Environmental Toxicology and Chemistry—Volume 38, Number 1—pp. 46–60, 2019
Received: 21 August 2018 | Revised: 24 September 2018 | Accepted: 3 October 2018

Critical Review

Critical Review: Grand Challenges in Assessing the Adverse Effects of Contaminants of Emerging Concern on Aquatic Food Webs

Elena Nilsen,[a,*] Kelly L. Smalling,[b] Lutz Ahrens,[c] Meritxell Gros,[c,d] Karina S.B. Miglioranza,[e] Yolanda Picó,[f] and Heiko L. Schoenfuss[g]

[a]US Geological Survey, Oregon Water Science Center, Portland, Oregon, USA
[b]US Geological Survey, New Jersey Water Science Center, Lawrenceville, New Jersey, USA
[c]Department of Aquatic Sciences and Assessment, Swedish University of Agricultural Sciences, Uppsala, Sweden
[d]Catalan Institute for Water Research, Girona, Spain
[e]Laboratory of Ecotoxicology and Environmental Pollution, Mar del Plata University, Mar del Plata, Argentina
[f]Environmental and Food Safety Research Group, Center of Research on Desertification (CIDe), Faculty of Pharmacy, University of Valencia, Valencia, Spain
[g]Aquatic Toxicology Laboratory, St. Cloud State University, St. Cloud, Minnesota, USA

Abstract: Much progress has been made in the past few decades in understanding the sources, transport, fate, and biological effects of contaminants of emerging concern (CECs) in aquatic ecosystems. Despite these advancements, significant obstacles still prevent comprehensive assessments of the environmental risks associated with the presence of CECs. Many of these obstacles center around the extrapolation of effects of single chemicals observed in the laboratory or effects found in individual organisms or species in the field to impacts of multiple stressors on aquatic food webs. In the present review, we identify 5 challenges that must be addressed to promote studies of CECs from singular exposure events to multispecies aquatic food web interactions. There needs to be: 1) more detailed information on the complexity of mixtures of CECs in the aquatic environment, 2) a greater understanding of the sublethal effects of CECs on a wide range of aquatic organisms, 3) an ascertaining of the biological consequences of variable duration CEC exposures within and across generations in aquatic species, 4) a linkage of multiple stressors with CEC exposure in aquatic systems, and 5) a documenting of the trophic consequences of CEC exposure across aquatic food webs. We examine the current literature to show how these challenges can be addressed to fill knowledge gaps. *Environ Toxicol Chem* 2019;38:46–60. © 2018 SETAC

Keywords: Aquatic toxicology; Ecological risk assessment; Endocrine disruptors; Mixtures; Pharmaceuticals; personal care products

INTRODUCTION

Contaminants of emerging concern (CECs) are ubiquitous in surface waters and include pharmaceuticals (stimulants, analgesics, antibiotics, antihistamines, and hormones), personal care products (fragrances, parabens), plasticizers, and flame retardants including perfluoroalkyl and polyfluoroalkyl substances (PFASs), among others. No commonly agreed definition exists for CECs; therefore, we are limiting the discussion of CECs for the purpose of this review to chemicals whose widespread use or ubiquitous presence in the environment in the past few decades has raised concern because analytical limitations and other impediments have hindered their study. Several groups of CECs are of particular concern because of their high rates of human usage, and because some can exert biological effects on nontarget organisms similar to the mode of action for which they were designed. These CECs include natural and synthetic hormones, pharmaceuticals and personal care products, endocrine-disrupting compounds (EDCs), and PFASs (e.g., perfluorooctanesulfonate [PFOS] and perfluorooctanoate). We recognize that this working definition is narrower than that used, for example, by the US Environmental Protection Agency (EPA 2008) and most notably excludes nanomaterials and some persistent organic pollutants. However, the diversity of CECs within and beyond our working definition requires exclusions to retain a focused review.

In recent years, substantial effort has been invested in understanding the presence of CECs in the aquatic environment

* Address correspondence to enilsen@usgs.gov
Published online 8 October 2018 in Wiley Online Library (wileyonlinelibrary.com).
DOI: 10.1002/etc.4290

© 2018 SETAC

(Burkhardt-Holm 2010; Windsor et al. 2017) and their effects on certain species (Tyler et al. 1998; Palace et al. 2009; Kidd et al. 2014). Major sources of CECs to the aquatic environment include small- and large-scale sewage treatment plants (STPs) from municipal and industrial sources as well as hospitals (Heberer 2002; Caliman and Gavrilescu 2009; Blum et al. 2017; Ebele et al. 2017; Gago-Ferrero et al. 2017). Some of these compounds are not completely removed by wastewater treatment processes; once they enter the receiving environment, they degrade slowly in water, sediment, and biofilms (Heberer 2002; Bolong et al. 2009; Kasprzyk-Hordern et al. 2009). Contaminants of emerging concern also enter aquatic environments from landfill leachates, surface runoff, atmospheric deposition, and application of biosolids and manure to agricultural land (Pal et al. 2010; Sui et al. 2015; Ebele et al. 2017).

After CECs are released, their distribution in the environment can be related to their physicochemical properties (e.g., water solubility, solid–liquid distribution, polarity, vapor pressure) and environmental conditions (e.g., pH, organic matter content; Pal et al. 2010; Wilkinson et al. 2017). Some CECs such as flame retardants are transported long range, which results in a global distribution even in remote regions (Liu and Wong 2013; Aus der Beek et al. 2016); other CECs have the potential to accumulate in aquatic food webs. Contaminants of emerging concern can be transformed by biotic (i.e., microorganisms and wildlife) or abiotic (e.g., ultraviolet) processes (Bletsou et al. 2015).

Studies are mostly devoted to lethal and sublethal effects of CECs at the individual level, with limited information on how these effects cascade through the food web via either direct or indirect effects. Very few investigations have focused on the effects of CECs on food webs, although clear detriment has been shown in an experimental lake with a synthetic estrogen (Kidd et al. 2014). To understand the potential impacts of CEC exposure on the structure and function of aquatic communities, more research at multiple levels of biological organization is needed.

In the present review, we identify knowledge gaps and explore the challenges associated with filling them. Although not unique to CECs alone, we posit that there are several critical gaps in comprehending the effects of CECs on food webs that individual and interdisciplinary fields of study have not yet tackled. We address first the challenges of defining and documenting the complex environmental mixtures of CECs and their effects in the aquatic environment (Figure 1). Organisms exposed to complex mixtures often exhibit subtle, sublethal effects that are challenging for toxicologists to identify accurately. The challenge of identifying sublethal effects is compounded by the multigenerational CEC exposure duration for organisms in the aquatic environment, which may delay the occurrence of adverse effects or may result in adaptive evolutionary change to historically exposed populations. Aquatic organisms are exposed to CECs often in concert with additional environmental stressors, resulting in the presence of multiple stressors (not only CEC mixtures but also geomorphological alterations, land-use changes, water abstraction, invasive species, and pathogens). We conclude by discussing the trophic consequences of multigenerational and population-level exposures, and the need for considering delayed effects over longer exposure periods and complex food web structures (Figure 1). These are the thorny challenges presenting obstacles to understanding CEC effects on food webs, and advances will not be achieved until we begin to address them. We acknowledge that these issues are not specific to CECs only; nevertheless, we assert that these issues have not been as completely (if at all) examined relative to CECs as they have been for more traditional contaminants. The present review has been structured around these grand challenges, with the goal of briefly summarizing the studies and data currently available and identifying the critical gaps that remain for each challenge. It is our hope that the present critical review will set the stage for motivated international research consortiums to grapple with these challenges and ultimately solve outstanding problems within this complex topic.

The present review is constrained to CECs in freshwater food webs, with limited mention of examples where freshwater food webs have direct linkages to estuarine and terrestrial food webs. The latter represent important gaps in understanding and should be addressed in detail in future work.

To deal with these challenges, expertise will be required from multiple fields including, among others, biology, ecology, chemistry, and toxicology. Work on these complex challenges will be difficult but not without precedent. Various scientific fields have together achieved wide-ranging and significant understanding of the occurrence and effects of CECs over the past 2 decades via laboratory, mesocosm, field-based, and modeling studies. However, we still lack basic comprehension regarding the effects of CECs on aquatic food webs. We encourage the application of novel approaches and innovative tools to explore these issues.

CHALLENGE 1: COMPLEXITY OF MIXTURES OF CECS IN THE AQUATIC ENVIRONMENT

High concentrations and low removal of some CECs in STPs are of concern and lead to their widespread distribution in the aqueous environment (Calisto and Esteves 2009) as persistent or "pseudo-persistent" contaminants (Daughton 2002) forming complex mixtures. Contaminants of emerging concern are widely used and continuously introduced into the environment. In addition, some CECs are designed to exert biological effects; thus it is expected that they will induce unwanted effects on nontarget organisms. These characteristics present problems that differ from studying the effects of other chemicals. Complex mixtures of CECs are distributed in surface waters worldwide in both heavily impacted and pristine environments, and nontarget organisms are exposed to these mixtures throughout their lives (Pal et al. 2010; Richardson 2010; Ahrens and Bundschuh 2014; Ebele et al. 2017; Richardson and Kimura 2017; Wilkinson et al. 2017).

As a result of the complexity of environmental mixtures, it is necessary to prioritize relevant CEC mixtures and their effects for further study. This is especially crucial because CEC mixtures in some cases lead to more bioaccumulation and stronger effects than would be expected with a single CEC (Ding et al. (2016)).

 © 2018 SETAC

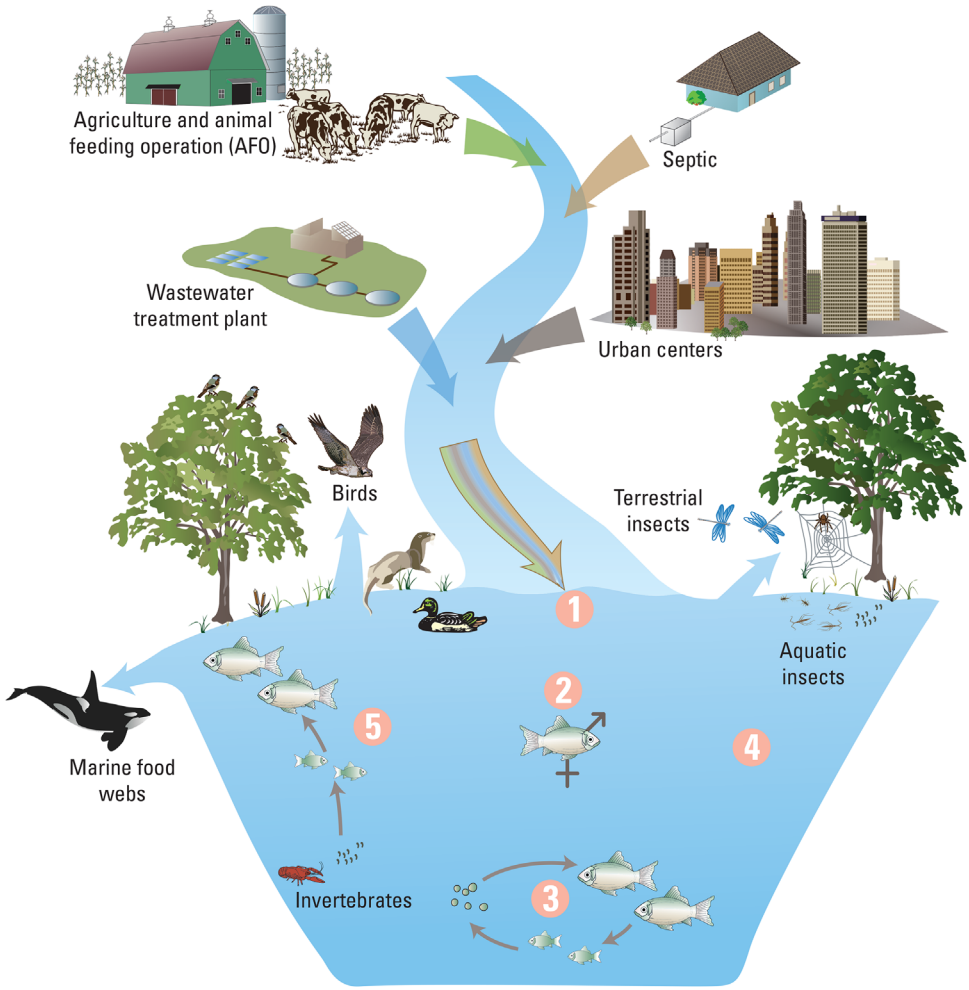

FIGURE 1: Conceptual image illustrating the grand challenges associated with contaminants of emerging concern (CECs) on aquatic food webs. (**1**) Complex contaminant mixtures; (**2**) sublethal effects of CEC exposure; (**3**) long-term, multigenerational exposures; (**4**) multiple stressors; and (**5**) linkages across populations and trophic levels. AFO = animal feeding operation.

For instance, Ding et al. (2016) found that co-exposure to roxithromycin and fluoxetine, and propranolol and fluoxetine, induced stronger antioxidant responses than single pharmaceutical exposures in crucian carp (*Carassius auratus*). Concentration addition models have been employed to predict toxicity of CEC mixtures on a simplified food web in laboratory settings (Watanabe et al. 2016; Oliveira et al. 2017). Altenburger et al. (2018) ran a mixture of micropollutants in several different early life stage, whole-organism assays as well as in vitro bioassays, and found concentration addition to be the most suitable. However, the ecotoxicology of complex CEC mixtures at environmentally relevant concentrations requires further study (Backhaus 2014), particularly at the lower concentration ranges (Orton et al. 2014; Di Nica et al. 2017). Mixture toxicity models such as concentration addition and independent action do not consider any interactions that occur at the toxicokinetic or toxicodynamic level (Backhaus 2014). The bioavailability and effects of complex chemical mixtures of CECs on aquatic food webs in natural matrices have not been adequately explored.

Few data exist regarding the bioaccumulation of CEC mixtures in biota, with most of the available information obtained only for some substances representative of groups of chemicals and in a limited number of species, often in a laboratory setting. Mixtures of polybrominated, flame-retardant congeners have been assessed in limited food webs in natural settings (Stapleton and Baker 2003; Nilsen et al. 2014). Concentrations of a suite of PFASs were found to be higher in piscivorous fish species compared with nonpiscivorous species in a lake in Ethiopia (Ahrens et al. 2016). In a semi-natural mesocosm experiment, it was shown that uptake rates of 5 pharmaceuticals were variable in 4 invertebrate taxa and one fish species (Lagesson et al. 2016). Another study investigated the bioaccumulation and trophic transfer of 23 pharmaceuticals in 14 lake species (Xie et al. 2017). These authors observed interesting patterns in uptake across species and found that fish accumulated higher levels of pharmaceuticals in the liver and brain compared with other tissues; however, they did not see evidence of trophic magnification.

Studies on effects of chemical mixtures in food webs are needed to adequately understand the relevance of trophic position and biomagnification in contaminant transfer within aquatic food webs (Lescord et al. 2015). Outcomes of

© 2018 SETAC

pharmaceutical mixtures have been investigated in single fish species in laboratory exposures (Schoenfuss et al. 2016; McCallum et al. 2017; Sehonova et al. 2017). Complex chemical mixtures in STP effluent have been shown to cause reproductive disruption in a single fish species (Vajda et al. 2008). Nevertheless, limited information is available on the results of environmentally relevant pharmaceutical and STP effluent mixtures at multiple trophic levels in the field. Algal growth was inhibited by mixtures of antimicrobial agents (Yang et al. 2008); however, effects of CEC mixtures on aquatic plants have received little attention (Llorca et al. 2017) and could be included in future food web studies. More information exists on the outcomes of CEC mixtures on fish (Lee et al. 2012; Reinling et al. 2017), bivalves (Ismail et al. 2014), and aquatic invertebrates (Ruh´ et al. 2016; Garcia-Galan et al. 2017); nonetheless, investigations are often limited to a single organism or trophic position. Furthermore, complex laboratory mixtures should be representative of environmental conditions to achieve environmental relevance. With increased reporting on the presence and concentrations of CECs in aquatic environments, there are enough data to apply mathematical tools (i.e., cluster analysis) to develop laboratory mixtures that reflect environmental realities (Elliott et al. 2018).

To confront the challenge of grasping the complexity of CEC mixtures in the aquatic environment, several knowledge gaps will need to be addressed. The continued development of sensitive and robust analytical methods for a wide variety of CECs in various biological matrices will be required. Also, limited information is available on the occurrence of CEC metabolites and transformation products in the environment (Farré et al. 2008), in part because of the lack of existing analytical methods. Nontarget screening using high-resolution mass spectrometry could help to fill this data gap to identify metabolites, transformation products, or new contaminants (Bletsou et al. 2015). Models to understand the occurrence, composition, and concentrations of CECs in environmental mixtures are also missing. New high-throughput screening techniques may address some of the difficulties with low doses, complex mixtures, and variable environmental conditions encountered when using current mixture models (Judson et al. 2015; Rodea-Palomares et al. 2016). These types of tools may assist in distinguishing information gained from studying simple chemical mixtures containing similar compounds (Yang et al. 2008) versus complex mixtures containing multiple classes of compounds with widely different characteristics (e.g., Baldwin et al. 2016). A road map could be developed to show how these screening techniques and tools could be used to deal with this challenge and streamline advances.

CHALLENGE 2: SUBLETHAL EFFECTS OF CECS ON AQUATIC ORGANISMS

Considerable information is available in the scientific literature on the acute toxic effects of CECs on aquatic organisms (Kent et al. 2006; Santos et al. 2010); however, a broader understanding of sublethal effects on a wide range of aquatic organisms is necessary. For most CECs, acute toxic effects occur at concentrations in the mg L^{-1} range (Kent et al. 2006), whereas concentrations detected in the environment typically range from the ng L^{-1} to μg L^{-1} levels (Kolpin et al. 2002; Aus der Beek et al. 2016; Baldwin et al. 2016; Gago-Ferrero et al. 2017). Furthermore, environmental exposure to CECs occurs at relatively low concentrations chronically over long periods of time (Daughton 2002; Hampel et al. 2016). Exposure to low concentrations of CECs may not cause overt toxicity but rather subtle changes in the health and physiology (e.g., behavior) of the organisms (Hampel et al. 2016). These subtle changes have the potential to cause adverse ecological outcomes in terms of population levels and biodiversity.

A variety of CECs including some pharmaceuticals and personal care products, flame retardants, and synthetic hormones are known or suspected EDCs, altering the normal function of hormones resulting in a variety of health effects. Endocrine-disrupting compounds may in some cases interfere with the activation of nuclear estrogen and androgen receptors. At this level of biological organization, disruption results in the reduction of gamete production (declining production of vitellogenin, occurrence of intersex, ovarian atrophy; Vajda et al. 2008; Blazer et al. 2014), mate availability (Martinovic et al. 2007), fecundity (Parrott and Blunt 2005; Vajda et al. 2008; Dammann et al. 2011; Vajda et al. 2011), and fertility (Parrott and Blunt 2005). Reduction in reproductive potential through endocrine disruption is most readily connected to a decrease in population size and subsequently to an adverse impact on the trophic cascade (Fleeger et al. 2003; Kidd et al. 2007; Miller et al. 2007). An example of endocrine disruption in snails caused by exposure to tributyltin has recently been extrapolated to vertebrates, including fish that were found to be as sensitive as snails to this compound and suffered endocrine effects at environmental concentrations (Lagadic et al. 2017).

Other important consequences of CEC exposure include behavioral effects mainly associated with psychoactive drugs. For example, fish exposed to anti-anxiety and serotonin reuptake inhibitor drugs have the potential to cause increased activity and boldness (Brodin et al. 2017), inhibition of aggressive behavior (Forsatkar et al. 2014; Greaney et al. 2015), changes in motor activity (Kellner et al. 2016), reduction in daytime activity levels (Melvin et al. 2015), and increased time (Bisesi et al. 2014) and decreased ability (Gaworecki and Klaine 2008) to capture prey items. Neurotoxic effects have also been reported for the antidepressant venlafaxine that targets neurological tissue (Bidel et al. 2016).

For many pharmaceuticals, scientific research has focused on understanding effects on wildlife based on the drug's therapeutic mode of action in humans (Corcoran et al. 2010). The evolutionary preservation of molecular targets of CECs in aquatic organisms suggests that they may exert a mode of action-mediated effects similar to those desired in humans (Huerta et al. 2016), which is a unique characteristic of CECs.

Various molecular techniques provide useful tools for evaluating sublethal effects of CECs on aquatic organisms, although some methods require further development. Changes in gene expression, which are frequently considered one of the first steps in altering the entire organism pathway, are a widely reported sublethal response to CEC exposure (Garca-Reyero

 © 2018 SETAC

et al. 2011). Genes associated with steroid production, sexual differentiation, immune–response growth, and development have been reported to be altered by CEC exposures (Jin et al. 2010; Caspillo et al. 2014; Baldigo et al. 2015). Few studies have assessed the immunological responses of aquatic organisms exposed to CECs. In fish species, this may be related to the difficulties in applying an appropriate methodology because nucleated erythrocytes complicate the use of flow cytometry—one of the workhorses of immune system analysis in other vertebrates (Schoenfuss et al. 2016). Limited research utilizing gene arrays has pinpointed gene expression changes consistent with immunotoxic responses in fish exposed to CECs (Burkina et al. 2015). Genotoxic alterations—such as DNA strand breaks in blood cells (increase of genetic damage index), chromosome breakage and segregational abnormalities (Rodrigues et al. 2017), DNA and oxidative damages (Pandey et al. 2017), erythrocyte DNA strand breaks (Barreto et al. 2017), and loss of DNA integrity (Rocco et al. 2010)—have been observed in fish exposed to pharmaceuticals. Molecular biomarkers reported in the literature highlight the broad array of biological effects of CECs on aquatic organisms at molecular, biochemical, histological, and behavioral levels.

To expand the current knowledge on biomarkers, the field of aquatic ecotoxicology has recently integrated "omics" technologies including metabolomics, transcriptomics, and proteomics as valuable tools to assess sublethal effects of CECs on aquatic organisms. Metabolomics refers to the comprehensive profiling of small-molecule metabolites in cells, tissues, or whole organisms to detect changes in their pattern brought on by external or internal stressors utilizing advanced analytical techniques in combination with multivariate statistical analysis (Campillo et al. 2015; Kovacevic et al. (2016)). For example, Kovacevic et al. (2016) suggested that subchronic exposure of *Daphnia magna* to a range of sublethal concentrations of triclosan, carbamazepine, and ibuprofen produced a general oxidative stress condition; and several types of amino acids (branched, aromatics, etc.) emerged as potential bioindicators. Transcriptomics is useful in field-based studies to differentiate individuals collected at contaminated sites from those collected at pristine sites, in addition to providing insights into the mechanisms of action underlying changes at higher levels of biological organization (Everroad et al. 2012; Jordan et al. 2012; Softeland et al. 2016). The determination of transcription levels of target genes involved in essential cellular functions and pathways could be of great interest in evaluating the molecular responses to CECs. Proteomics has also become a useful tool to identify and understand processes involved in the bioactivity of CECs because it provides unique evidence about structure, function, posttranslational modifications, interactions, and abundance of proteins (Gouveia et al. 2017; Sánchez-Marń et al. 2017). Benzotriazole and its associated derivatives—as emphasized by proteomics—exhibit hepatotoxicity in the rare Chinese adult male minnow (*Gobiocypris rarus*) and the underlying mechanism associated with the damage (Liang et al. 2017). Proteomics also has the potential to determine the mechanisms of action of CECs on aquatic vertebrates during the early developmental period (Parolini et al. 2018).

The role that omics technologies can play in the establishment of sublethal effects of CECs and the identification of biomarkers is unquestionable. However, improvements are needed to produce quantitative and reproducible omics data (Simons 2018). Furthermore, there is no consensus as yet on which molecular responses are relevant indicators of exposure or recovery (Choudhuri et al. 2018), especially in field-based transcriptomics studies that are challenged by the influence of confounding factors (Giraudo et al. 2017b). Thus it is crucial to develop guidelines for interpreting field omics data that will allow researchers to better isolate omics signatures responsive to confounding environmental factors from those responsive to CECs.

To address the challenge of understanding sublethal effects of CECs on a wide range of aquatic organisms, several knowledge gaps will need to be addressed. Understanding interspecific differences regarding the chemical mode of action is a formidable challenge to establish sublethal effects of CECs because species can vary widely in their susceptibilities to chemicals (Brodin et al. 2014; Giraudo et al. 2017a). Understanding the sublethal effects of chronic exposure to low doses of CECs is still incomplete. Immunological responses of aquatic organisms exposed to CECs are not well known. There is a notable disconnect between the generation of omics data and its incorporation to risk assessment strategies. Finally, there is an urgent need for ecotoxicologists and computational scientists to develop easy-to-operate adverse outcome pathway (AOP) models for effective synthesis of omics data to predict population-level effects and support quantitative ecological risk assessment.

CHALLENGE 3: DURATION OF CEC EXPOSURE WITHIN AND ACROSS GENERATIONS IN AQUATIC SPECIES

Life cycle and multigenerational exposures have long been recognized standards in toxicological studies (Organisation for Economic Co-operation and Development 2012); however, the costs and logistical difficulties associated with rearing and maintaining aquatic organisms for months or even years under constant exposure conditions are daunting. Nevertheless, mounting evidence suggests that life cycle (embryonic to maturity continuous exposure) and multigenerational exposures (exposures extending beyond one life cycle) are required to fully assess the effects of CECs on aquatic life (reviewed in Parrott et al. 2017). The significance of extending exposures past a generation was underscored by the 17α-ethinylestradiol (EE2) exposure study in the Canadian Experimental Lakes Area leading to the population collapse of a fathead minnow population after exposure of 2 generations (Kidd et al. 2007). Because the collapse was associated with a lack of young-of-the-year recruitment, this effect would not have been evident without the prolonged duration (3 consecutive summers) of the exposure. Even beyond the immediate effect of the exposure, food web consequences became apparent only after several years of exposure (Palace et al. 2009). Similarly, Schwindt et al. (2014) documented population-level disruption in mesocosm

© 2018 SETAC

exposure experiments in which fathead minnows were exposed to EE2 over 3 generations.

Two discrete questions related to the effects of CEC exposure of a population require long-term contact to be answered: 1) To what extent are exposure effects discontinuous across ontogeny? and 2) How does species sensitivity change over generations? The first question encompasses exposure outcomes that may occur as a result of contact during discrete vulnerable life stages such as development, migration, or maturation and may impact survival and reproductive output at a later time. The second question incorporates epigenetic and evolutionary changes in the exposed organisms that do not manifest themselves until subsequent generations. In addition, both questions contain underlying methodological challenges related to sensitive windows of exposure (Van Aerle et al. 2002; Burggreen and Mueller 2015), mechanistically linking delayed effects to original exposure (Mihaich et al. 2017) and extrapolating from individual (and often laboratory) exposure effects to population consequences (Parrott and Blunt 2005; Miller et al. 2007; Schwindt and Winkelman 2016).

Ontogenetic disconnect between exposure occurrence and effect has been a hallmark of many EDCs (Parrott et al. 2017). Parrott et al. (2017) found that changes in organ development or organ differentiation in embryonic and larval organisms result in reduced survival and fitness toward and during maturity. Biased sex ratios, a commonly reported outcome of exposure to estrogenic CECs during embryonic organogenesis or larval sexual differentiation, may reduce fitness in adults, often independent of the continuation of exposure past a sensitive window (Van Aerle et al. 2002; Burggreen and Mueller 2015). Fetal development, a time at which receptor sensitivity is heightened, appears to be a particularly sensitive window for enhanced effects of CEC exposure (Kopras et al. 2014). Nonetheless, establishing sensitive windows of exposure has been complicated by the diversity of life history strategies among aquatic species (Palace et al. 2009). For example, species that migrate seasonally between habitats may experience exposure during the sensitive developmental periods; however, this exposure may be temporally and geographically discontinuous to effects observed later in ontogeny (Bangsgaard et al. 2006). Similarly, asynchronous spawners may produce offspring during a broad range of environmental conditions that may impact bioavailability of pollutants. Thus timing, duration, dose, and developmental stage all influence the severity of observed adverse effects (Patisaul and Adewale 2009). Bioaccumulation resulting in maternal transfer and direct effects on offspring ontogeny appears to be a less common phenomenon for CECs than reported for many legacy contaminants. However, PFOS has been known to bioaccumulate in aquatic organisms (Du et al. 2009; Haddad et al. 2017) and may lead to adverse outcome for first filial generation offspring of exposed parental organisms (Du et al. 2009). Similarly, some pharmaceuticals have been reported to bioaccumulate in tissues with little knowledge of the long-term consequences (Zhao et al. (2017)). Given the chemical diversity of pharmaceuticals, it is not surprising that bioaccumulation varies for these CECs. For example, the antidepressant sertraline and its metabolite (norsertraline) bioaccumulate in fish tissues (Schultz et al. 2011; Arnnok et al. 2017), whereas the antidepressant venlafaxine does not (Schultz et al. 2011). In this context, bioaccumulation must be assessed on both a compound and tissue-specific level. To illustrate, bioaccumulation of antidepressants in whole-body preparations may be less predictive of adverse effects than the knowledge that they bioaccumulate in the pharmaceuticals' target tissue—the central nervous system (Schultz et al. 2011; Arnnok et al. 2017). Similarly, Zhao et al. (2017) reported bioconcentration for several pharmaceuticals to be highest in the liver and the brain and lower in muscle tissues. An understanding of pharmaceutical bioaccumulation across size classes of organisms displaying ontogenetic feeding shifts is lacking (Haddad et al. 2017). This may be of concern when CECs are incorporated into the gamete through maternal transfer and impact the developing embryo through its nutrient source. For example, turtle sex ratios are dependent on environmental temperature and shifts in critical sex-determining temperatures have been linked to maternal estrogenic exposure and transfer to the developing gamete (Bull et al. 1988). Similarly, malformations in zebrafish embryos were linked to maternal PFOS transfer to embryological development (Du et al. 2009). Little is known about maternal transfer of pharmaceuticals but several studies have reported bioaccumulation of these CECs in exposed organisms (Schultz et al. 2011; Clairardin et al. 2013; Zhao et al. (2017)), suggesting that maternal transfer to eggs is plausible and has been observed for other pollutants (Marsh-Matthews et al. 2001).

A review of recent studies on endocrine-active CECs concludes that there is little expectation that organisms would become more sensitive in subsequent exposure generations (Parrott et al. 2017). However, in some instances, multigenerational (more than 2 generations) studies have documented exacerbated adverse effects in the second and third generations of exposure. For example, exposures to estrogenic CECs including 4-nonylphenol (Yokota et al. 2001; Watanabe et al. 2017), 17β-estradiol (Cripe et al. 2009, 2010), and EE2 (Nash et al. 2004) resulted in histopathological changes to reproductive organs with increasing severity in the second exposed generation and ultimate reproductive failure (Nash et al. 2004).

Organismal plasticity (i.e., phenotypic alterations during development to better suit environmental needs) and epigenetic changes as a result of CEC exposure have been reported in the literature (reviewed by Rissman and Adli 2014; Wilkinson et al. 2015); however, their linkage with adverse population-level outcomes that may affect the trophic cascade are far from certain. Epigenetic effects are related to changes in gene expression, may persist beyond the exposed generation (Skinner et al. 2011; Singh and Li 2012), and are distinct from mutagenic effects where genetic information is altered directly. Du et al. (2009) showed epigenetic changes in offspring of PFOS-exposed zebrafish that persisted into the second filial generation despite a lack of exposure.

Whereas epigenetic changes may be reversible after several generations of reduced pollutant exposure, adaptive changes in response to persistently polluted environments may irreversibly alter exposed species (Bridges and Semlitsch 2001; Matson et al. 2006; Bickham 2011). Adaptive changes have been documented

 © 2018 SETAC

previously for environmental pollutants such as nitrogenous compounds (Egea-Serrano et al. 2009), polyaromatic hydrocarbons (Diamond et al. 1995) and pesticides (Cothran et al. 2013; Hua et al. 2015). A whole genome analysis of multiple resident killifish populations along the Eastern Seaboard of the United States (Reid et al. (2016)) identified multiple populations that developed tolerance to persistent industrial pollutant mixtures independently and repeatedly. Adaptive tolerance was linked to the aryl hydrocarbon receptor-based signaling pathway and incurred likely adverse changes to other pathways including estrogen and hypoxia signaling. Reid et al. (2016) suggested that the ability for species to develop adaptive tolerance may scale with population size, positioning many aquatic organisms near the base of the aquatic food web in particular danger.

The adequate assessment of the environmental impacts of CECs across generations will require multigenerational exposure studies in controlled laboratory and field settings. Rigorous laboratory research using the established Organisation for Economic Co-operation and Development (2012) framework may provide a starting point for experimental designs well suited to test the long-term exposure effects of CECs individually and in mixtures. Common garden experiments (Egea-Serrano and Tejedo 2014), mesocosm studies (Elliott et al. 2014; Schwindt et al. 2014), and staged environmental analyses (Reid et al. 2016) may need to be combined to address issues of adaptive changes in exposed populations and cross-species effects of CEC exposures. Advancements in molecular tools such as in vitro cell-based testing platforms for gene expression in liver tissue (Rodd et al. 2017) may provide new avenues to assess long-term adaptive responses in organisms over time. These tools are in the early stages of development and their applicability to environmentally realistic scenarios remains to be explored and evaluated.

CHALLENGE 4: INTEGRATION OF MULTIPLE STRESSORS WITH CEC EXPOSURE IN AQUATIC SYSTEMS

Aquatic organisms reside in waters receiving both point (e.g., STP effluent) and nonpoint (e.g., agricultural and storm water runoff) sources of CECs, thereby encountering multiple stressors either simultaneously or sequentially (Eggen et al. 2004). These stressors include exposure to complex mixtures of contaminants, as previously discussed in the Challenge 1 section, and changing environmental conditions such as rising temperatures, altered stream flows, increased radiation, varying pH, low oxygen concentrations, turbidity, salinity, diseases, and parasites, among others. Consideration of the combined effects of multiple stressors is needed to gain understanding of the full extent of CEC effects on aquatic food webs.

Although more attention has been focused on traditional contaminants, there are a few examples of studies assessing the interplay between exposure to CECs and physical stressors. Exposures to chemical surfactants combined with heat stress and desiccation were shown to have synergistic effects (i.e., greater than the sum of individual effects) in earthworms (*Dendrobaena octaedra*) and springtail (*Folsomia candida*) (Holmstrup et al. 2010). The toxicity to *D. magna* of a pharmaceutical mixture was found to be pH-dependent—such that site-specific pH measurements were needed to avoid overestimation or underestimation of environmental toxicity of mixtures of ionizable pharmaceuticals in natural aquatic environments (Boström and Berglund 2015)—and was temperature- and radiation-dependent (Kim et al. 2010) under controlled laboratory conditions. Triclosan exposure and grazing pressure were observed to have negative synergistic effects on diatom-size class and mortality because the effects of triclosan were higher than expected when periphyton were subject to grazing by snails (*Radix ovata*) (Guasch et al. 2016). Toxicities of mixtures to *Chironomus dilutus* were tested at different temperatures in a controlled setting (Harwood et al. 2009). The combined stress of exposure to both a pharmaceutical mixture and to elevated nitrate concentrations was negatively correlated with abundances of aquatic invertebrate species including snails (*Lithoglyphus naticoides*), the Diptera species *Polypedilum nubeculosum*, and the crustacean *Limnomysis benedeni* in the Danube River (Rico et al. 2016). Biodegradation of venlafaxine by stream biofilms was found to be hindered by the presence of a mixture of 4 CECs (erythromycin, sulfisoxazole, diclofenac, and imidacloprid) under constant stream flows in an artificial stream facility (Acuna and Petrovic 2018). Interestingly, the reduction in biodegradation capacity in the presence of a CEC mixture was less acute under intermittent stream flows compared with constant stream flows. Hence Acuna and Petrovic (2018) found a combined effect of water flow conditions and CEC exposure on biodegradation. In another study, a combination of chemical analysis of more than 400 compounds, bioanalysis using 8 different biological endpoints, and mixture toxicity modeling was used to assess effects of a complex mixture of wastewater-derived CECs on zebrafish embryos under low flow conditions (Neale et al. 2017). This type of multifaceted approach is rare and provides a deeper understanding of a system than using a single device for bioassessment, and could be expanded to study multiple organisms within a food web.

Climate change may alter contaminant toxicity directly through variations in temperature (Müller et al. 2012), pH, and oxygen (Carere et al. 2011) and/or by increased concentrations of micropollutants caused by lower stream flows and subsequent decreased dilution of STP effluents (Bundschuh et al. 2011). Indirect effects can result from actions taken to adapt to a changing climate (Stahl et al. 2013) such as increased pesticide applications (Delcour et al. 2015). Elevated water temperatures can increase the biotransformation of contaminants to either more bioactive metabolites or less toxic metabolites (Escher and Fenner 2011), and enhanced air temperatures can lead to increases in concentrations and transport of airborne contaminants (Noyes et al. 2009). In parallel, chemical exposures can impair the response and adaptation of species to physical stressors related to climate change, especially for populations occupying the fringes of their physiological tolerance range (Noyes et al. 2009). The combination of stressors related to climate change and contaminant exposure poses significant extinction risks, particularly for small populations with relatively few individuals (Brown et al. 2015).

© 2018 SETAC

One important data gap in comprehending multiple stressors is the consideration of human-induced habitat changes in concert with contamination. The few studies on this topic are mostly focused on legacy contaminants but results could transfer to understanding the effects of CECs as well. For example, accumulation of legacy EDCs such as polychlorinated biphenyls (PCBs) in impoundments behind dams has been shown to negatively impact fish reproductive health (Feist et al. 2005), and the same could be true for CECs. Concomitant effects of dam impoundments such as changes in water temperatures and total dissolved gas should be considered in concert with toxicity. Contaminant transport after dam removal can affect downstream food webs (Davis et al. 2017) and should be examined as part of any dam removal proposal. Coho salmon (*Oncorhynchus kisutch*) spawner mortality has been attributed to chemical contaminants in land-based runoff that may contain CECs to urban streams in Puget Sound, along the northwestern US state of Washington (Spromberg and Scholz 2011). Spatial analyses found a strong positive correlation of chemically induced Coho salmon spawner mortality with land cover including roads, impervious surfaces, and commercial property within a watershed (Feist et al. 2011). Chemical stressors—especially CECs in combination with human-induced habitat changes caused by, among other occurrences, water withdrawals, deforestation, and dam operations/removal—have rarely been taken into account.

To comprehensively assess the effects of multiple stressors on aquatic food webs, novel tools, models, and innovative study designs need to be developed. Although early attempts to assess effects of multiple stressors on aquatic organisms relied on weight-of-evidence approaches (Lowell et al. 2000), steady-state and non–steady-state bioaccumulation models are needed to understand how multiple stressors alter trophic structure and feeding ecology (McLeod et al. 2016). Segner et al. (2014) posit that to assess the effects of multiple stressors, study design must shift from a focus on stressor characteristics to a focus on the properties of the biological receptor (i.e., the fish or aquatic invertebrate being studied). Future research could employ this strategy to test the interplay among multiple stressors such as climate factors, bioavailability, and toxicity of CECs—with an emphasis on the properties of the aquatic species being studied. The use of outdoor flow-through mesocosms that approximate realistic ecological conditions, while allowing for control of multiple stressors, provides a powerful tool to examine indirect multiple stressor effects and biotic interactions (e.g., Moran et al. 2010; Magbanua et al. 2016; Bruder et al. 2017). Eco-epidemiological analysis of existing monitoring datasets has been shown to quantify multiple stressor effects on aquatic ecosystems (Posthuma et al. 2016). New high-throughput screening techniques may address some of the difficulties with low concentrations, complex mixtures, and variable environmental conditions encountered when using current mixture models (Rodea-Palomares et al. 2016). Manciocco et al. (2014) recommended an international "open-access" database for tissue contamination and temperature. Cross-disciplinary efforts are needed to design the tools to improve understanding of the mechanisms and modes of action of effects of multiple stressors on aquatic species and food webs. This type of repository containing data for multiple stressors could stimulate global cooperation in pursuit of understanding effects of multiple stressors.

CHALLENGE 5: TROPHIC CONSEQUENCES OF CEC EXPOSURE ACROSS AQUATIC FOOD WEBS

Effectively evaluating the direct and indirect trophic consequences of CECs requires the extrapolation of toxicity data across different levels of biological organization from the individual, to the population, to the community, and finally to the ecosystem. Limited knowledge is available on the effects of CECs at broader ecological scales (Boxall et al. 2012; Windsor et al. 2017) caused, in part, by the complexity of environmental mixtures, the incomplete information on effects at multiple trophic levels, the interactions between CECs and other stressors, and the importance of bottom-up food web processes. Recently, studies have filled some data gaps in predicting toxicity of EE2 at several levels of the food web (Hallgren et al. 2014; Kidd et al. 2014). In these 2 studies, prey fish biomass decreased in response to EE2, which resulted in an increase in zooplankton. Kidd et al. (2014) also reported a decline in biomass of the top predator (lake trout) most likely because of an indirect effect from EE2 reduction of prey abundance. These studies indicate that CECs have the potential to directly and indirectly alter the structure and function of food webs. To advance our knowledge of food web interactions, the exposure and potential indirect effects of CECs should be addressed, including community-level interactions such as predator–prey relationships, resource competition, successional processes, and trophic cascades (Kramer et al. 2011; Windsor et al. 2017).

To move beyond effects at the individual species level, scientists have begun to bridge the gap between individual effects and food web responses by utilizing a combination of mechanistic toxicology (effects at the individual level) and conservation biology (bottom-up or top-down food web interactions; Ankley et al. 2010; Macneale et al. (2010); Kramer et al. 2011). Macneale et al. (2010) combined such disciplines to highlight information gaps and to discuss how future research should assess the effects of pesticides on aquatic food webs in support of Pacific salmon conservation. These authors examined multiple lines of evidence that indicated pesticides can impact primary productivity, invertebrate abundance, and prey community structure by eliminating energetically favorable species; nevertheless, they were unable to unequivocally link the bottom-up effects of pesticide mixtures to higher predators such as salmon. Kidd et al. (2014) found a significant reduction in forage fish abundance resulting in a decline in the top predator. Notably this was the first and currently only study to address the indirect effects of a CEC on the aquatic food web. However, as with toxicity studies, single contaminant exposures do not represent real-world scenarios. Understanding trophic consequences involves the incorporation of population dynamic modeling into toxicity assessments allowing the exploration of

 © 2018 SETAC

the relationships among species life histories, exposure patterns, and sensitivities to CECs (Forbes et al. 2016).

Approaches such as the application of eco-epidemiological models that link chemical stressors to effects on assemblages (De Zwart et al. 2006; Posthuma et al. 2016), the use of modern molecular and genetic tools (Van Straalen 2003; Eggen et al. 2004), the AOP concept (Ankley et al. 2010; Kramer et al. 2011), energy allocation models (Fischer et al. 2013), and/or principles in community ecotoxicology (Rohr et al. 2006; Clements and Rohr 2009) all show promise in connecting responses within and across biological levels of organization. The eco-epidemiological framework described by De Zwart et al. (2006) provides a measure of impact and statistical estimates of potential stressor effects on fish assemblages using a combination of exposure and population modeling. Adverse outcome pathways offer a unique structure for organizing information, identifying uncertainties and research priorities, and improving the avenues necessary to advance our understanding of CECs at multiple levels of organization (Ankley et al. 2010). Modeling the effects at the individual level using AOPs can provide the key that links toxicity information to top-down processes including effects on population dynamics, community diversity, and ecosystem responses (Kramer et al. 2011). Community ecotoxicology is focused on the movement of contaminants across ecosystems and their subsequent impacts on communities (e.g., species richness and diversity), which have the potential to incorporate food web responses at different levels of organization. Gessner and Tlili (2016) characterize these types of approaches as simply incorporating ecological principles into the design and execution of ecotoxicological studies, which echoes the conclusions of Rosi-Marshall and Royer (2012).

The current risk assessment paradigm has little ecological relevance to the population, the community, or the ecosystem for most contaminants including CECs because of the limited data on direct and indirect effects of CECs on multiple levels of biological organization. The scarcity of information on trophic consequences and effects beyond the individual can yield inaccurate estimates of risk to sensitive populations or communities (Forbes et al. 2011). Unfortunately, assessments on individuals do not drive regulatory action or societal change; tools must evolve to model effects at higher levels of biological structure (Arnold et al. 2014). Existing population models integrate potential effects of exposure on individual survival, growth, and reproduction, in combination with life histories, to predict impacts beyond the individual. Population models have been used extensively to answer conservation biology and resource management questions but their use in chemical risk assessments is unfortunately limited (Forbes et al. 2016). However, there is a continued need for increased long-term monitoring of populations of interest for different regions, taxa, and exposure potential to increase confidence and applicability at multiple trophic levels.

To truly understand risks to the food web we must look beyond sublethal effects of single compounds or simple mixtures at the individual level and incorporate all pathways from the source to the receptor, including a detailed comprehension of environmentally relevant chemical mixtures as well as food web structure and dynamics. This also includes an understanding of the impacts of CECs relative to other stressors at multiple levels of organization. Extrapolating from individual-level effects in laboratory tests to population effects and community responses is challenging. For example, small fish models offer utility for pharmaceutical risk assessments for regulatory decision-making; however, they are not considered protective of all fish because of differences in physiologies, behaviors, and life histories (Brown et al. 2015) and cannot be extrapolated to a food web. Toxicity data of this type are also limited to a handful of species. Sublethal endpoints relative to population modeling must include measures of survival, growth, and fecundity (Kramer et al. 2011; Chambers et al. 2014) but more information is required to identify the mechanistic causes of CEC toxicities. Furthermore, environmental reservoirs host complex mixtures of CECs and other contaminants making toxicity information for key species beneficial to help prioritize chemicals that could pose the greatest threat no matter the trophic level (Boxall et al. 2012). At present, the only data on effects at the population level occur as a result of "sudden and catastrophic events," according to Arnold et al. (2014).

The available toxicity information and sublethal responses to CECs are biased toward the freshwater environment, with limited data available on the marine and terrestrial ecosystems (Gerbersdorf et al. 2015). To further complicate matters, movement of persistent CECs across ecosystem boundaries (i.e., between freshwater and terrestrial/marine environments) should also be considered. Anadromous fish have the potential to accumulate CECs in the ocean and deposit them in freshwater environments during spawning. For instance, sockeye salmon (*Oncorhynchus nerka*) transport hydrophobic contaminants such as PCBs upstream after spawning, where these pollutants may affect multiple trophic positions including their offspring (Krummel et al. 2003). If contaminant transport can be related to energy flows between ecosystem boundaries, additional information is needed beyond the aquatic environment.

To understand the trophic consequences of exposure to CECs, a transdisciplinary approach must be conceived encompassing chemistry, toxicology, conservation biology, and community ecology to develop the tools and avenues necessary to scale up beyond the individual To establish links between CEC exposure, indirect effects, and bottom-up or top-down cascades, a research emphasis needs to be placed on several key hypotheses including: 1) altered behavior at any level leads to survival variations and changes in trophic structure; 2) changes in sex ratios, fecundity, and fertility of lower trophic level species result in declines at higher trophic levels; 3) overt (lethal) toxicity at lower trophic levels (invertebrates) reduces food availability, subsequently reducing population size at higher trophic levels; and 4) bioaccumulation and biomagnification can decrease fecundity and fertility, creating adverse health outcomes at higher trophic levels.

CONCLUSIONS

Proliferation of synthetic chemicals in the environment is driving global change at a faster rate than other more commonly

© 2018 SETAC

cited stressors such as rising atmospheric CO_2 concentrations, nutrient pollution, habitat destruction, and biodiversity loss (Bernhardt et al. 2017). In the present review, we propose 5 grand challenges that need to be addressed to advance our understanding of CECs from single-exposure events to multispecies aquatic food web interactions. Limited information is available on the effects of CECs at broader ecological scales in part because of the complexity of environmental mixtures, the incomplete data on effects at multiple trophic levels, the lack of multigenerational studies, the interactions between CECs and other stressors, and the importance of bottom-up food web processes. The knowledge gaps can seem daunting; however, novel tools, models, and innovative study designs are being developed to comprehensively assess the effects CECs and other stressors on aquatic food webs, and ecological principles can drive improved design and execution of ecotoxicological research. Breakthroughs in analytical and high-throughput screening techniques can serve as powerful tools to face challenges presented by low doses, complex mixtures, and variable environmental conditions. New modeling strategies can assist in prediction of food web transfer and fate of CEC mixtures. Omics approaches may help unravel interspecific differences regarding chemical mode of action to understand sublethal effects of CECs and species susceptibilities to chemicals—and many of these techniques have untapped potential. Several examples have shown that some of the most extreme effects of CECs cannot be resolved without employing longer duration, multigenerational exposure experiments under laboratory conditions. Finally, we must utilize innovative methods designed to extrapolate toxicity data across multiple trophic levels to evaluate the direct and indirect trophic consequences of CECs from the individual, to the population, to the community, and to the ecosystem. The greatest challenges in these experiments are linking delayed effects with the original exposure and extrapolating effects on individuals to population-level consequences, given the diversity of life history strategies. To address these grand challenges, future CEC research will incorporate transdisciplinary, multidisciplinary systems to move beyond the individual compounds and receptors to include numerous levels of biological organization in concert with multiple stressors over a range of exposure durations and life histories. We have the tools and creativity to accomplish these ambitious goals as a scientific community, and to achieve a broader understanding of the effects of CECs on aquatic food webs.

Acknowledgment—Funding for the present review was provided by the US Geological Survey Oregon Water Science Center, and the US Geological Survey Toxic Substances Hydrology Program. H.L. Schoenfuss received support from the Great Lakes Restoration Initiative through the US Fish and Wildlife Service CEC team, as well as additional funding from a National Science Foundation grant (CBET 1336062). We thank W. Hapke, C. Smith, and J. Morace for helpful discussions, and J. Olsen and C. Wegner for graphics and library support. This review was greatly improved by thoughtful input from several peer reviewers.

Disclaimer—Use of trade or product names is for descriptive purposes only and does not imply endorsement by the US government. The findings and conclusions in this review are those of the authors and do not necessarily represent the views of the US Fish and Wildlife Service but do represent the views of the US Geological Survey.

Data Accessibility—Data, associated metadata, and calculation tools are accessible from the corresponding author (enilsen@usgs.gov).

REFERENCES

Acuna V, Petrovic M. 2018. Effects of chronic pollution and water flow intermittency on stream biofilms biodegradation capacity. *Environ Pollut* 233:1131–1137.

Ahrens L, Bundschuh M. 2014. Fate and effects of poly- and perfluoroalkyl substances in the aquatic environment: A review. *Environ Toxicol Chem* 33:1921–1929.

Ahrens L, Gashaw H, Sjoholm M, Gebrehiwot SG, Getahun A, Derbe E, Bishop K, Akerblom S. 2016. Poly- and perfluoroalkylated substances (PFASs) in water, sediment and fish muscle tissue from Lake Tana, Ethiopia, and implications for human exposure. *Chemosphere* 165:352–357.

Altenburger R, Scholze M, Busch W, Escher BI, Jakobs G, Krauss M, Krüger J, Neale PA, Ait-Aissa S, Almeida AC, Seiler TB, Brion F, Hilscherová K, Hollert H, Novák J, Schlichting R, Serra H, Shao Y, Tindall A, Tolefsen KE, Umbuzeiro G, Williams TD, Kortenkamp A. 2018. Mixture effects in samples of multiple contaminants—An inter-laboratory study with manifold bioassays. *Environ Int* 114:95–106.

Ankley GT, Bennett RS, Erickson RJ, Hoff DJ, Hornung MW, Johnson RD, Mount DR, Nichols JW, Russom CL, Schmieder PK, Serrano JA, Tietge JE, Villeneuve DL. 2010. Adverse outcome pathways: A conceptual framework to support ecotoxicological research and risk assessment. *Environ Toxicol Chem* 29:730–741.

Arnnok P, Singh RR, Burakham R, Pérez-Fuentetaja A, Aga DS. 2017. Selective uptake and bioaccumulation of antidepressants in fish from effluent-impacted Niagara River. *Environ Sci Technol* 51:10652–10662.

Arnold KE, Brown AR, Ankley GT, Sumpter JP. 2014. Medicating the environment: Assessing risks of pharmaceuticals to wildlife and ecosystems. Philos Trans R Soc Lond B Biol Sci. [2018 June 23]. Available from: https://doi.org/10.1098/rstb.2013.0569

Aus der Beek T, Weber F-A, Bergmann A, Hickmann S, Ebert I, Hein A, Küster A. 2016. Pharmaceuticals in the environment—Global occurrences and perspectives. *Environ Toxicol Chem* 35:823–835.

Backhaus T. 2014. Medicines, shaken and stirred: A critical review on the ecotoxicology of pharmaceutical mixtures. Philos Trans R Soc Lond B Biol. [cited 2018 May 19]. Available from: https://doi.org/10.1098/rstb.2013.0585

Baldigo BP, George SD, Phillips PJ, Hemming JDC, Denslow ND, Kroll KJ. 2015. Potential estrogenic effects of wastewaters on gene expression in Pimephales promelas and fish assemblages in streams of southeastern New York. *Environ Toxicol Chem* 34:2803–2815.

Baldwin AK, Corsi SR, De Cicco LA, Lenaker PL, Lutz MA, Sullivan DJ, Richards KD. 2016. Organic contaminants in Great Lakes tributaries: Prevalence and potential aquatic toxicity. *Sci Total Environ* 554-555: 42–52.

Bangsgaard K, Madsen SS, Korsgaard B. 2006. Effect of waterborne exposure to 4-tert-octylphenol and 17β-estradiol on smoltification and downstream migration in Atlantic salmon, *Salmo salar*. *Aquat Toxicol* 80:23–32.

Barreto A, Luis LG, Soares AMVM, Pa'ga P, Santos LHMLM, Delerue-Matos C, Hylland K, Loureiro S, Oliveira M. 2017. Genotoxicity of gemfibrozil in the gilthead seabream (*Sparus aurata*). *Mutat Res-Genetic Toxicol Environ Mutagen* 821:36–42.

Bernhardt ES, Rosi EJ, Gessner MO. 2017. Synthetic chemicals as agents of global change. *Front Ecol Environ* 15:84–90.

Bickham JW. 2011. The four cornerstones of evolutionary toxicology. *Ecotoxicology* 20:497–502.

 © 2018 SETAC

Bidel F, Di Poi C, Budzinski H, Pardon P, Callewaert W, Arini A, Basu N, Dickel L, Bellanger C, Jozet-Alves C. 2016. The antidepressant venlafaxine may act as a neurodevelopmental toxicant in cuttlefish (*Sepia officianalis*). *Neurotoxicology* 55:142–153.

Bisesi JH, Bridges W, Klaine SJ. 2014. Effects of the antidepressant venlafaxine on fish brain serotonin and predation behavior. *Aquat Toxicol* 148:130–138.

Blazer VS, Iwanowicz DD, Walsh HL, Sperry AJ, Iwanowicz LR, Alvarez DA, Brightbill RA, Smith G, Foreman WT, Manning R. 2014. Reproductive health indicators of fishes from Pennsylvania watersheds: Association with chemicals of emerging concern. *Environ Monit Assess* 186:6471–6491.

Bletsou AA, Jeon J, Hollender J, Archontaki E, Thomaidis NS. 2015. Targeted and non-targeted liquid chromatography-mass spectrometric workflows for identification of transformation products of emerging pollutants in the aquatic environment. *Trends Anal Chem* 66:32–44.

Blum KM, Andersson PL, Renman G, Ahrens L, Gros M, Wiberg K, Haglund P. 2017. Non-target screening and prioritization of potentially persistent, bioaccumulating and toxic domestic wastewater contaminants and their removal in on-site and large-scale sewage treatment plants. *Sci Total Environ* 575:265–275.

Bolong N, Ismail AF, Salim MR, Matsuura T. 2009. A review of the effects of emerging contaminants in wastewater and options for their removal. *Desalination* 238:229–246.

Boström ML, Berglund O. 2015. Influence of pH-dependent aquatic toxicity of ionizable pharmaceuticals on risk assessments over environmental pH ranges. *Water Res* 72:154–161.

Boxall ABA, Rudd MA, Brooks BW, Caldwell DJ, Choi K, Hickmann S, Innes E, Ostapyk K, Staveley JP, Verslycke T, Ankley GT, Beazley KF, Belanger SE, Berninger JP, Carriquiriborde P, Coors A, Deleo PC, Dyer SD, Ericson JF, Gagné F, Giesy JP, Gouin T, Hallstrom L, Karlsson MV, Larsson DGJ, Lazorchak JM, Mastrocco F, McLaughlin A, McMaster ME, Meyerhoff RD, Moore R, Parrott JL, Snape JR, Murray-Smith R, Servos MR, Sibley PK, Straub JO, Szabo ND, Topp E, Tetreault GR, Trudeau VL, Van der Kraak G. 2012. Pharmaceuticals and personal care products in the environment: What are the big questions? *Environ Health Perspect* 120:1221–1229.

Bridges CM, Semlitsch RD. 2001. Genetic variation in insecticide tolerance in a population of southern leopard frogs (*Rana sphenocephala*): Implications for amphibian conservation. *Copeia* 2001:7–13.

Brodin T, Nordling J, Lagesson A, Kaminder J, Hellström G, Christensen B, Fick J. 2017. Environmental relevant levels of benzodiazepine (oxazepam) alters important behavioral traits in a common planktivorous fish (*Rutilus rutilus*). *J Toxicol Environ Health A* 80:963–970.

Brodin T, Piovano S, Fick J, Klaminder J, Heynen M, Jonsson M. 2014. Ecological effects of pharmaceuticals in aquatic systems—Impacts through behavioral alterations. *Philos Trans R Soc Lond B Biol Sci* 369:20120580.

Brown AR, Owen SF, Peters J, Zhang Y, Soffker M, Paull GC, Hosken DJ, Wahab MA, Tyler CR. 2015. Climate change and pollution speed declines in zebrafish populations. *Proc Natl Acad Sci U S A* 112:E1237–E1246.

Bruder A, Salis RK, Jones PE, Matthaei CD. 2017. Biotic interactions modify multiple-stressor effects on juvenile brown trout in an experimental stream food web. *Glob Chang Biol* 23:3882–3894.

Bull JJ, Gutzke WH, Crews D. 1988. Sex reversal by estradiol in three reptilian orders. *Gen Comp Endocrinol* 70:425–428.

Bundschuh M, Zubrod JP, Schulz R. 2011. The functional and physiological status of *Gammarus fossarum* (Crustacea, Amphipoda) exposed to secondary treated wastewater. *Environ Pollut* 159:244–249.

Burggreen WW, Mueller CA. 2015. Developmental critical windows and sensitive periods as a three-dimensional construct in time and space. *Physiol Biochem Zool* 88:91–102.

Burkhardt-Holm P. 2010. Endocrine disruptors and water quality: A state-of-the-art review. *Int J Water Resour Dev* 26:477–493.

Burkina V, Zlabek V, Zamaratskaia G. 2015. Effects of pharmaceuticals present in aquatic environment on Phase I metabolism in fish. *Environ Toxicol Phar* 40:430–444.

Caliman FA, Gavrilescu M. 2009. Pharmaceuticals, personal care products and endocrine disrupting agents in the environment—A review. *Clean-Soil Air Water* 37:277–303.

Calisto V, Esteves VI. 2009. Psychiatric pharmaceuticals in the environment. *Chemosphere* 77:1257–1274.

Campillo JA, Sevilla A, Albentosa M, Bernal C, Lozano AB, Cánovas M, León VM. 2015. Metabolomic responses in caged clams, *Ruditapes decussatus*, exposed to agricultural and urban inputs in a Mediterranean coastal lagoon (Mar Menor, SE Spain). *Sci Total Environ* 524:136–147.

Carere M, Miniero R, Cicero MR. 2011. Potential effects of climate change on the chemical quality of aquatic biota. *Trends Anal Chem* 30: 1214–1221.

Caspillo NR, Volkova K, Hallgren S, Olsson PE, Porsch-Hällström I. 2014. Short-term treatment of adult male zebrafish (*Danio rerio*) with 17α-ethinylestradiol affects the transcription of genes involved in development and male sex differentiation. *Comp Biochem Phys C* 164:35–42.

Chambers JE, Greim H, Kendall RJ, Segner H, Sharpe RM, Van der Kraak G. 2014. Human and ecological risk assessment of a crop protection chemical: A case study with the azole fungicide epoxiconazole. *Crit Rev Toxicol* 44:176–210.

Choudhuri S, Patton GW, Chanderbhan RF, Mattia A, Klaassen CD. 2018. From classical toxicology to Tox21: Some critical conceptual and technological advances in the molecular understanding of the toxic response beginning from the last quarter of the 20th century. *Toxicol Sci* 161:5–22.

Clairardin SG, Paitz RT, Bowden RM. 2013. In ovo inhibition of steroid metabolism by bisphenol-A as a potential mechanism of endocrine disruption. *Proc Roy Soc B-Biol Sci* 280:20131773.

Clements WH, Rohr JR. 2009. Community response to contaminants: Using basic ecological principles to predict ecotoxicological effects. *Environ Toxicol Chem* 28:1789–1800.

Corcoran J, Winter MJ, Tyler CR. 2010. Pharmaceuticals in the aquatic environment: A critical review of the evidence for health effects in fish. *Crit Rev Toxicol* 40:287–304.

Cothran RD, Brown JM, Relyea RA. 2013. Proximity to agriculture is correlated with pesticide tolerance: Evidence for the evolution of amphibian resistance to modern pesticides. *Evol Appl* 6:832–841.

Cripe GM, Hemmer BL, Goodman LR, Fournie JW, Raimondo S, Vennari JC, Danner RL, Smith K, Manfredonia BR, Kulaw DH, Hemmer MJ. 2009. Multigenerational exposure of the estuarine sheepshead minnow (*Cyprinodon variegatus*) to 17b-estradiol. I. Organism-level effects over three generations. *Environ Toxicol Chem* 28:2397–2408.

Cripe GM, Hemmer BL, Raimondo S, Goodman LR, Kulaw DH. 2010. Exposure of three generations of the estuarine sheepshead minnow (*Cyprinodon variegatus*) to the androgen 17ß-trenbolone: Effects on survival, development, and reproduction. *Environ Toxicol Chem* 29: 2079–2097.

Dammann AA, Shapell NW, Bartell SE, Schoenfuss HL. 2011. Comparing biological effects and potencies of estrone and 17β-estradiol in mature fathead minnows, *Pimephales promelas*. *Aquat Toxicol* 105:559–568.

Daughton CG. 2002. Environmental stewardship and drugs as pollutants. *Lancet* 360:1035–1036.

Davis RP, Sullivan SMP, Stefanik KC. 2017. Reductions in fish-community contamination following lowhead dam removal linked more to shifts in food-web structure than sediment pollution. *Environ Pollut* 231:671–680.

Delcour I, Spanoghe P, Uyttendaele M. 2015. Literature review: Impact of climate change on pesticide use. *Food Res Int* 68:7–15.

De Zwart D, Dyer SD, Posthuma L, Hawkins CP. 2006. Predictive models attribute effects on fish assemblages to toxicity and habitat alteration. *Ecol Appl* 16:1295–1310.

Diamond SA, Oris JT, Guttman SI. 1995. Adaptation to fluoranthene exposure in a laboratory population of fathead minnows. *Environ Toxicol Chem* 14:1393–1400.

Ding JN, Lu GH, Li Y. 2016. Interactive effects of selected pharmaceutical mixtures on bioaccumulation and biochemical status in crucian carp (*Carassius auratus*). *Chemosphere* 148:21–31.

Di Nica V, Villa S, Finizio A. 2017. Toxicity of individual pharmaceuticals and their mixtures to Aliivibrio fischeri: Experimental results for single compounds and considerations of their mechanisms of action and potential acute effects on aquatic organisms. *Environ Toxicol Chem* 36:807–814.

Du Y, Shi X, Liu C, Yu K, Zhou B. 2009. Chronic effects of water-borne PFOS exposure on growth, survival and hepatotoxicity in zebrafish: A partial life-cycle test. *Chemosphere* 74:723–729.

Ebele AJ, Abou-Elwafa Abdallah M, Harrad S. 2017. Pharmaceuticals and personal care products (PPCPs) in the freshwater aquatic environment. *Emerg Contam* 3:1–16.

Egea-Serrano A, Tejedo M. 2014. Contrasting effects of nitrogenous pollution on fitness and swimming performance of Iberian waterfrog,

© 2018 SETAC

Pelophylax perezi (Seoane, 1885), larvae in mesocosms and field enclosures. *Aquat Toxicol* 146:144–153.

Egea-Serrano A, Tejedo M, Torralva M. 2009. Populational divergence in the impact of three nitrogenous compounds and their combination on larvae of the frog *Pelophylax perezi* (Seoane, 1885). *Chemosphere* 76:869–877.

Eggen R, Behra R, Burkhardt-Holm P, Escher B, Schweigert N. 2004. Challenges in ecotoxicology. *Environ Sci Technol* 38:A59–A64.

Elliott SM, Brigham ME, Kiesling RL, Schoenfuss HL, Jorgenson ZG. 2018. Environmentally relevant chemical mixtures of concern in U.S. tributaries to the Great Lakes. *Integr Environ Assess Manag* 14:509–518.

Elliott SM, Kiesling RL, Jorgenson ZG, Rearick DC, Schoenfuss HL, Fredricks KT, Gaikowski MP. 2014. Fathead minnow and bluegill sunfish life-stage responses to 17β-estradiol exposure in outdoor mesocosms. *J Am Water Resour Assoc* 50:376–387.

Escher BI, Fenner K. 2011. Recent advances in environmental risk assessment of transformation products. *Environ Sci Technol* 45:3835–3847.

Everroad RC, Yoshida S, Tsuboi Y, Date Y, Kikuchi J, Moriya S. 2012. Concentration of metabolites from low-density planktonic communities for environmental metabolomics using nuclear magnetic resonance spectroscopy. *J Vis Exp* 62:e3163.

Farré ML, Pérez S, Kantiani L, Barceló D. 2008. Fate and toxicity of emerging pollutants, their metabolites and transformation products in the aquatic environment. *Trends Anal Chem* 27:991–1007.

Feist BE, Buhle ER, Arnold P, Davis JW, Scholz NL. 2011. Landscape ecotoxicology of coho salmon spawner mortality in urban streams. *PLoS One* 6:e23424.

Feist GW, Webb MAH, Gundersen DT, Foster EP, Schreck CB, Maule AG, Fitzpatrick MS. 2005. Evidence of detrimental effects of environmental contaminants on growth and reproductive physiology of white sturgeon in impounded areas of the Columbia River. *Environ Health Perspect* 113:1675–1682.

Fischer BB, Pomati F, Eggen RI. 2013. The toxicity of chemical pollutants in dynamic natural systems: The challenge of integrating environmental factors and biological complexity. *Sci Total Environ* 449:253–259.

Fleeger JW, Carman KR, Nisbet RM. 2003. Indirect effects of contaminants in aquatic ecosystems. *Sci Total Environ* 317:207–233.

Forbes VE, Calow P, Grimm V, Hayashi T, Jager T, Katholm A, Palmqvist A, Pastorok R, Salvito D, Sibly R, Spromberg J, Stark J, Stillman RA. 2011. Adding value to ecological risk assessment with population modeling. *Hum Ecol Risk Assess* 17:287–299.

Forbes VE, Galic N, Schmolke AM, Vavra J, Pastorok R, Thorbeck P. 2016. Assessing the risks of pesticides to threatened and endangered species using population modeling: A critical review and recommendations for future work. *Environ Toxicol Chem* 35:1904–1913.

Forsatkar MN, Nematollahi MA, Amiri BM, Huang WB. 2014. Fluoxetine inhibits aggressive behavior during parental care in male fighting fish (*Betta splendens*, Regan). *Ecotoxicology* 23:1794–1802.

Gago-Ferrero P, Gros M, Ahrens L, Wiberg K. 2017. Impact of on-site, small and large scale wastewater treatment facilities on levels and fate of pharmaceuticals, personal care products, artificial sweeteners, pesticides, and perfluoroalkyl substances in recipient waters. *Sci Total Environ 601-602*:1289–1297.

Garcia-Galan MJ, Sordet M, Bulete A, Garric J, Vulliet E. 2017. Evaluation of the influence of surfactants in the bioaccumulation kinetics of sulfamethoxazole and oxazepam in benthic invertebrates. *Sci Total Environ* 592:554–564.

García-Reyero N, Lavelle CM, Escalon BL, Martinovic D, Kroll KJ, Sorensen PW, Denslow ND. 2011. Behavioral and genomic impacts of a wastewater effluent on the fathead minnow. *Aquat Toxicol* 101:38–48.

Gaworecki KM, Klaine SJ. 2008. Behavioral and biochemical responses of hybrid striped bass during and after fluoxetine exposure. *Aquat Toxicol* 88:207–213.

Gerbersdorf SU, Cimatoribus C, Class H, Engesser KH, Heblbich S, Hollert H, Lange C, Kranert M, Metger J, Nowak W, Seiler TB, Steger K, Steinmetz H, Wieprecht S. 2015. Anthropogenic trace compounds (ATCs) in aquatic habitats—Research needs on sources, fate, detection and toxicity to ensure timely elimination strategies and risk management. *Environ Int* 79:85–105.

Gessner MO, Tlili A. 2016. Fostering integration of freshwater ecology with ecotoxicology. *Freshwater Biol* 61:1991–2001.

Giraudo M, Cottin G, Esperanza M, Gagnon P, De Silva AO, Houde M. 2017a. Transcriptional and cellular effects of benzotriazole UV stabilizers UV-234 and UV-328 in the freshwater invertebrates Chlamydomonas reinhardtii and *Daphnia magna*. *Environ Toxicol Chem* 36:3333–3342.

Giraudo M, Douville M, Cottin G, Houde M. 2017b. Transcriptomic, cellular and life-history responses of *Daphnia magna* chronically exposed to benzotriazoles: Endocrine-disrupting potential and molting effects. *PLoS One* 12:e0171763.

Gouveia D, Chaumot A, Charnot A, Almunia C, François A, Navarro L, Armengaud J, Salvador A, Geffard O. 2017. Ecotoxico-proteomics for aquatic environmental monitoring: First in situ application of a new proteomics-based multibiomarker assay using caged amphipods. *Environ Sci Technol* 51:13417–13426.

Greaney NE, Mannion KL, Dzieweczynski TL. 2015. Signaling on Prozac: Altered audience effects on male-male interactions after fluoxetine exposure in Siamese fighting fish. *Behav Ecol Sociobiol* 69: 1925–1932.

Guasch H, Ricart M, López-Doval J, Bonnineau C, Proia L, Morin S, Muñoz I, Roman AM, Sabater S. 2016. Influence of grazing on triclosan toxicity to stream periphyton. *Freshwater Biol* 61:2002–2012.

Haddad SP, Du B, Scott WC, Saari GN, Breed C, Kelly M, Broach L, Chambliss K, Brooks BW. 2017. Ontogenetic dietary shifts and bioaccumulation of diphenhydramine in *Mugil cephalus* from an urban estuary. *Mar Environ Res* 127:155–162.

Hallgren P, Nicolle A, Hansson L-A., Brönmark C, Nikoleris L, Hyder M, Persson A. 2014. Synthetic estrogen directly affects fish biomass and may indirectly disrupt aquatic food webs. *Environ Toxicol Chem* 33:930–936.

Hampel M, Blasco J, Martín Díaz ML. 2016. Biomarkers and effects. In Blasco J, Chapman PM, Campana O, Hampel M, eds, *Marine Ecotoxicology*. Academic, New York, NY, USA, pp 121–165.

Harwood AD, You J, Lydy MJ. 2009. Temperature as a toxicity identification evaluation tool for pyrethroid insecticides: Toxicokinetic confirmation. *Environ Toxicol Chem* 28:1051–1058.

Heberer T. 2002. Occurrence, fate, and removal of pharmaceutical residues in the aquatic environment: A review of recent research data. *Toxicol Lett* 131:5–17.

Holmstrup M, Bindesbol AM, Oostingh GJ, Duschl A, Scheil V, Kohler HR, Loureiro S, Soares AM, Ferreira AL, Kienle C, Gerhardt A, Laskowski R, Kramarz PE, Bayley M, Svendsen C, Spurgeon DJ. 2010. Interactions between effects of environmental chemicals and natural stressors: A review. *Sci Total Environ* 408:3746–3762.

Hua J, Jones DK, Mattes BM, Cothran RD, Relyea RA, Hoverman JT. 2015. Evolved pesticide tolerance in amphibians: Predicting mechanisms based on pesticide novelty and mode of action. *Environ Pollut* 206: 56–63.

Huerta B, Margiotta-Casaluci L, Rodríguez-Mozaz S, Scholze M, Winter MJ, Barceló D, Sumpter JP. 2016. Anti-anxiety drugs and fish behavior: Establishing the link between internal concentrations of oxazepam and behavioral effects. *Environ Toxicol Chem* 35:2782–2790.

Ismail NS, Muller CE, Morgan RR, Luthy RG. 2014. Uptake of contaminants of emerging concern by the bivalves *Anodonta californiensis* and *Corbicula fluminea*. *Environ Sci Technol* 48:9211–9219.

Jin Y, Chen R, Liu W, Fu Z. 2010. Effect of endocrine disrupting chemicals on the transcription of genes related to the innate immune system in the early developmental stage of zebrafish (*Danio rerio*). *Fish Shellfish Immun* 28:854–861.

Jordan J, Zare A, Jackson LJ, Habibi HR, Weljie AM. 2012. Environmental contaminant mixtures at ambient concentrations invoke a metabolic stress response in goldfish not predicted from exposure to individual compounds alone. *J Proteome Res* 11:1133–1143.

Judson RS, Magpantay FM, Chickarmane V, Haskell C, Tania N, Taylor J, Xia M, Huang R, Rotroff DM, Filer DL, Houck KA, Martin MT, Sipes N, Richard AM, Mansouri K, Setzer RW, Knudsen TB, Crofton KM, Thomas RS. 2015. Integrated model of chemical perturbations of a biological pathway using 18 in vitro high-throughput screening assays for the estrogen receptor. *Toxicol Sci* 148:137–154.

Kasprzyk-Hordern B, Dinsdale RM, Guwy AJ. 2009. The removal of pharmaceuticals, personal care products, endocrine disruptors and illicit drugs during wastewater treatment and its impact on the quality of receiving waters. *Water Res* 43:363–380.

Kellner M, Porseryd T, Hallgren S, Porsch-Hällström I, Hansen HS, Olsén KH. 2016. Waterborne citalopram has anxiolytic effects and increases locomotor activity in the three-spine stickleback (*Gasterosteus aculeatus*). *Aquat Toxicol* 173:19–28.

 © 2018 SETAC

Kent K, Weston AA, Caminada D. 2006. Ecotoxicology of human pharmaceuticals. *Aquat Toxicol* 76:122–159.

Kidd K, Blanchfield PJ, Mills KH, Palace VP, Evans RE, Lazorchak JM, Flick RW. 2007. Collapse of a fish population after exposure to a synthetic estrogen. *Proc Natl Acad Sci U S A* 104:8897–8901.

Kidd KA, Paterson MJ, Rennie MD, Podemski CL, Findlay DL, Blanchfield PJ, Liber K. 2014. Direct and indirect responses of a freshwater food web to a potent synthetic oestrogen. *Philos Trans R Soc Lond B Biol Sci*. [cited 2018 May 3]. Available from: https://doi.org/10.1098/rstb.2013.0578.

Kim J, Park J, Kim PG, Lee C, Choi K, Choi K. 2010. Implication of global environmental changes on chemical toxicity-effect of water temperature, pH, and ultraviolet B irradiation on acute toxicity of several pharmaceuticals in *Daphnia magna*. *Ecotoxicology* 19:662–669.

Kolpin DW, Furlong ET, Meyer MT, Thurman EM, Zaugg SD, Barber LB, Buxton HT. 2002. Pharmaceuticals, hormones, and other organic wastewater contaminants in U.S. streams, 1999-2000: A national reconnaissance. *Environ Sci Technol* 36:1202–1211.

Kopras E, Potluri V, Bermudez ML, Williams K, Belcher S, Kasper S. 2014. Actions of endocrine-disrupting chemicals on stem/progenitor cells during development and disease. *Endocr Relat Cancer* 21:1–12.

Kovacevic V, Simpson AJ, Simpson MJ. 2016. 1H NMR-based metabolomics of *Daphnia magna* responses after sub-lethal exposure to triclosan, carbamazepine and ibuprofen. *Comp Biochem Phys D* 19:199–210.

Kramer VJ, Etterson MA, Hecker M, Murphy CA, Roesijadi G, Spade DJ, Spromberg JA, Wang M, Ankley GT. 2011. Adverse outcome pathways and ecological risk assessment: Bridging to population-level effects. *Environ Toxicol Chem* 30:64–76.

Krummel EM, Macdonald RW, Kimpe LE, Gregory-Eaves I, Demers MJ, Smol JP, Finney B, Blais JM. 2003. Delivery of pollutants by spawning salmon—Fish dump toxic industrial compounds in Alaskan lakes on their return from the ocean. *Nature* 425:255–256.

Lagadic L, Katsiadaki I, Biever R, Guiney PD, Karouna-Renier N, Schwartz T, Meador JP. 2017. Tributyltin: Advancing the science on assessing endocrine disruption with an unconventional endocrine-disrupting compound. In De Voogt P, ed, Reviews of Environmental Contamination and Toxicology, Vol 245. Springer, Cham, Switzerland, pp 65–127.

Lagesson A, Fahlman J, Brodin T, Fick J, Jonsson M, Bystrom P, Klaminder J. 2016. Bioaccumulation of five pharmaceuticals at multiple trophic levels in an aquatic food web—Insights from a field experiment. *Sci Total Environ* 568:208–215.

Lee H, De Silva AO, Mabury SA. 2012. Dietary bioaccumulation of perfluorophosphonates and perfluorophosphinates in juvenile rainbow trout: Evidence of metabolism of perfluorophosphinates. *Environ Sci Technol* 46:3489–3497.

Lescord GL, Kidd KA, De Silva AO, Williamson M, Spencer C, Wang X, Muir DC. 2015. Perfluorinated and polyfluorinated compounds in lake food webs from the Canadian high Arctic. *Environ Sci Technol* 49:2694–2702.

Liang X, Zha J, Martyniuk CJ, Wang Z, Zhao J. 2017. Histopathological and proteomic responses in male Chinese rare minnow (*Gobiocypris rarus*) indicate hepatotoxicity following benzotriazole exposure. *Environ Pollut* 229:459–469.

Liu JL, Wong MH. 2013. Pharmaceuticals and personal care products (PPCPs): A review on environmental contamination in China. *Environ Int* 59:208–224.

Llorca M, Farré M, Eljarrat E, Díaz-Cruz S, Rodŕguez-Mozaz S, Wunderlin D, Barcelo D. 2017. Review of emerging contaminants in aquatic biota from Latin America: 2002–2016. *Environ Toxicol Chem* 36:1716–1727.

Lowell R, Culp J, Dube M. 2000. A weight-of-evidence approach for Northern River risk assessment: Integrating the effects of multiple stressors. *Environ Toxicol Chem* 19:1182–1190.

Macneale KH, Kiffney PM, Scholtz N. 2010. Pesticides, aquatic food webs and the conservation of Pacific salmon. *Front Ecol Environ* 8:475–482.

Magbanua FS, Townsend CR, Hageman KJ, Piggott JJ, Matthaei CD. 2016. Individual and combined effects of fine sediment and glyphosate herbicide on invertebrate drift and insect emergence: A stream mesocosm experiment. *Freshwater Sci* 35:139–151.

Manciocco A, Calamandrei G, Alleva E. 2014. Global warming and environmental contaminants in aquatic organisms: The need of the etho-toxicology approach. *Chemosphere* 100:1–7.

Marsh-Matthews W, Skierkowski P, DeMarais A. 2001. Direct evidence for mother-to-embryo transfer of nutrients in the livebearing fish Gambusia geiseri. *Copeia* 2001:1–6.

Martinovic D, Hogarth WT, Jones R, Sorensen PW. 2007. Environmental estrogens suppress hormones, behavior, and reproductive fitness in male fathead minnows. *Environ Toxicol Chem* 26:271–278.

Matson CW, Lambert MM, McDonald TJ, Autenrieth RL, Donnelly KC, Islamzadeh A, Politov DI, Bickham JW. 2006. Evolutionary toxicology: Population-level effects of chronic contaminant exposure on the marsh frogs (*Rana ridibunda*) of Azerbaijan. *Environ Health Perspect* 114:547–552.

McCallum ES, Krutzelmann E, Brodin T, Fick J, Sundelin A, Balshine S. 2017. Exposure to wastewater effluent affects fish behaviour and tissue-specific uptake of pharmaceuticals. *Sci Total Environ* 605-606:578–588.

McLeod AM, Paterson G, Drouillard KG, Haffner GD. 2016. Ecological implications of steady state and nonsteady state bioaccumulation models. *Environ Sci Technol* 50:11103–11111.

Melvin SD, Buck DR, Fabbro LD. 2015. Diurnal activity patterns as a sensitive behavioural outcome in fish: Effect of short-term exposure to treated sewage and a sub-lethal PPCP mixture. *J Appl Toxicol* 36:1173–1182.

Mihaich EM, Schafers C, Dreier DA, Hecker M, Ortego L, Kawashima Y, Dang Z-C., Solomon K. 2017. Challenges in assigning endocrine-specific modes of action: Recommendations for researchers and regulators. *Integr Environ Assess Manag* 13:280–292.

Miller DH, Jensen KM, Villeneuve DL, Kahl MD, Makynen EA, Durhan EJ, Ankley GT. 2007. Linkage of biochemical responses to population-level effects: A case study with vitellogenin in the fathead minnow (*Pimephales promelas*). *Environ Toxicol Chem* 26:521–527.

Moran R, Harvey IAN, Moss B, Feuchtmayr H, Hatton K, Heyes TOM, Atkinson D. 2010. Influence of simulated climate change and eutrophication on three-spined stickleback populations: A large scale mesocosm experiment. *Freshwater Biol* 55:315–325.

Müller R, Seeland A, Jagodzinski LS, Diogo JB, Nowak C, Oehlmann J. 2012. Simulated climate change conditions unveil the toxic potential of the fungicide pyrimethanil on the midge *Chironomus riparius*: A multi-generation experiment. *Ecol Evol* 2:196–210.

Nash JP, Kime DE, Van der Ven LTM, Wester PW, Brion F, Maack G, Stahlschmidt-Allner P, Tyler CR. 2004. Long-term exposure to environmental concentrations of the pharmaceutical ethynylestradiol causes reproductive failure in fish. *Environ Health Perspect* 112: 1725–1733.

Neale PA, Munz NA, Aït-Aïssa S, Altenburger R, Brion F, Busch W, Escher BI, Hilscherova K, Kienle C, Novak J, Seiler TB, Shao Y, Stamm C, Hollender J. 2017. Integrating chemical analysis and bioanalysis to evaluate the contribution of wastewater effluent on the micropollutant burden in small streams. *Sci Total Environ* 576:785–795.

Nilsen EB, Zaugg S, Alvarez DA, Morace JL, Waite IR, Counihan TD, Hardiman JM, Torres L, Patiño R, Mesa M, Grove R. 2014. Contaminants of legacy and emerging concern in largescale suckers (*Catostomus macrocheilus*) and the foodweb in the lower Columbia River, Oregon and Washington, USA. *Sci Total Environ* 484:344–352.

Noyes PD, McElwee MK, Miller HD, Clark BW, Van Tiem LA, Walcott KC, Erwin KN, Levin ED. 2009. The toxicology of climate change: Environmental contaminants in a warming world. *Environ Int* 35:971–986.

Oliveira IB, Groh KJ, Schonenberger R, Barroso C, Thomas KV, Suter MJ. 2017. Toxicity of emerging antifouling biocides to non-target freshwater organisms from three trophic levels. *Aquat Toxicol* 191:164–174.

Organisation for Economic Co-operation and Development. 2012. Guidance document on standardised test guidelines for evaluating chemicals for endocrine disruption. Series on Testing and Assessment, No. 150. ENV/JM/MONO (2012) 22. Paris, France.

Orton F, Ermler S, Kugathas S, Rosivatz E, Scholze M, Kortenkamp A. 2014. Mixture effects at very low doses with combinations of anti-androgenic pesticides, antioxidants, industrial pollutant and chemicals used in personal care products. *Toxicol Appl Pharm* 278:201–208.

Pal A, Gin KYH, Lin AYC, Reinhard M. 2010. Impacts of emerging organic contaminants on freshwater resources: Review of recent occurrences, sources, fate and effects. *Sci Total Environ* 408:6062–6069.

Palace VP, Evans RE, Wautier KG, Mills KH, Blanchfield PJ, Park BJ, Baron CL, Kidd KA. 2009. Interspecies differences in biochemical, histopathological, and population responses in four wild fish species exposed to ethynylestradiol added to a whole lake. *Can J Fish Aquat Sci* 66:1920–1935.

Pandey PK, Ajima MNO, Kumar K, Poojary N, Kumar S. 2017. Evaluation of DNA damage and physiological responses in Nile tilapia *Oreochromis niloticus* (Linnaeus, 1758) exposed to sub-lethal diclofenac (DCF). *Aquat Toxicol* 186:205–207.

© 2018 SETAC

Parolini M, Bini L, Magni S, Rizzo A, Ghilardi A, Landi C, Armani A, Del Giacco L, Binelli A. 2018. Exposure to cocaine and its main metabolites altered the protein profile of zebrafish embryos. *Environ Pollut* 232:603–614.

Parrott JL, Bjerregaard P, Brugger KE, Gray LE, Jr., Iguchi T, Kadlec SM, Weltje L, Wheeler JR. 2017. Uncertainties in biological responses that influence hazard and risk approaches to the regulation of endocrine active substances. *Integr Environ Assess Manag* 13:293–301.

Parrott JL, Blunt BR. 2005. Life-cycle exposure of fathead minnows (*Pimephales promelas*) to an ethinylestradiol concentration below 1 ng/L reduces egg fertilization success and demasculinizes males. *Environ Toxicol* 20: 131–141.

Patisaul HB, Adewale HB. 2009. Long-term effects of environmental endocrine disruptors on reproductive physiology and behavior. *Front Behav Neurosci* 3:10.

Posthuma L, Dyer SD, De Zwart D, Kapo K, Holmes CM, Burton GA Jr. 2016. Eco-epidemiology of aquatic ecosystems: Separating chemicals from multiple stressors. *Sci Total Environ* 573:1303–1319.

Reid NM, Proestou DA, Clark BW, Warren WC, Colbourne JK, Shaw JR, Karchner SI, Hahn ME, Nacci D, Oleksiak MF, Crawford DL, Whitehead A. 2016. The genomic landscape of rapid repeated evolutionary adaptation to toxic pollution in wild fish. *Science* 354:1305–1308.

Reinling J, Houde M, Verreault J. 2017. Environmental exposure to a major urban wastewater effluent: Effects on the energy metabolism of northern pike. *Aquat Toxicol* 191:131–140.

Richardson SD. 2010. Environmental mass spectrometry: Emerging contaminants and current issues. *Anal Chem* 82:4742–4774.

Richardson SD, Kimura SY. 2017. Emerging environmental contaminants: Challenges facing our next generation and potential engineering solutions. *Environ Technol Innov* 8:40–56.

Rico A, Van den Brink PJ, Leitner P, Graf W, Focks A. 2016. Relative influence of chemical and non-chemical stressors on invertebrate communities: A case study in the Danube River. *Sci Total Environ* 571:1370–1382.

Rissman EF, Adli M. 2014. Minireview: Transgenerational epigenetic inheritance: Focus on endocrine disrupting compounds. *Endocrinology* 155:2770–2780.

Rocco L, Frenzilli G, Zito G, Archimandritis A, Peluso C, Stingo V. 2010. Genotoxic effects in fish induced by pharmacological agents present in the sewage of some Italian water treatment plants. *Environ Toxicol* 18:25.

Rodd AL, Messier NJ, Vaslet CA, Kane AB. 2017. A 3D fish liver model for aquatic toxicology: Morphological changes and Cyp1a induction in PLHC-1 microtissues after repeated benzo(a)pyrene exposures. *Aquat Toxicol* 186:134–144.

Rodea-Palomares I, Gonzalez-Pleiter M, Gonzalo S, Rosal R, Leganes F, Sabater S, Casellas M, Muñoz-Carpena R, Fernández-Piñas F. 2016. Hidden drivers of low-dose pharmaceutical pollutant mixtures revealed by the novel GSA-QHTS screening method. *Sci Adv* 2:e1601272.

Rodrigues S, Antunes SC, Correia AT, Nunes B. 2017. Rainbow trout (*Oncorhynchus mykiss*) pro-oxidant and genotoxic responses following acute and chronic exposure to the antibiotic oxytetracycline. *Ecotoxicology* 26:104–107.

Rohr JR, Kerby JL, Sih A. 2006. Community ecology as a framework for predicting contaminant effects. *Trends Ecol Evol* 21:606–613.

Rosi-Marshall EJ, Royer TV. 2012. Pharmaceutical compounds and ecosystem function: An emerging research challenge for aquatic ecologists. *Ecosystems* 15:867–880.

Ruh´A, Acuna V, Barcelo D, Huerta B, Mor JR, Rodriguez-Mozaz S, Sabater S. 2016. Bioaccumulation and trophic magnification of pharmaceuticals and endocrine disruptors in a Mediterranean river food web. *Sci Total Environ* 540:250–259.

Sánchez-Marń P, Fernández-González LE, Mantilla-Aldana L, Diz AP, Beiras R. 2017. Shotgun proteomics analysis discards alkali labile phosphate as a reliable method to assess vitellogenin levels in Mytilus galloprovincialis. *Environ Sci Technol* 51:7572–7580.

Santos LH, Araujo AN, Fachini A, Pena A, Delerue-Matos C, Montenegro MC. 2010. Ecotoxicological aspects related to the presence of pharmaceuticals in the aquatic environment. *J Hazard Mater* 175:45–95.

Schoenfuss HL, Furlong ET, Phillips PJ, Scott T, Kolpin DW, Cetkovic-Cvrlje M, Lesteberg KE, Rearick DC. 2016. Complex mixtures, complex responses: Assessing pharmaceutical mixtures using field and laboratory approaches. *Environ Toxicol Chem* 34:953–965.

Schultz MM, Painter MM, Bartell SE, Logue A, Furlong ET, Werner SL, Schoenfuss HL. 2011. Selective uptake and biological consequences of environmentally relevant antidepressant pharmaceutical exposures on male fathead minnows. *Aquat Toxicol* 104:38–47.

Schwindt AR, Winkelman DL. 2016. Estimating the effects of 17α-ethinylestradiol on stochastic population growth rates of fathead minnows: A population synthesis of empirically derived vital rates. *Ecotoxicology* 25:1364–1375.

Schwindt AR, Winkelman DL, Keteles K, Murphy M, Vajda AM. 2014. An environmental oestrogen disrupts fish population dynamics through direct and transgenerational effects on survival and fecundity. *J Appl Ecol* 51:582–591.

Segner H, Schmitt-Jansen M, Sabater S. 2014. Assessing the impact of multiple stressors on aquatic biota: The receptor's side matters. *Environ Sci Technol* 48:7690–7696.

Sehonova P, Plhalova L, Blahova J, Doubkova V, Prokes M, Tichy F, Fiorino E, Faggio C, Svobodova Z. 2017. Toxicity of naproxen sodium and its mixture with tramadol hydrochloride on fish early life stages. *Chemosphere* 188:414–423.

Simons K. 2018. How can omic science be improved? *Proteomics* 18:5–6.

Singh S, Li SS. 2012. Epigenetic effects of environmental chemicals bisphenol-A and phthalates. *Int J Mol Sci* 13:10143–10153.

Skinner MK, Manikkam M, Guerrero-Bosagna C. 2011. Epigenetic transgenerational actions of endocrine disruptors. *Reprod Toxicol* 31:337–343.

Softeland L, Berntssen MHG, Kirwan JA, Storseth TR, Viant MR, Torstensen BE, Waagbo R, Olsvik PA. 2016. Omega-3 and alpha-tocopherol provide more protection against contaminants in novel feeds for Atlantic salmon (*Salmo salar* L.) than omega-6 and gamma tocopherol. *Toxicol Reports* 3:211–224.

Spromberg JA, Scholz NL. 2011. Estimating the future decline of wild coho salmon populations resulting from early spawner die-offs in urbanizing watersheds of the Pacific Northwest, USA. *Integr Environ Assess Manag* 7:648–656.

Stahl RG, Jr., Hooper MJ, Balbus JM, Clements W, Fritz A, Gouin T, Helm R, Hickey C, Landis W, Moe SJ. 2013. The influence of global climate change on the scientific foundations and applications of environmental toxicology and chemistry: Introduction to a SETAC international workshop. *Environ Toxicol Chem* 32:13–19.

Stapleton HM, Baker JE. 2003. Comparing polybrominated diphenyl ether and polychlorinated biphenyl bioaccumulation in a food web in Grand Traverse Bay, Lake Michigan. *Arch Environ Contam Toxicol* 45:227–234.

Sui Q, Cao X, Lu S, Zhao W, Qiu Z, Yu G. 2015. Occurrence, sources and fate of pharmaceuticals and personal care products in the groundwater: A review. *Emerg Contam* 1:14–24.

Tyler CR, Jobling S, Sumpter JP. 1998. Endocrine disruption in wildlife. *Crit Rev Toxicol* 28:319–361.

US Environmental Protection Agency. 2008. Contaminants of Emerging Concern including Pharmaceuticals and Personal Care Products. [cited 2018 September 3]. Available from: https://www.epa.gov/wqc/contaminants-emerging-concern-including-pharmaceuticals-and-personal-care-products

Vajda AM, Barber LB, Gray JL, Lopez EM, Bolden AM, Schoenfuss HL. 2011. Demasculinization of male fish by wastewater treatment plant effluent. *Aquat Toxicol* 103:213–221.

Vajda AM, Barber LB, Gray JL, Lopez EM, Woodling JD, Norris DO. 2008. Reproductive disruption in fish downstream from an estrogenic wastewater effluent. *Environ Sci Technol* 42:3407–3414.

Van Aerle R, Pounds N, Hutchinson TH, Maddix S, Tyler CR. 2002. Window of sensitivity for the estrogenic effects of ethinylestradiol in early life-stages of fathead minnow, *Pimephales promelas*. *Ecotoxicology* 11:423–434.

Van Straalen NM. 2003. Ecotoxicology becomes stress ecology. *Environ Sci Technol* 37:A324–A330.

Watanabe H, Horie Y, Takanobu H, Koshio M, Flynn K, Iguchi T, Tatarazako N. 2017. Medaka extended one-generation reproductive test evaluating 4-nonylphenol. *Environ Toxicol Chem* 36:3254–3266.

Watanabe H, Tamura I, Abe R, Takanobu H, Nakamura A, Suzuki T, Hirose A, Nishimura T, Tatarazako N. 2016. Chronic toxicity of an environmentally relevant mixture of pharmaceuticals to three aquatic organisms (alga, daphnid, and fish). *Environ Toxicol Chem* 35:996–1006.

Wilkinson J, Hooda PS, Barker J, Barton S, Swinden J. 2017. Occurrence, fate and transformation of emerging contaminants in water: An overarching review of the field. *Environ Pollut* 231:954–970.

 © 2018 SETAC

Wilkinson JL, Hooda PS, Barker J, Barton S, Swinden J. 2015. Ecotoxic pharmaceuticals, personal care products, and other emerging contaminants: A review of environmental, receptor-mediated, developmental, and epigenetic toxicity with discussion of proposed toxicity to humans. *Crit Rev Environ Sci Technol* 46:336–381.

Windsor FM, Ormerod SJ, Tyler CR. 2017. Endocrine disruption in aquatic systems: Up-scaling research to address ecological consequences. *Biol Rev Camb Philos Soc* 93:626–641.

Xie Z, Lu G, Yan Z, Liu J, Wang P, Wang Y. 2017. Bioaccumulation and trophic transfer of pharmaceuticals in food webs from a large freshwater lake. *Environ Pollut* 222:356–366.

Yang LH, Ying GG, Su HC, Stauber JL, Adams MS, Binet MT. 2008. Growth-inhibiting effects of 12 antibacterial agents and their mixtures on the freshwater microalga Pseudokirchneriella subcapitata. *Environ Toxicol Chem* 27:1201–1208.

Yokota H, Seki M, Maeda M, Oshumi Y, Tadokoro H, Honjo T, Kobayashi K. 2001. Life-cycle toxicity of 4-nonylphenol to medaka (*Oryzias latipes*). *Environ Toxicol Chem* 20:2552–2560.

Zhao J-L., Furlong ET, Schoenfuss HL, Kolpin DW, Bird KL, Feifarek DJ, Schwab EA, Ying G-G. 2017. Uptake and disposition of select pharmaceuticals by bluegill exposed at constant concentrations in a flow-through aquatic exposure system. *Environ Sci Technol* 51:4434–4444.

© 2018 SETAC