTP discharges of down-the-drain chemicals (Figure 1). The framework starts with a generic prospective assessment using conservative assumptions to identify potential ecological risks, followed by a higher resolution prospective assessment as necessary and then by a targeted retrospective assessment if warranted. The prospective assessment in this framework is informed by the type of wastewater treatment used, the population size served by the WWTP, and the available effluent dilution in the receiving water. Our framework is intended to facilitate the process by which ecological risks from domestic WWTP effluents of down-the-drain chemical mixtures can be prioritized in terms of whether single chemicals or the mixture as a whole represent a potential risk, and then verified using retrospective ecologically based assessments.

The framework presented is founded on the principle that screening prospective assessments should provide testable hypotheses regarding predicted ecological risks of specific chemicals or the mixture as a whole, which can be evaluated using more refined prospective assessments as well as site-specific retrospective assessments. The prospective risk assessment necessarily incorporates numerous site-specific uncertainties, and does not consider site-specific characteristics of the receiving waterbody and other mechanisms that may decrease the exposure or bioavailability of the chemicals of concern. Site-specific retrospective assessments help address these uncertainties and provide useful information that can feed into risk management and the refinement of prospective or retrospective risk analyses. For those prospective scenarios in which ecological risk is hypothesized, we present a process for retrospectively evaluating the findings that include a feedback loop for improving future assessments of mixtures. The combination of prospective and retrospective assessments provides an “ecological reality check” (Burton et al. 2012) that can be used to identify the types of WWTP effluent scenarios for which the mixture assessment process can be simplified.

### MATERIALS AND METHODS

#### Scenario examined

We illustrate the framework using a hypothetical domestic wastewater scenario that consists of a small municipality with wastewater originating from domestic sources exclusively. We recognize that more complex scenarios than these occur, including the addition of industrial wastewater input and storm-water runoff. This scenario does not intend to be an actual mixture risk assessment of real-world WWTP effluents in general; however, we believe that the framework we present can be used to address a wide range of conditions.

In this scenario we evaluated a WWTP that receives domestic wastewater from a fairly small population (10,000 people) and is operating such that conventional pollution measures such as biochemical oxygen demand or ammonia do not signal an acute or chronic risk to aquatic life in the receiving waterbody. For illustrative purposes, we assumed a design flow (worst case) of 2000 m³/d for this scenario and a 10-fold dilution of the effluent in the receiving stream.

We qualitatively evaluated 3 types of general wastewater treatment processes on relative loadings of representative down-the-drain chemicals and the types of chemical mixtures that might be observed in the treated effluent. The 3 types of treatment considered are: 1) trickling filter secondary treatment, 2) activated sludge secondary treatment, and 3) advanced oxidation following activated sludge secondary treatment (advanced oxidation process). The latter is reported to be an effective means of degrading or removing many types of chemicals that are not otherwise amenable to biological treatment (Saweson et al. 2010; US Environmental Protection Agency 2010). We recognize that within each of these types of treatment there is a range of effectiveness depending on their design (e.g., hydraulic retention time, sludge retention time) and other aspects of the treatment process (e.g., headwork equalization, clarification, and type of disinfection). The 3 treatment types used in the present study are meant to illustrate a range of treatment effects on down-the-drain chemical mixtures that might be used to help simplify prospective mixture assessments on regional or national scales. Site-specific contaminant removal information could provide more accurate risk predictions using our framework.

#### Selection of chemicals for the prospective assessment

To select representative down-the-drain chemicals, we found it helpful to first identify key activities that could result in the disposal of chemicals down the drain (Figure 2). Activities involving the domestic use of chemicals and their subsequent disposal to a WWTP include laundry care, home care, health care, personal care, and food. Laundry care, health care, and

![FIGURE 2: Associations between domestic releases of chemicals from product use and household articles and chemical classes considered in the scenario.](image)
food correspond to different types of product classes for purposes of this scenario: detergents, human drugs, and food additives, respectively. In addition, 3 product types were identified for personal care activities: shampoos, soaps, and sunscreens (Figure 2). We recognize that there are additional products or classes, but the ones included are considered representative for the purpose of illustrating our framework.

For each of the product classes identified in Figure 2, chemicals were selected based on the availability of usage information, from which loadings and effluent concentrations could be derived, and which had published effects data. Where feasible, we attempted to include chemicals that have been reported in domestic wastewater fairly frequently (Diamond et al. 2011; Dickenson et al. 2011; Petrie et al. 2015) and/or have been considered high priority by some researchers in terms of potential ecological risk (Diamond et al. 2011; Maruya et al. 2014). Table 1 lists the chemical classes and specific chemicals considered in this scenario.

**Deriving chemical concentrations of the mixtures for the prospective assessment**

Chemical loading rates were derived as per capita per day use rates and expressed as grams per capita per day. The underlying consumption data for the pharmaceuticals are based on the European Union country with the maximum per capita use for each medicinal product based on 2008 usage data (Eurostat 2015). For substances used in cleaning products, product consumption rates using the Environmental Safety Check tool (Pickup et al. 2016) were used in combination with typical concentrations of the substance in the respective product. In the case of personal care products, annual sunscreen consumption rates and typical concentrations of the nanoparticles zinc oxide (ZnO) and titanium oxide (TiO) were obtained from the German association of personal care product makers (The German Cosmetic, Toiletry, Perfumery and Detergent Association 2016).

The loading rate for these substances is calculated by dividing the annual sunscreen consumption rate by 120 d/yr, thereby accounting for the limited portion of the year in which these products are used. Data for caffeine and acesulfame consumption were obtained from the open literature. Loading rates used for all of the chemicals evaluated in the prospective assessment are presented in Table 1 and referenced in the Supplemental Data, Table S1.

The loadings of readily degradable down-the-drain chemicals such as shorter chain linear alkyl surfactants are known to be affected by the size and complexity of the sewershed serving the WWTP (Human and Environmental Risk Assessment 2013). A recent update entitled “Environmental Safety of the Use of Major Surfactant Classes in North America” (Cowan et al. 2014) indicated that in-sewer losses of alkyl sulfates, alkyl ethoxylate sulfates, and linear alkyl benzene sulfonates (LAS) typically ranged from 50 to >90%. In our scenario, the population being served is small such that the sewershed is assumed to be fairly short and linear. Given this type of sewershed, we assumed that there is little opportunity for degradation of surfactants and other down-the-drain chemicals. Assuming no loss in transit of down-the-drain chemicals from the home to the WWTP is probably a conservative assumption, which is appropriate for a screening prospective assessment (The German Cosmetic, Toiletry, Perfumery and Detergent Association 2016). Site-specific information regarding concentrations of contaminants entering the WWTP could be used in higher tier prospective assessments to more accurately characterize loading rates.

Removal (degradation, sorption) of chemicals in the WWTP and, therefore, the concentration of chemicals discharged to the receiving waterbody depends on the type of wastewater treatment and its operation performance. Several sources were consulted to estimate removal rates of each of the chemicals in our scenario for the 3 treatment types. In addition to using published removal rate information for these chemicals, we relied on physicochemical properties of the selected chemicals and respective use class examined in the mixture assessment scenario, associated per capita use, and removal rates based on literature values for trickling filter (TF), activated sludge (AS), and activated sludge with advanced oxidation (AOP) treatment.

### Table 1: Chemicals and respective use class examined in the mixture assessment scenario, associated per capita use, and removal rates based on literature values for trickling filter (TF), activated sludge (AS), and activated sludge with advanced oxidation (AOP) treatment

| Chemical Name | Chemical Class | Per capita use rate (g/cap/d) | TF  | AS  | AOP |
|---------------|----------------|-------------------------------|-----|-----|-----|
| Linear alkyl surfactants (LAS) | Surfactant | 6.0E-01 | 85 | 90 | 95 |
| Benzalkonium chloride (BAC) | Quaternary amine | 8.2E-03 | 80 | 90 | 95 |
| Zinc acetate (ZnAc) | Inorganic | 3.2E-03 | 70 | 74 | 90 |
| Galaxolide (HHCB) | Fragrances | 2.0E-02 | 40 | 56 | 80 |
| Zinc oxide (ZnO) | UV blocker | 6.5E-02 | 50 | 74 | 85 |
| Titanium oxide (TiO) | UV blocker | 9.8E-02 | 40 | 87 | 90 |
| Ethynylestradiol (EE2) | Human drug | 1.8E-06 | 30 | 82 | 88 |
| Ibuprofen (IBU) | Human drug | 1.9E-02 | 85 | 90 | 92 |
| Carbamazepin (CMZ) | Human drug | 1.4E-03 | 15 | 22 | 22 |
| Sulfomethoxazole (SMX) | Human drug | 5.0E-03 | 40 | 58 | 95 |
| Methylisothiazolinone (MI) | Disinfection product/Antimicrobial | 6.0E-04 | 35 | 50 | 80 |
| Acesulfame (AcS) | Sweetener | 1.5E-02 | 20 | 27 | 60 |
| Caffeine (CAF) | Food additive | 3.0E-01 | 80 | 84 | 95 |
| 1-H-Benzotriazole (BTZ) | Corrosion Inhibitor | 3.0E-03 | 0 | 0 | 65 |

*See Supplemental Data for more details.*
chemicals, which were obtained from various sources, as noted in the Supplemental Data, Table S2. Table S3 in the Supplemental Data summarizes the removal rates reported in the literature for the chemicals for each of the 3 types of wastewater treatment. Both trickling filter and activated sludge treatment types have variable effectiveness at removing the selected chemicals, but overall, trickling filter technology has been shown to be less effective at removing some personal care and pharmaceutical chemicals than activated sludge treatment (Guškowska et al. 2008; Gardner et al. 2013; Kasprzyk-Hordern et al. 2009). For example, little carbamazepine is removed or degraded using typical trickling filter treatments, but removal rates are generally higher using activated sludge treatments (Kasprzyk-Hordern et al. 2009).

The addition of more advanced treatment such as advanced oxidation process following activated sludge treatment often provides higher removal rates (approaching 100%) than activated sludge treatment alone for many down-the-drain chemicals, including those identified in our scenario (Saweson et al. 2010; Drewes et al. 2009). Thus more advanced treatment such as advanced oxidation often degrades or removes many readily biodegradable chemicals as well as most of the other chemicals selected in this scenario (see removal rates in the Supplemental Data, Table S3). It should be acknowledged that the actual mixtures of chemicals in effluents from more advanced treatment may in fact be more complex than those from WWTPs with lower levels of treatment because of a potentially greater presence of transformation byproducts. However, it has been generally shown that the toxicity or endocrine effects of wastewaters after advanced treatment is low, and therefore the risk to aquatic life is likely to be lower than for those effluents receiving less advanced treatment (Boxall et al. 2004; Bundschuh and Schulz 2011).

Average removal rates based on the range of literature values reported for each treatment type and chemical are listed in Table 1. Average removal rates were used with the loading rates described above and are shown in Table 1 to derive predicted effluent concentrations for each chemical and treatment type (Table 2) using the formula:

\[
\text{Predicted environmental concentration} = \text{per capita use rate} \times \text{number of inhabitants} \times \frac{1 - \text{WWTP}_{\text{Removal}}}{\text{downstream river flow}}
\]

where per capita use rate is expressed in g/capita/d for each chemical, number of inhabitants is 10,000 people in our scenario, 1–WWTP\text{Removal} is the removal rate for each chemical and treatment type expressed as a decimal between 0.000 and 1.000, and downstream river flow, which in our scenario is the effluent flow of 2000 m\textsuperscript{3}/d diluted 1:10 with the river under dry weather conditions, or 20,000 m\textsuperscript{3}/d. Table 2 presents predicted environmental concentrations for each chemical in the receiving stream for each type of treatment given the Equation 1 above.

### Effects data for prospective assessment

Aquatic effects data for standard toxicity endpoints were obtained for each of the selected chemicals from various published sources. The data were collected for 3 species groups (algae, daphnia, and fish), representing primary producers, primary consumers, and predators. As an additional data source, the Environmental Safety Check tool (Pickup et al. 2016) was utilized, which provides environmental safety evaluations of cleaning and maintenance products. Supporting documentation was also accessed via the web link (Pickup et al. 2016). The LAS and quaternary amine benzalkonium chloride (BAC) are mixtures in themselves and consist of several homologs and (in the case of LAS) isomers. Because the exact composition of these mixtures in the environment is not known, effects data for these substances were chosen such that they reflect an average composition of the substances as used in commercial products. Toxicity evaluations for LAS and BAC have been performed by

| Chemical | PEC: TF | PEC: AS | PEC: AOP | Tier 1 PNEC | Tier 2 Fish chronic | Tier 2 Crustacea chronic | Tier 2 Algae chronic |
|----------|---------|---------|----------|-------------|---------------------|-------------------------|----------------------|
| LAS      | 0.0450  | 0.0300  | 0.0000   | 0.27        | 0.27                | 0.29                    | 2.4                  |
| BAC      | 0.0008  | 0.0004  | 0.0000   | 0.00114     | 0.17                | 0.0114                 | ND                   |
| ZnAc     | 0.0005  | 0.0004  | 0.0002   | 0.0078      | 2.1                 | 0.28                    | 0.0078               |
| HHCB     | 0.0059  | 0.0044  | 0.0020   | 0.0044      | 0.068               | 0.044                   | 0.201                |
| ZnO      | 0.0163  | 0.0085  | 0.0033   | 0.0206      | 100                 | 0.206                   | 0.017                |
| TrO      | 0.0293  | 0.0063  | 0.0015   | 0.184       | 29                  | 0.0184                  | ND                   |
| EE2      | 6.13E-07| 1.58E-07| 8.76E-08 | 3E-07       | 3.0E-07             | 0.07                    | ND                   |
| IBU      | 0.0014  | 0.0009  | 0.0009   | 7.1         | 25                  | 14                      | 34                   |
| CMZ      | 0.0006  | 0.0005  | 0.0005   | 0.025       | 25                  | 0.025                   | >100                 |
| SMX      | 0.0015  | 0.0011  | 0.0001   | 0.0018      | 800                 | 8                       | 90                   |
| MI       | 0.0002  | 0.0002  | 0.0000   | 0.001       | 0.477               | 0.093                   | 0.03                 |
| AcS      | 0.006   | 0.0055  | 0.0026   | 1           | >1000               | >1000                   | ND                   |
| CAF      | 0.03    | 0.0240  | 0.0075   | 0.087       | 8.7                 | 18.2                    | 6.25                 |
| BTZ      | 0.0015  | 0.0000  | 0.0000   | 0.1         | 18                  | 1.58                    | 10                   |

*See Table 1 for definitions of chemical acronyms.

PEC = predicted environmental concentration; TF = trickling filter; AS = activated sludge; AOP = advanced oxidation process; PNEC = predicted no-effect concentration; ND = data not available.
Human and Environmental Risk Assessment (2013) and Environmental Safety Check (Pickup et al. 2016), respectively.

For the tier 1 assessment, predicted no-effect concentrations (PNECs) were derived as threshold values following the European Union guidance (European Commission 2003) by applying assessment factors to the lowest reported aquatic toxicity effects data as the PNEC. The effects data used, the sources of information, and the assessment factors used are summarized in the Supplemental Data, Table S4. These PNEC values are equivalent to the reference toxicity values used in traditional single-compound risk assessments. The PNEC values were used as the preferred endpoint for this prospective mixture risk framework because they are likely to be a reasonable worst-case effect endpoint for initial tier 1 screening.

For the tier 2 prospective assessment, the lowest chronic PNEC was determined for species in each of 3 groups (fish, algae, and crustaceans). If chronic toxicity values were not available, assessment factors were applied consistent with European Union guidance (European Commission 2003) to derive a species PNEC. The lowest PNEC for each species group was used to derive risk characterization ratios (RCRs) for each chemical (see Calculation of prospective risk section below). We acknowledge that for some chemicals it is not known whether the European Union assessment factors adequately incorporate potential endocrine disruption effects (Vestel et al. 2016). Differentiating between species groups provides enhanced diagnostic resolution and may help identify the species group most at risk. This information should be helpful in targeting the retrospective assessment.

For ZnO, the tier 1 threshold values reflects the toxicity of the Zn²⁺ ion, which is considered to be the cause of toxic effects. The tier 2 threshold values for ZnO and TiO are based on tests with nanomaterials, which are the actual ingredients of the sunscreen products. According to Notter et al. (2014), the nanoparticle form of these substances contributes to the effect.

Calculation of prospective risk

The mixture risk assessment for aquatic life was calculated using the approach shown in Figure 1. In the initial tier 1 screening assessment, an RCR was computed for each chemical and treatment type based on its predicted environmental concentration, and the PNEC value was obtained as described in Effects data for prospective assessment. The RCRs for individual chemicals were then summed, providing a cumulative RCR (European Commission 2003) as a measure for approximating the risk of the mixture for each treatment type. This process of risk additivity corresponds to the concept of “concentration addition,” that is, assuming all materials have a similar mode of action. Although we recognize this is not necessarily true, there is a general consensus that this is a suitable screening level approach (Dyer et al. 2011). If the cumulative RCR (sum of individual RCRs) is < 1.0 at tier 1, the mixture risk is considered to be acceptably low and further steps, including retrospective analyses, are not warranted.

If the cumulative RCR (sum of individual RCR) ≥ 1.0 in the tier 1 assessment for a given treatment type and mixture, then a tier 2 assessment is recommended in which a more refined examination of effects information based on trophic level is used. For tier 2, a cumulative RCR was computed for 3 organism groups (fish, daphnia, and algae) for each chemical and treatment type based on its predicted environmental concentration and published or estimated chronic toxicity values. The RCRs of individual chemicals were then summed separately for each organism group assuming concentration addition, which is thought to be a conservative assumption in most cases (Umweltbundesamt 2014; Drescher and Boedeker 1995). The summed hazard quotients (cumulative RCRs) for each organism group were then compared among treatments. For a few chemicals (primarily for the algae group) we were unable to identify robust toxicity data. In these cases, a chemical PNEC was not available and was not included in the cumulative RCR calculation for each wastewater treatment type. Note that the chronic toxicity values collected in tier 2 are not necessarily the toxicity estimates on which the PNEC of tier 1 is based.

Based on the various treatment types and the chemicals selected for this scenario, we attempted to identify those conditions under which risk was simplified in terms of the number of chemicals driving the overall mixture risk. The incremental contribution of each chemical to the cumulative RCR was examined in the initial tier 1 assessment and for each organism group in the tier 2 assessment by type of treatment, to evaluate the relative importance of each chemical to the overall mixture risk. We also calculated the maximum cumulative risk ratio (MCR; Price and Han 2011) for each organism group and treatment type, to evaluate whether mixture toxicity was driven in large part by a few chemicals (and therefore a simpler mixture) or by many chemicals (more complex mixture). The MCR was modified (Price et al. 2012) to be a generic ratio based on the chronic toxicity values available for each chemical. The MCR was calculated as the quotient of the sum of the individual RCR values (HI) over the maximum hazard quotient (MHQ) within that mixture (Equation 2). Importantly, the MCR provides an indicator as to whether chemicals in a mixture are operating under an independent suite of modes of actions, and therefore allowing one or a few materials to drive the mixture assessment.

\[ \text{MCR} = \frac{\text{HI}}{\text{MHQ}} \]  

(2)

The use of the MCR as well as the tier 2 assessment based on organism groups should be helpful in the retrospective assessment. Information regarding the complexity of the mixture’s risk and the type of biota at greatest risk is obtained using this prospective approach, which provides specific hypotheses that can be directly tested in the retrospective assessment.

RESULTS OF PROSPECTIVE RISK ANALYSIS OF HYPOTHETICAL SCENARIO

The predicted environmental concentrations of chemicals for the hypothetical scenario and the 3 different treatments are shown in Table 1. Generally the highest concentrations are predicted for the effluent of the trickling filter treatment, with
concentrations ranging from 1 ng/L (ethinylestradiol [EE2]) to approximately 0.15 mg/L (caffeine). In the activated sludge treatment, environmental concentrations ranged from <1 ng/L for EE2 to 0.024 mg/L for caffeine. With the advanced oxidation treatment, concentrations for all chemicals were ≤0.003 mg/L (galaxolide).

Under the tier 1 assessment of this hypothetical mixture, the cumulative RCR was greater than 1.0 for all 3 treatment types (Figure 3). Thus, unacceptable mixture risk was hypothesized for all 3 treatment types even though no one chemical exceeded a hazard quotient of 1.0 in either the activated sludge or the advanced oxidation treatment. Using a more refined approach in tier 2 for this scenario, far less risk was indicated for all 3 treatment types and especially for the trickling filter treatment (Table 3). The cumulative RCR was highest in the trickling filter treatment over the 3 organism groups (RCR ≥ 4) as expected because of low removal rates of several chemicals such as BAC and galaxolide (HHCB; Table 3). The activated sludge treatment yielded a lower cumulative RCR (<1) than the trickling filter treatment, as expected given the better removal rates, especially for LAS, BAC, and ZnO. The advanced oxidation treatment resulted in a cumulative RCR that was approximately 0.2 in the tier 2 assessment using the exposure and effects data identified in this scenario. Thus, for the chemical mixture used in this scenario, evaluating effects data based on organism groups separately decreased the cumulative RCR by more than 50% for

**TABLE 3:** Risk characterization ratios (RCRs) by treatment type for each of 3 organism groups in the tier 2 prospective risk assessment

| Chemical | Trickling filter | Activated sludge (AS) | AS + advanced oxidation process (AOP) |
|----------|------------------|------------------------|---------------------------------------|
|          | Fish  | Daphnia | Algae | Fish  | Daphnia | Algae | Fish  | Daphnia | Algae |
| LAS      | 0.167 | 0.155   | 0.019 | 0.111 | 0.103   | 0.013 | 0.000 | 0.000   | 0.000 |
| BAC      | 0.005 | 0.072   |       | 0.002 | 0.036   |       | 0.000 | 0.000   |       |
| ZnAc     | 0.000 | 0.002   | 0.061 | 0.000 | 0.001   | 0.053 | 0.000 | 0.001   | 0.020 |
| HHCB     | 0.087 | 0.135   | 0.030 | 0.064 | 0.099   | 0.022 | 0.029 | 0.045   | 0.010 |
| ZnO      | 0.000 | 0.789   | 0.956 | 0.000 | 0.410   | 0.497 | 0.000 | 0.158   | 0.191 |
| TiO      | 0.001 | 1.590   |       | 0.000 | 0.344   |       | 0.000 | 0.079   |       |
| EE2      | 2.046 | 0.000   |       | 0.526 | 0.000   |       | 0.292 | 0.000   |       |
| IBU      | 0.000 | 0.000   | 0.000 | 0.000 | 0.000   | 0.000 | 0.000 | 0.000   | 0.000 |
| CMZ      | 0.000 | 0.024   | 0.010 | 0.000 | 0.022   | 0.010 | 0.000 | 0.022   | 0.010 |
| SMX      | 0.000 | 0.000   | 0.000 | 0.000 | 0.000   | 0.000 | 0.000 | 0.000   | 0.000 |
| Mi       | 0.000 | 0.002   | 0.007 | 0.000 | 0.002   | 0.005 | 0.000 | 0.000   | 0.002 |
| AcS      | 0.000 | 0.002   |       | 0.000 | 0.000   |       | 0.000 | 0.000   |       |
| CAF      | 0.003 | 0.002   | 0.005 | 0.003 | 0.001   | 0.004 | 0.001 | 0.000   | 0.001 |
| BTZ      | 0.000 | 0.001   | 0.000 | 0.000 | 0.001   | 0.000 | 0.000 | 0.001   | 0.000 |
| Sum RCR  | 2.310 | 2.831   | 1.087 | 0.707 | 1.060   | 0.603 | 0.323 | 0.316   | 0.234 |

*See Table 1 for definitions of chemical acronyms. Blank cells indicate lack of relevant toxicity data with which to calculate an RCR.*
the 3 treatment types, and an acceptable risk was predicted for the activated sludge and advanced oxidation effluent mixtures.

In the tier 1 assessment, the number of chemicals contributing to the mixture risk, as indicated by the MCR, was greatest for the trickling filter treatment followed by the activated sludge and the advanced oxidation treatments (Table 4). The types of chemicals contributing to the cumulative RCR for each treatment differed between the trickling filter treatment and the activated sludge and advanced oxidation treatments (Table 4). The substances LAS, BAC, ZnO, HHCB, and carbamazepine all contributed similarly to the RCR in the trickling filter treatment, whereas only HHCB, EE2, and carbamazepine contributed to most of the cumulative RCR for the activated sludge and advanced oxidation treatments. The decreased contribution of LAS, BAC, and ZnO to the cumulative RCR in the activated sludge treatment, and more so in the advanced oxidation treatment, was because of the greater removal rates of these chemicals compared with the trickling filter treatment. By contrast, the values of MCR for the tier 2 assessment ranged between 1.1 and 2.0 (Table 3), indicating that more than one-third of the predicted risk is accounted for by one chemical.

At least 90% of the cumulative risk in our scenario was accounted for by 3 chemicals, although the specific chemicals varied with the species group. Under the activated sludge treatment, our modeled results suggest that EE2, HHCB, and LAS comprise most (99%) of the cumulative RCR for fish; TiO, ZnO and HHCB, or BAC comprised more than 92% of the cumulative risk for daphnids; and ZnO, zinc actinium, and HHCB accounted for 98% of the cumulative risk for algae for the different treatments (Table 4). These results can provide useful information regarding the need to incorporate additional prospective assessment refinements and/or certain retrospective assessment analyses.

With the advanced oxidation treatment, modeled results suggest that whereas the aggregated risk will be reduced for all species...
groups, the relative importance of the major chemical drivers remains more or less unchanged from that of the activated sludge treatment (Table 4). The lower RCR and MCR values for the tier 2 assessment compared with those obtained using the more conservative tier 1 assessment indicates that the top 3 chemicals explain most of the expected mixture effects, that is, fewer chemicals are responsible for the overall risks when effects are attributed by organism group (Figure 4).

Because the tier 2 assessment indicated acceptable risks for the activated sludge and advanced oxidation treatments given the scenario used, it may not be necessary to further refine the risk assessment in this case (see Figure 1). In the case of the trickling filter treatment, or, if a mixture risk had been identified for the activated sludge or advanced oxidation treatments in the tier 2 assessment (e.g., if predicted environmental concentrations were much higher because effluent flow was not diluted in-stream as much as assumed in our scenario), further prospective assessment refinements or a retrospective assessment would be warranted in those cases.

In the tier 2 assessment, the risk of the chemical mixture is based on concentration addition for each organism group, which assumes that the chemicals in our hypothetical scenario have the same mechanism of action. Concentration addition has been shown to yield typically a conservative (i.e., more protective) component-based mixture effect prediction (Drescher and Boedeker 1995). However, the key chemicals that drive the risk in our scenario for both the activated sludge and the advanced oxidation process treatments do not have the same mechanism of action, and therefore concentration addition would tend to overestimate the potential mixture risk. The HHCB is a musk fragrance that interferes with Cyp isoenzymes, EE2 is a synthetic hormone that is an estrogen receptor agonist, carbamazepine is an anticonvulsant drug that reduces polysynaptic responses and blocks the post-tetanic potentiation, and sulfomethoxazole (SMX) is an antimicrobial agent that inhibits the synthesis of dihydrofolic acid, an intermediate step in the formation of tetrahydrofolic acid. Following the approach of Price et al. (2012), deviations from dose additivity are expected in this case, suggesting that the tier 2 prospective risk assessment could be further refined by examining chemicals on the basis of independent action (i.e., response addition; Price et al. 2012; Altenburger et al. 2004). However, this would require not only mode-of-action information but also complete dose–response knowledge, which might not be available. The refinement and further simplification of screening prospective assessments for down-the-drain chemicals may benefit from this finding if the types of chemicals that were key risk factors in our hypothetical study are fairly ubiquitous in municipal WWTP effluents.

Although the screening prospective results are hypothetical, there may be some overarching patterns that could serve to simplify the mixture assessment process for down-the-drain chemicals in WWTP discharges in general. First, certain types of chemicals such as LAS and BAC may be regarded as unlikely to contribute to a potential risk in activated sludge WWTP effluents, because they are readily biodegradable. Several other types of down-the-drain chemicals such as ibuprofen, acsesulfame, caffeine, and ben佐otrazole were also very minor contributors to the cumulative risk in all cases in this scenario. These hypothetical results suggest that mixture risk of down-the-drain chemicals in WWTP effluents might be simplified by taking into account the type of treatment process used (and ideally its performance) and evaluating predicted effects by organism group, as in the tier 2 assessment. Furthermore, results of the tier 2 assessment can help identify the hypothesized chemical drivers that can then be further examined in a retrospective assessment.

**USING A RETROSPECTIVE ASSESSMENT TO REFINE THE PROSPECTIVE ASSESSMENT**

Once a prospective risk assessment has identified a potential risk, it is important to assess whether the identified chemical(s) actually are causing or could cause effects in the receiving environment. This of course assumes that the chemicals of interest are present in the effluent and a retrospective examination is warranted to support the prospective risk analysis. In retrospective analyses, biological conditions downstream of effluent discharges of interest are examined and used to identify whether an impairment exists, and, if so, whether chemicals in the effluent can be linked as a potential cause. Although the term “impairment” may carry legal and/or regulatory connotations, our use of this term is intended to be more generic, indicating a negative deviation of an expected condition. Retrospective analyses take into account pollutant fate and bioavailability in the receiving waterbody as well as the actual ecosystem. In addition, the retrospective analyses can potentially identify unexpected environmental interactions that can lead to enhancement or elimination of predicted impairments (Kapo et al. 2008).

The properties of the chemicals and characteristics of the receiving environment will greatly affect their fate. Many will degrade (e.g., biodegradation, photolysis), sometimes into chemicals that still have toxic properties, whereas others will partition to organic sediments and inorganic constituents, altering their bioavailability. These processes are influenced by water quality (e.g., pH, total suspended sediments, and dissolved organic carbon), flow (e.g., mass transfer, sedimentation), physical habitat (e.g., depth, temperature, light), and the characteristics of the biological communities present. In addition, effects of chemicals can manifest differently depending on environmental conditions or interactions, and these can be difficult to predict prospectively.

The traditional retrospective assessment approach makes inferences about the effects of the effluent via a comparison with a “reference condition” (European Centre for Ecotoxicology and Toxicology of Chemicals 2011). Reference conditions can consist of sites upstream or outside the influence of an effluent discharge or can consist of a highly defined ecological suite of conditions that must be met such as attainment of in-stream habitat quality and a reduced (or absent) presence of other discharges and human activity. The simplest approach for retrospectively assessing an effluent is using an upstream/downstream approach. This can work especially well when the effluent outfall has minimal confounding factors and appropriate
reference sites are available. However, wastewater outfalls are often (almost always) situated where there are influences of other activities and contaminant inputs upstream such as agricultural and urban runoff. In addition, the volume of discharged wastewater by itself will make a difference in flow conditions upstream and downstream and thereby alter habitat conditions, particularly in the case of small streams with relatively little baseflow. Site conditions will vary temporally (daily/annually), which may alter environmental conditions and exposure. Separating the effects of wastewater is therefore often complicated by the cumulative impacts of other stressors upstream or natural variability at the sites. The site selection and design of sampling must therefore be carefully considered and based on testable hypotheses. Discharge points of stormwater and sewer overflow should also be taken into account when one is selecting study sites, because they can be upstream of the treated wastewater discharge point.

The major advantage of retrospective assessment in the receiving environment is its ecological relevance and integration of the potential interactions into the assessment. This of course is also the major challenge because it becomes difficult to associate specific responses in the environment to individual components of the effluent and to separate them from natural variability. To make linkages across levels of biological organization (adverse outcome pathways [AOPs] within an organism as well as populations and communities) and different trophic levels within an ecosystem, we can conduct a variety of laboratory (effluent) tests to establish or infer causation or at least propose linkages. In the framework presented just below we have highlighted the need to test hypotheses at different levels and stages in the assessment.

The proposed retrospective assessment framework is summarized in Figure 5 and is intended to outline an approach to test the predictions of the prospective assessment in waterbodies.
receiving wastewater effluents. In the current context the proposed approach is therefore not intended to independently define or monitor effects in the environment (a different question). The presence of a specific chemical or effect in the retrospective analyses does not in itself validate or directly confirm the prospective assessment. We promote a weight-of-evidence approach to inform the risk assessment and management. Testing is intended to inform a risk management decision that could range from doing nothing, refining the assessment, establishing specific monitoring, or taking remedial actions (Diamond et al. 2010, 2011).

To test the prospective risk assessment predictions it is necessary to test the effluent and then work toward the more complex receiving environment responses. The first task would be to determine whether there is evidence of chemical mixture exposure as predicted by the prospective risk assessment. The screening prospective assessment of the trickling filter effluent presented in this scenario, for example, indicated that the effluent may contain a mixture of several chemicals that both singly and in combination could present a risk to aquatic life (Figure 3 and Table 2). In this case, a retrospective assessment using the framework shown in Figure 5 could be used to determine whether there is evidence of chemical mixture exposure. Although the figure suggests a linear approach, in the process of going from effluent analysis to measurements downstream of a domestic WWTP discharge, any or all of the steps shown may be used simultaneously or in a different order depending on the site-specific situation and the information available (see, for example, Diamond et al. 2015). Use of more of the steps shown in Figure 5 may lead to a more informed risk management outcome.

The process begins with testable hypotheses indicating the potential for adverse effects because of a chemical and/or mixture as a result of domestic discharge. An initial analysis could be based on verifying the presence of the suspect chemical(s), ensuring that they are bioavailable and have measurable adverse effects on organism health (e.g., AOP testing or whole-effluent toxicity [WET] studies). Because many WWTPs routinely measure WET, it may be possible to examine relationships between WET results and prospective risk assessment results for chemicals eliciting effects that can be measured in WET tests (e.g., direct effects on survival, growth, or reproduction). In some cases, an analytical verification of exposure may be all that is needed to solidify a linkage between the prospective risk assessment and the actual mixture risk. Although laboratory analyses such as WET may indicate whether there is an in-stream risk to aquatic life, it is often useful to also assess the ecological condition of in-stream biota downstream of a WWTP discharge, if feasible. The combination of laboratory and in-stream analyses may provide better evidence of linkages with prospective risk assessment than either approach alone.

The prospective risk assessment gives information on which chemicals may be of interest. Direct analytical chemical confirmation is preferred; however, a surrogate measure of the chemical(s) of interest when analytical techniques are not available or appropriate can be used. Alternative methods could be applied using for example cell assays (e.g., total or trace levels of estrogens, antiandrogens; Escher et al. 2014) or bile of fish exposed to effluents (e.g., rapidly metabolized pharmaceuticals; Togunde et al. 2012). If the specific chemicals of interest are present, this can inform the risk assessment and/or risk management directly. If the chemicals cannot be measured in the effluent, this would also inform the risk management that the detection limits of the methods should be considered carefully. For example, some estrogens (e.g., EE2) may be biologically active at concentrations below most analytical methods available for wastewater effluent.

To assess whether there is evidence of biological exposure (bioavailability) in an effluent, specific standardized tests should be applied to verify that the effluents cause effects. Chronic toxicity endpoints using appropriate test species can be useful for evaluating effects of certain chemicals that prospectively suggest a potential effect cannot be ruled out (e.g., ZnO in the trickling filter effluent [Table 2]) or, when known, a specific response in an AOP (e.g., SMX resistance in the trickling filter effluent). For example, if the endpoint of concern is endocrine disruption through estrogen agonists (e.g., EE2 in the trickling filter effluent in the prospective assessment), a test to determine that fish have altered vitellogenin or reduced egg-laying capacity would be appropriate, especially if it links directly to the assessment endpoints in the prospective risk assessment. In the case where the WWTP uses activated sludge treatment (either with or without advanced oxidation), none of the chemicals prospectively evaluated were likely to result in a risk to aquatic life given the RCRs calculated and the mechanism of action of the few chemicals that had the greatest contribution to the MCR (Table 3). In this case, a retrospective assessment following the framework in Figure 5 could be used to confirm that chemical mixture effects are not observed. This assessment may include not only effluent chemical measurements, but higher order biological endpoints such as chronic effluent toxicity tests using appropriate test species and biological assemblage analyses. These results can also directly inform (provide feedback to) the risk assessment and/or risk management.

Further analysis can address whether field studies indicate a biological response of interest that is consistent with AOPs or predictions associated with a chemical or chemical mixtures as predicted by the prospective risk assessment. It is possible that there are effects/changes observed but they are not associated with those predicted. This can be the case because the effects observed are unrelated to the chemicals being assessed or the predictions were inaccurate or incomplete. For example, eutrophication responses or habitat impairment may be responsible for changes rather than the suspected estrogen agonist. A chemical that acts as an estrogen agonist may also have effects through other pathways that were not considered in the prospective risk assessment. For example, the discharge of antimicrobial agents such as SMX may induce antimicrobial resistance in the receiving system, resulting in unanticipated biological effects (Proia et al. 2016). Also, it may be that multiple outcome pathways have not been explored or established. Complex effluents may contain many chemicals that act through the same outcome pathway (e.g., multiple estrogen agonists) or
interfere with it (e.g., antiestrogens). The responses again inform the process but do not explicitly confirm or contradict the prediction. It is then desirable to determine whether the exposure has led to adverse outcomes at the population or community level. The effects at the higher levels of biological organization are usually more ecologically relevant and are therefore useful as endpoints in assessing risk. Unfortunately, these types of responses are much more difficult to link directly to specific chemicals or mixtures in effluents. At any point the information can inform the risk assessment that could be refined or could be used in risk management decisions. It is expected that as more knowledge is acquired about mechanisms and additional outcome pathways are established, the linkages between specific chemicals (and mixtures) and effects in the environment will become stronger. However a weight-of-evidence approach will still be required, and field-based retrospective assessments will help to focus and reduce uncertainty in prospective assessments.

CONCLUSIONS

Prospective and retrospective risk assessments are 2 important and compatible tools to assess and manage the risk of wastewater effluents entering the environment. Prospective risk assessments contain many uncertainties that are difficult to quantify, especially in complex mixtures such as domestic wastewaters. They rely on minimal data to make extrapolations to complex effluents and environments. However, prospective risk assessments make specific predictions that can be further tested or acted upon. The retrospective risk assessment provides additional information that integrates the complexity of real mixtures and ecosystems. It therefore also contains large uncertainties, especially at higher levels of biological organization where it is difficult to establish direct cause and effect relationships, but it can be applied to test specific hypotheses as well as monitor for change in the environment. It may never be possible to fully account for all chemicals and their potential diverse modes of action on organisms in natural ecosystems, but, given the potential for mixture effects, it is critical to move assessment approaches in this direction. Our ability to quantify chemicals and understand their fate in wastewater systems as well as the receiving environment has improved greatly over recent years, but our ability to predict their effects, especially as mixtures, remains fairly poor. Similarly, although our ability to test for acute and chronic effects in biota exposed to wastewaters has advanced, extrapolation of those results to ecosystems remains challenging. Endocrine disruption is an example of a response that would not have been readily detected using traditional end-of-pipe testing. Although we can add tests to predict some of these responses, there are many potential modes of action for which testing is not yet possible or even contemplated. A combination of approaches (prospective and retrospective) and weight of evidence is therefore required to ensure environmental protection.

Prospective and retrospective assessment tools should be used together within a weight-of-evidence approach to inform risk assessment and risk management of chemicals and effluents. Research needs to continue to fill knowledge gaps that will strengthen our ability to reduce uncertainty in risk predictions. An enhanced ability to make reliable linkages between specific chemicals and an environmental effect of concern (across multiple levels of biological organization) would greatly improve our estimations of risk and our confidence in risk management options.

Supplemental Data—The Supplemental Data are available on the Wiley Online Library at DOI: 10.1002/etc.4013.

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Data availability—Data, associated metadata, and calculation tools are available in the Supplemental Data.

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