The AAPS Journal, Vol. 16, No. 2, March 2014 (© 2014)
DOI: 10.1208/s12248-014-9561-3

Review Article

Theme: Pharmaceuticals and Personal Care Products in the Environment
Guest Editors: James P. Laurenson, Raanan A. Bloom, and Nakissa Sadrieh

Ethinyl Estradiol and Other Human Pharmaceutical Estrogens in the Aquatic Environment: A Review of Recent Risk Assessment Data

James P. Laurenson,[1,4] Raanan A. Bloom,[1] Stephen Page,[2] and Nakissa Sadrieh[3]

Received 10 September 2013; accepted 2 January 2014; published online 28 January 2014

Abstract. Interest in pharmaceuticals in the environment has increased substantially in recent years. Several studies in particular have assessed human and ecological risks from human pharmaceutical estrogens, such as 17α-ethinyl estradiol (EE2). Regulatory action also has increased, with the USA and other countries developing rules to address estrogens and other pharmaceuticals in the environment. Accordingly, the Center for Drug Evaluation and Research at the US Food and Drug Administration has conducted a review and analysis of current data on the long-term ecological exposure and effects of EE2 and other estrogens. The results indicate that mean-flow long-term predicted environmental concentrations (PECs) of EE2 in approximately 99% or more of US surface water segments downstream of wastewater treatment plants are lower than a predicted no-effect concentration (PNEC) for aquatic chronic toxicity of 0.1 ng/L. Exceedances are expected to be primarily in localized, effluent-dominated water segments. The median mean-flow PEC is more than two orders of magnitude lower than this PNEC. Similar results exist for other pharmaceutical estrogens. Data also suggest that the contribution of EE2 more broadly to total estrogenic load in the environment from all sources (including other human pharmaceutical estrogens, endogenous estrogens, natural environmental estrogens, and industrial chemicals), while highly uncertain and variable, appears to be relatively low overall. Additional data and a more comprehensive approach for data collection and analysis for estrogenic substances in the environment, especially in effluent-dominated water segments in sensitive environments, would more fully characterize the risks.

KEY WORDS: aquatic ecology; environmental impact; estrogens; regulatory science; toxicity.

INTRODUCTION

Since the early 2000s, there has been a significant increase in the number of published scientific and policy reports on pharmaceuticals and personal care products in the environment. Indeed, about 15,000 such reports have been published over the past 20 years, with about half published in just the past four years (1,2). Topics range from the development of analytical chemistry methods capable of detection at below nanograms per liter levels to measures of sublethal behavioral effects in aquatic organisms. Pharmaceutical estrogens such as 17α-ethinyl estradiol (EE2), which like most pharmaceuticals are released into the environment predominantly via the human excretion → wastewater treatment plant (WWTP) → surface water pathway, have perhaps received the most attention due to the potential adverse endocrine disrupting ecological effects associated with their presence at very low levels. More broadly, a new joint report on the state of the science of endocrine disrupting chemicals (EDCs) by the World Health Organization and the United Nations Environment Programme concludes that human and environmental exposure to EDCs, including hormonally active pharmaceuticals in the environment, needs to be addressed (3).

The Center for Drug Evaluation and Research (CDER) at the US Food and Drug Administration (FDA) has been evaluating the impact of pharmaceuticals in the environment since the early 1970s, following enactment of the National Environmental Policy Act of 1969 (NEPA). In 1997, CDER examined the available aquatic toxicity data for synthetic estrogens in human drugs as part of a revised set of regulations under NEPA (4). CDER concluded that, at the expected level of exposure at the time, little potential existed for significant effects on the environment. Since then, several reviews and actions have addressed the significance of the human and ecological health risks from estrogens in the environment (5–9). The US Environmental Protection Agency

[1] Office of Pharmaceutical Science, Center for Drug Evaluation and Research, US Food and Drug Administration, 10903 New Hampshire Avenue, Silver Spring, Maryland 20903, USA.
[2] American Institutes for Research, 1000 Thomas Jefferson Street, NW, Washington, District of Columbia 20007-3835, USA.
[3] Office of Cosmetics and Colors, Center for Food Safety and Applied Nutrition, US Food and Drug Administration, 4300 River Road, College Park, Maryland 20740, USA.
[4] To whom correspondence should be addressed. (e-mail: james.laurenson@fda.hhs.gov)

(EPA) recently finalized a rule under the Safe Drinking Water Act (SDWA) requiring public water systems to monitor EE2 and six other hormones (all endogenous) as part of the Unregulated Contaminant Monitoring (UCM) program (10). Similarly, the European Parliament recently voted to add EE2 and two other pharmaceuticals to the European Union's watch list under the Water Framework Directive, EU's main water policy instrument for setting antipollution strategies (11). Some pharmaceuticals in the environment are being considered for assessment as part of the EPA Endocrine Disruptor Screening Program (EDSP) (12). EPA also recently proposed adding pharmaceutical wastes to the Universal Waste Rule (13) and began updating a series of guidelines to derive ambient water quality criteria (AWQC) for aquatic life, using EE2 to illustrate recommendations for data use (14,15).

Most of these initiatives addressing pharmaceuticals in the environment have focused on aquatic life rather than risks to human health (e.g., via drinking water). Indeed, most of the studies examined to date have concluded that human exposure to pharmaceutical estrogens in drinking water in the USA is negligible, especially compared to other estrogenic substances in water and to the estrogenicity of food and other sources (5,7,8,16–18). This area continues to be assessed by FDA and others (19–21), in part because of increased pharmaceutical use in the US population and of exposure to other "contaminants of emerging concern" such as from water reuse for potable water purposes (18,22,23).

As part of its assessment of the impact of pharmaceuticals in the environment, CDER conducted a review and analysis of the current state of knowledge regarding the aquatic chronic toxicity of EE2 and other prescribed estrogens to ecological communities. This paper presents an overview of that study. The study was organized and conducted using FDA and EPA ecological risk assessment guidelines, which take into consideration factors that are typically used in CDER environmental assessments (EAs) within the regulatory framework established under NEPA. This framework and the risk assessment guidelines are described briefly below. Given the many abbreviations, acronyms, and terms used in these assessments, a glossary is provided in Table I.

REGULATORY FRAMEWORK

As required by NEPA, FDA assesses environmental impacts resulting from approval of individual drug applications. The applications submitted to FDA ordinarily are required to have either an EA or a claim for exclusion from the requirement to prepare an EA ("categorical exclusion"). Most categorical exclusions are for when the specific application either (1) increases use of the active moiety, but the estimated concentration of the substance at the point of entry into the aquatic environment—the expected introduction concentration (EIC)—will be below 1 part per billion (ppb or milligrams per liter) or (2) does not increase use of the active moiety (24). If any of the categorical exclusion criteria are not met, the applicant must submit an EA. Since 1997, when the latest regulations were revised (4), most individual drug applications for pharmaceutical estrogens—including EE2—have qualified for one or more categorical exclusions based on information existing at the time. Regardless of whether categorical exclusion criteria are met, FDA may require an EA if "extraordinary circumstances" indicate that the specific proposed action (e.g., drug application approval) could significantly affect the quality of the human environment. This provision generally has not been used for new estradiol and EE2 drug applications because the applications are typically competing for shares of a mature market and thus approval of individual applications generally do not significantly change existing environmental concentrations or risks. Consequently, approval of a specific application would not be expected to result in a significant impact. Therefore, CDER undertook this study in an attempt to assess the level of potential ecological impact from *all* sources of EE2 and other human pharmaceutical estrogens used in approved drugs.

ECOLOGICAL RISK ASSESSMENT

Assessing the presence of pharmaceutical estrogens in the aquatic environment, the subsequent exposure to aquatic organisms, and the resulting impact of exposure, is a complex process. This complexity is due in part to the multiple estrogenic substances arising from various sources, the difficulties of developing toxicity profiles for the various substances and organisms, and the unknown and multifaceted aspects of human metabolism, wastewater treatment, and environmental fate. To help address these complexities and limitations, this analysis was organized and conducted using FDA and EPA ecological risk assessment guidelines (24,25). In general, these guidelines recommend four main steps:

1. *Problem formulation.* This step defines the problem and presents the plan for analyzing and characterizing risk from specific hazards, such as pharmaceutical estrogens, to specific assessment endpoints, such as self-sustaining populations of aquatic organisms. This step includes a review and an understanding of the peer-reviewed literature and the development of a hypothesis. Selection of a toxicological endpoint is based on the potential of the stressor to negatively impact survival of local populations and, by extension, aquatic community dynamics.
2. *Effects characterization*. This step examines stressor–response relationships and benchmark exposure concentrations, the evidence for causality, and the relationship between measures of effect and assessment endpoints. A key goal of this step for purposes of this analysis is to develop a predicted no effect concentration (PNEC) for aquatic chronic toxicity for use during the risk characterization. EPA generally describes such values as being based on the most sensitive, scientifically acceptable toxicity endpoint available for a given taxon (26). These values have been established to generally protect ≥95% of species within an aquatic community under the assumption that some species loss (≤5%) is tolerable due to functional redundancies among species (25,26).
3. *Exposure characterization*. This step assesses the sources of stressors, the distribution of stressors in the environment in terms of measured environmental concentrations (MECs) and predicted environmental concentrations (PECs), and the extent of co-occurrence between the stressor and organisms identified in the assessment endpoints.

Table I. Glossary

	Definition
Abbreviations/ acronyms	
AF	Assessment factor
ANDA	Abbreviated new drug application
API	Active pharmaceutical ingredient
AWQC	Ambient Water Quality Criteria (USA)
AWQG	Ambient Water Quality Guideline (British Columbia)
CDER	Center for Drug Evaluation and Research
CWA	Clean Water Act
E1	Estrone
E2	17β-estradiol
E2-eq	E2 equivalent factor
EE2	17α-ethinyl estradiol
E3	Estriol
EA	Environmental assessment
EC_{50}	Median effective concentration
EDC	Endocrine disrupting chemical
EDSP	Endocrine Disruptor Screening Program
EEC	Expected environmental concentration
E-FAST	Exposure and Fate Assessment Screening Tool
EIC	Expected introduction concentration
ELISA	Enzyme-linked immunosorbent assay
EPA	Environmental Protection Agency
FDA	Food and Drug Administration
GC-MS/MS	Gas chromatography, tandem mass spectrometry
GREAT-ER	Geography Referenced Regional Exposure Assessment Tool for European Rivers
IND	Investigational new drug
LC-MS/MS	Liquid chromatography, tandem mass spectrometry
LC_{50}	Median lethal concentration
LOEC	Lowest observed effect concentration
log K_{ow}	logarithm of the octanol/water partition coefficient
MEPBC	The Ministry of Environment for the Province of British Columbia
MEC	Measured environmental concentration
MS	Mass spectrometry
NCCT	National Center for Computational Toxicology
NCGC	NIH Chemical Genomics Center
NEPA	National Environmental Policy Act
NIH	National Institutes of Health
NIEHS	National Institutes of Environmental Health Sciences
NDA	New drug application
NOEC	No observed effect concentration
NTP	National Toxicology Program
NWIS	National Water Information System
OECD	Organisation for Economic Co-operation and Development
PEC	Predicted environmental concentration
P*h*ATE	Pharmaceutical Assessment and Transport Evaluation
P*h*RMA	Pharmaceutical Research and Manufacturers of America
PNEC	Predicted no effect concentration
RIA	Radioimmunoassay
RQ	Risk quotient
SDWA	Safe Drinking Water Act
SPE	Solid-phase extraction
SSD	Species sensitivity distribution
STORET	STOrage and RETrieval
UCM	Unregulated Contaminant Monitoring
USGS	United States Geological Survey
WWTP	Wastewater treatment plant
Terms	
Conjugated estrogen	Mixture of sodium salts of sulfate esters of estrogenic compounds
E2-equivalent (E2-eq)	Measurement of estrogen-induced activity using E2 activity as a referenced standard where estrogenic activity of E2 is equal to 1
Effluent	Water runoff exiting waste water treatment plants
Endogenous estrogens	Estrogens of human or animal origin
Environmental estrogens	Estrogenic substances, including endogenous estrogens and natural and synthetic substances with estrogenic properties, in the environment
Esterified estrogen	Mixture of sodium salts of esters of estrogenic compounds
Estrogenicity/ estrogenic activity	Refers to the estrogenic biological activity of a substance or the degree to which a substance elicits an estrogenic biological response
Ethinyl estradiol (EE2)	Synthetic derivative of estradiol, the endogenous human estrogen
Mycoestrogens	Estrogenic substances of fungal origin
Phytoestrogens	Estrogenic substances of botanical origin
Risk quotient (RQ)	The ratio of the PEC to a PNEC and an indicator of the level of environmental risk
Surface water	Water collected on the ground, in streams, rivers, lakes, and ponds

4. *Risk characterization*. This step involves examining all available evidence, including laboratory and field studies, to characterize ecological risks using a weight-of-evidence approach. Risk (or hazard) quotients (RQs), which compare exposure concentrations (MECs and/or PECs) with PNECs, are used to present risk estimates. An RQ less than one generally indicate that the contaminant alone is unlikely to cause adverse ecological effects. If a simple screening-level analysis is conducted, an RQ of one or higher only indicates that additional analysis is needed, not that adverse effects are likely. Risk characterization concludes with discussion of the confidence in the risk estimates and of the likely magnitude and severity of ecological effects.

Problem Formulation

A review of the literature suggests that environmental levels of estrogenic substances downstream of WWTPs are causing adverse effects in natural fish populations (14,27,28). Reports in

these studies of feminized male fish and the absence of male fish led many investigators to hypothesize that estrogenic chemicals, perhaps pharmaceutical EE2 and/or other estrogens released from WWTPs, might be responsible for the effects. Such effects could result in failure of the fish species to successfully reproduce and in local extirpation of one or more species, which is of particular concern for threatened or endangered species and sensitive ecosystems. Thus, the problem, or hypothesis, to address is whether existing levels of pharmaceutical estrogenic chemicals in rivers and streams might be responsible for significant adverse effects in natural populations or might pose a significant risk at some future time. An assessment endpoint suggested by the observations also was selected. To evaluate this assessment endpoint, several measurement endpoints (or measures of effects) were selected.

Selected Pharmaceutical Estrogens

Estrogens of pharmaceutical origin are prescribed for indications such as oral contraceptives, hormone replacement therapies, motor deficits associated with menopause, hypoestrogenism, and the management of some pre- and postmenopausal symptoms. Pharmaceuticals prescribed for such indications include EE2, 17β-estradiol (E2), and various conjugated and esterified estrogens. The primary urinary and fecal metabolites of pharmaceutical and natural estrogens include estrone (E1), E2, EE2, and estriol (E3). As a result of their usage and subsequent excretion, pharmaceutical estrogens are introduced through WWTP effluents into US streams, rivers, and lakes where exposure to aquatic organisms is possible. These introductions are considered as "pseudo-persistent" since these pharmaceuticals and metabolites are introduced into the environment on a continuous basis.

EE2 is a highly potent estrogen receptor agonist and a derivative of the natural hormone E2. Oral contraceptives containing EE2 recently ranked among the top 15 US active pharmaceutical ingredients (APIs) in terms of frequency of use (daily dose equivalents) (16). Greater than 150 new and generic drug products containing EE2 have been approved by FDA since the first approval of Estinyl in 1943 (29). Many of these drugs have been discontinued, and over the years the dosage amount of EE2 in birth control formulations has greatly decreased, with dosages today generally ranging from 20 to 30 μg/day. Production values for EE2-containing drugs also are relatively low compared to many other APIs and indeed production has seen a five-year downward trend. Nevertheless, oral contraceptives currently are the leading method of contraception in the USA. From 2006 to 2008, this method was used by 10.7 million females aged 15–44 years (17% of females aged 15–44) (30). Sales amounts for EE2-containing drugs in the USA remain relatively low, with API levels below 100 kg/year (31). For comparison, approximately 1 million kg/year of the antibiotic amoxicillin are sold.

E2 is an endogenous sex hormone that also is indicated for motor deficits associated with menopause, hypoestrogenism, and the management of some postmenopausal symptoms. Sales for E2 have declined slightly from 550 kg/year in 2006 to 497 kg/year in 2011 (31).

Conjugated and esterified estrogens are indicated for the management of symptoms associated with vulvar and vaginal atrophy, motor deficits associated with menopause, hypoestrogenism, and the management of some postmenopausal symptoms. In 2011, 378 and 21 kg/year were produced for conjugated estrogens and esterified estrogens, respectively (31). Use of these estrogens for postmenopausal hormone therapy has declined significantly since 2002 after the Women's Health Initiative clinical trial of conjugated equine estrogens plus medroxyprogesterone acetate was terminated when health risks were shown to exceed the benefits of combined hormone therapy (32).

To compare the estrogenic activity of different estrogens and other estrogenic substances and thus select which of these substances to assess in this study, a reference estrogen approach was used (6,7,28). In this approach, the average excretion rate per person for each estrogen is multiplied by a rough estimate of the fraction resulting from prescriptions—reported as an overestimate in the cited study—and by a standard equivalence factor for relative estrogenic activity for that estrogen, using human endogenous estrogen, E2, as the reference. The E2-equivalence factor (E2-eq) for each estrogen is based on fish chronic reproductive toxicity and other data. The excretion rates, prescription fractions, specific E2-eqs, and products of this approach are shown in Table II. As seen in this table, EE2 presents the majority of the excreted prescription-based estrogenicity. EE2 also is known to be more persistent in the environment (33). Therefore, EE2 will be the focus of this paper. One caveat to this approach is that the E2-eq potency factors are subject to variability and uncertainty depending on the specific effect measured, target organism, and other factors. Regarding the EE2 E2-eq factor, while some estimated factors range up to 31, they also range as low as 0.17 (5,7,28,34,35). The value of 20 is the mean of the more sensitive *in vivo* vitellogenin induction studies and thus believed to be the most representative value to use (7).

Assessment and Measurement Endpoints

Compounds with known potent estrogenic activity have been associated with the broader class of EDCs, which are defined as exogenous agents that interfere with the production, release, transport, metabolism, binding, action, or elimination of the natural hormones in the body responsible for the maintenance of homeostasis and the regulation of developmental processes (36). Estrogens in the environment, including those of pharmaceutical origin, can be EDCs because they may influence coordinated gene expression patterns under control of the endocrine system, possibly resulting in adverse effects on development and reproduction (37–39). Moreover, because the use of a diffuse endocrine system to coordinate developmental and reproductive processes is highly conserved evolutionarily among vertebrates and invertebrates, there is a broad taxonomic spectrum of organisms that may be potentially threatened by exposure to EDCs (14).

Effects from EDCs can occur at very low concentrations over long periods of time. Typically, however, for CDER EAs, only short-term acute ecotoxicity effects testing data are available, such as median lethal or effective concentrations (LC_{50} and EC_{50}, respectively). These short-term values generally are divided by an assessment factor (AF) of 1,000 to result in PNECs for chronic toxicity, or as illustrated in CDER guidance, the toxicity data are divided by an expected environmental concentration (EEC) proxy—a PEC, MEC, or EIC—and the result compared to the AF (24). Either way, extrapolations related to acute-to-chronic toxicity, species-to-species differences, and laboratory-to-field toxic effect levels are being directly or indirectly used to

Table II. Primary Urinary and Fecal Metabolites of Prescription and Natural Estrogens

Metabolite	Average excretion (μg/day/person)[a]	Fraction from prescriptions[b]	E2-equivalence (E2-eq)[a]	E2-eq average excretion from prescriptions (μg/day/person)
Estrone (E1)	19	0.2	0.33	1
17β-estradiol (E2)	7.7	0.1	1	0.8
17α-ethinyl estradiol (EE2)	0.41	1	20	8
Estriol (E3)	81	0.00075	0.033	0.002

[a] From Anderson *et al.* (6)
[b] Estimated using ratios of prescribed estrogens to dietary and naturally produced estrogens from Table I of Caldwell *et al.* (8), and reported as overestimates due to the omission of phytoestrogen contributions

determine whether environmental concentrations may cause a significant impact. Recent research for hormonally active chemicals such as EDCs, however, indicate that a larger AF would be needed for extrapolating acute to chronic values in the approach noted above. The CDER guidance recommends that chronic toxicity testing be conducted if either (1) sublethal effects at the maximum expected environmental concentration indicate that chronic toxicity testing should be performed or (2) the logarithm of the octanol/water partition coefficient (log K_{ow}) of a compound—a measure of a substance's lipophilicity—is ≥ to 3.5. Thus, EE2, with its hormonal activity at environmentally relevant concentrations and with a log K_{ow} of 4.15 (40), was examined for chronic toxicity.

Based on an initial literature review for EE2, fish were identified as the most sensitive species in aquatic communities, which was expected for a synthetic hormone that binds with estrogen receptors on estrogen-sensitive tissues. Therefore, the assessment endpoint selected for this study is successful fish reproduction that allows for self-sustaining populations, which is in agreement with a recent EPA analysis of EE2 (14). Commonly selected measurement endpoints for this assessment endpoint include the induction of vitellogenin (an egg yolk precursor protein normally expressed only in females) in male fish, a reduction in intensity or absence of secondary sexual (e.g., breeding coloration and tubercles) and sex-specific behaviors (e.g., courtship and defense of spawning sites), the presence of ovarian tissue and eggs in the testes (testis-ova or intersex), skewed sex ratios from male to female, and complete sex reversal of males.

Effects Characterization

This step involved developing the biological effect (or hazard) levels associated with EE2 and other estrogen exposures. As is typically the case when attempting to characterize the inherent effects of a specific environmental chemical or other stressor, the stressor is simultaneously associated with a large number of other stressors that have the same effects. As such, any field data linking a specific measurement endpoint in fish with a specific pharmaceutical estrogen concentration downstream of a WWTP generally would be insufficient to assign causation to the pharmaceutical estrogen. Characterizing the actual dose–response relationship would be additionally difficult. Consequently, laboratory measures of the same effects in organisms exposed to known concentrations of EE2 and other pharmaceutical estrogens also were sought to assess whether these estrogens could contribute to or are the principal causes of effects in the field, and if so then what the dose–response relationship would be.

A focused literature search approach was used for this effort. In addition, data from the EPA ECOTOX database (41) were obtained for effects of EE2 on aquatic organisms. Studies were selected and reviewed for endpoints related to growth, survival, reproduction, and development and viewed at the species and effect level. Laboratory tests for the biological effects of EE2 relevant to aquatic ecosystems were available on fish, amphibians, invertebrates, and algae. Several studies also were found for effects in the field. These studies were used to develop a PNEC for this report. Table III presents this PNEC along with other

Table III. EE2 PNECs for Chronic Toxicity in Aquatic Life

PNEC (ng/L)	Derivation	Source and year
0.5	Based on studies reporting a multi-species LOEC of 1.0 ng/L for reproduction and egg production, along with an added AF of 2	British Columbia (42), 2009
0.35	Based on available chronic toxicity data, no observed effect concentration data (NOEC), and a species sensitivity distribution (SSD) approach, which fits a distribution to the NOECs from available studies across multiple taxa to determine the hazardous concentration of EE2 at which 5% of all the species tested are affected (referred to as the HC5)	Caldwell *et al.* (43), 2008
0.1	Based on a LOEC of 0.19 ng/L that corresponds to a 25% increase in mortality of fertilized zebrafish eggs in a two-generation study (44), with an added AF of 2	CDER-commissioned literature review (see text), 2011
0.1	Used the same SSD methods as Caldwell *et al.* in 2008 (above), but based on the most current chronic reproductive toxicity data available and argument that the robustness of the data supports an AF of 1	Caldwell *et al.* (7), 2012
0.035	Developed using an SSD approach to obtain an HC5 of 0.07 ng/L, which is less than the above HC5 of 0.1 ng/L due apparently to the use of two different data sets. Also, EC applied an AF of 2, while Caldwell *et al.* argued that an AF of 1 was sufficient	European Commission (proposed) (45), 2012

PNECs developed for EE2 chronic toxicity. These values are discussed below.

In 2011, CDER commissioned a study (as noted in the "Acknowledgements") to examine the available toxicity data and apply the methodology described previously in this paper for a PNEC for assessing long-term EE2 exposure impacts. That study concluded that the most relevant data on EE2 were from Soares *et al.* (44), which demonstrated a lowest observed effect concentration (LOEC) of 0.19 ng/L that corresponded to a 25% increase in mortality of fertilized zebrafish eggs in a two-generation study. The 0.19 ng/L value was rounded to 0.2 ng/L and an AF of 2 applied, as was done during British Columbia (BC) and European Commission (EC) PNEC development, described below. The result was a PNEC of 0.1 ng/L for aquatic chronic toxicity. As seen in Table III, this is equal to the PNEC developed by Caldwell *et al.* (7), lower than both a previously established PNEC by Caldwell *et al.* (43) and a PNEC established by the Ministry of Environment for the Province of British Columbia (MEPBC) (46), and higher than the PNEC recently proposed by the EC (45). Finalization of the EC value currently is on hold pending further review (47).

Exposure Characterization

This step involved examining the literature and several databases for distributions of MECs and PECs for EE2 and other estrogens. Based on the initial studies reviewed, the degree of variability and uncertainty in the measured data appears to be significant. Furthermore, precisely defining the lower and mid-points of the EE2 statistical distribution in surface waters and wastewater effluents is difficult given that most such waters have not been sampled, and for those that have, the samples often are below the analytic detection limits for EE2. Simulation modeling, on the other hand, can estimate likely distributions of EE2 concentrations over time and space, although these often are difficult to corroborate against measured values, particularly at the lower ends of the distributions. Nonetheless, the literature reviewed for this paper identified an upper end (e.g., approximately 99th percentile) of the distribution of EE2 measurements in surface waters, with maximum values that are caveated with the knowledge that episodic releases of untreated wastewater or low or effluent dominated stream flow might result in higher levels.

The relative contribution of estrogenic substances from human and other sources in terms of E2-eq also was examined. Concentration data were converted based on estrogen potency relative to E2 (i.e., E2-eq) as described previously. Adjusted values were also corrected to reflect removal rates from passing through WWTPs (6).

Measured Concentrations of EE2 and Other Estrogens

WWTP effluents and the locations where effluents are introduced to surface waters are expected to have higher concentrations of estrogens than other areas where in-stream dilution and loss processes have reduced the levels of the compounds. Therefore, to properly assess the impact that pharmaceutical estrogens and specifically EE2 may be having on aquatic systems, it is necessary to determine the current state of EE2 levels across all waters.

A fairly recent paper by Hannah *et al.* (48) compiled all of the approximately 1,650 MECs for US and non-US WWTP effluents and surface waters that were available at the time across 52 peer-reviewed English language papers published through the end of 2006. The results of these studies varied widely. For example, a study using a detection limit of 0.5 ng/L to analyze surface water samples from ten sites along Boulder Creek, Colorado reported no detectable levels of EE2 in any of the samples (49). Similarly, a study with a lower detection limit, 0.05 ng/L, analyzed three samples from the Colorado River and the Sacramento River Delta, detecting EE2 in only one sample, at a concentration of 0.067 ng/L (50). More recent studies, not included in Hannah *et al.* (48), reflect the earlier data. For example, a study utilizing a detection limit of 1 ng/L analyzed levels in 19 surface water samples, finding only one detectable level, 1.4 ng/L (51), while Washington State, utilizing a range of detection limits, analyzed 266 samples from lakes and streams and detected EE2 in 66 samples, with a maximum concentration measuring 4 ng/L (52).

Hannah *et al.* (48) developed a cumulative probability distribution of the data, with concentrations ranging from nondetectable to 273 ng/L, a 90th percentile concentration of 1.7 ng/L, and approximately 70% of the measurements as nondetectable with limits of detection ranging from 0.01 to 30 ng/L. Due to concerns that mass spectrometry (MS) measurement can lead to an overestimation of the concentration of EE2 from an overlap of the EE2 peak in the chromatogram with impurities of similar mass-to-charge ratio (e.g., fulvic and humic acids), the authors examined a subset of samples ($n=360$) that were analyzed by gas chromatography/tandem mass spectrometry (GC-MS/MS) or liquid chromatography/tandem mass spectrometry (LC-MS/MS) with an additional cleanup step following the extraction. This subset resulted in concentrations ranging from nondetectable to 4.6 ng/L, a 90th percentile concentration of 0.43 ng/L, and 87% of measurements as nondetectable, with limits of detection ranging from 0.1 to 1 ng/L. A comparison of the analytical methods using split samples would provide a useful contribution to this discussion.

In addition to data from the literature, two databases were examined: the EPA "STOrage and RETrieval" database (STORET) (53) and the US Geological Survey (USGS) National Water Information System database (NWIS) (54). Together, these sources contain data for 286 samples from various US surface waters. Only three of the samples had EE2 concentrations above the level of detection, and these were from the same location and on the same day.

As seen by these data, EE2 concentrations in US surface waters are highly variable, with the majority of the samples below detection levels. While these data do not represent national averages for EE2 concentrations, they do indicate that for most ecosystems, EE2 concentrations in surface water are lower than PNECs. Considering the limitations of the analytical methodologies used to measure EE2 surface water levels and the variability reported, more data are needed to better define spatial and temporal concentrations of EE2.

Modeling Approaches to Determining EE2 Concentrations

Several computer models have been developed and used to estimate PECs of EE2 in surface waters. The use of models

has helped to overcome many of the limitations associated with only using traditional measurements. One such limitation is the often cost prohibitive chemical analysis of water samples, which can impact the range of locations incorporated into a study. Another is the limits of detection imposed by current analytical methods. By utilizing appropriate assumptions and data about per capita EE2 usage, per capita wastewater outputs, metabolic and wastewater treatment removal rates, and instream dilution and loss processes in water bodies, models can provide relatively accurate estimates of the concentration distributions of EE2 in wastewaters and surface waters. Two models used to generate data (Pharmaceutical Assessment and Transport Evaluation (P*h*ATE) and Geography Referenced Regional Exposure Assessment Tool for European Rivers (GREAT-ER)) have been described previously (55,56) and are summarized below.

The P*h*ATE model developed by the Pharmaceutical Research and Manufacturers of America (P*h*RMA), was used by Hannah *et al.* (48) to predict the concentration of chemicals, particularly estrogens, in stream and other surface water segments of 11 US watersheds. P*h*ATE generates data based on input parameters that account for water volume, flow, and proximity to WWTPs. Only water segments located below a WWTP (i.e., segments potentially impacted by WWTP effluents) were used in the data analyses. An in-water depletion rate of zero was used for the model runs as a conservative assumption in light of limited available data to estimate this parameter. Using P*h*ATE, Hannah *et al.* (48) indicated that under mean flow conditions representing chronic or long-term exposure scenarios, the median, 90th percentile, 99th percentile, and maximum PECs for EE2 would be about 0.00064, 0.0075, 0.1, and 0.46 ng/L, respectively, according to Figure 1 of Hannah *et al.* Thus, approximately 1% of affected water segments (approximately 99th percentile) downstream of WWTPs exceed 0.1 ng/L. While the P*h*ATE PECs were compared extensively with MECs and theoretical maximum concentrations in Hannah *et al.* (48) for model validation purposes, a recent paper by EPA scientists on WWTP influent allows for a more recent comparison (57). In particular, the EPA paper provides a 99th percentile estimate for EE2 in influent of 6 ng/L, which when reduced due to treatment (e.g., 82% removal for secondary treatment in Hannah *et al.*) and dilution (e.g., at least tenfold) would result in a stream concentration approximately equal to or < 0.1 ng/L.

The GREAT-ER was developed as a model for environmental risk assessment and management of chemicals in European river basins (58). Hannah *et al.* (48) used GREAT-ER to estimate EE2 concentrations in European watersheds under mean flow conditions. Specifically, these authors estimated that the median, 90th percentile, 99th percentile, and maximum PECs for EE2 would be about 0.054, 0.13, 0.5, and 0.62 ng/L, respectively, with about 23% of water segments exceeding 0.1 ng/L. This percentage is substantially higher that the approximately 1% exceedance of 0.1 ng/L obtained using P*h*ATE because the two models and respective inputs were developed and used for waters and EE2 uses that are specific to the two different areas and populations (USA and Europe). For example, a key model input that differs significantly between these two model applications is the approximately 50% lower per capita use of EE2 in the USA compared to the EU. Also, the average per capita water use in the US watersheds is approximately 50% higher than in the EU watersheds, resulting in greater dilution, while at the same time there is greater in-stream dilution in US watersheds compared with EU watersheds.

Several other relevant models exist that could be used to assess EE2. For example, the American Cleaning Institute developed the web-based model, iSTREEM, to predict environmental concentrations of chemicals, including pharmaceuticals, from consumer products in river segments receiving wastewater discharges (59). EPA designed the Exposure and Fate Assessment Screening Tool (E-FAST) to estimate environmental concentrations of chemicals from consumer products, including antimicrobial pesticides, released into surface waters for use in screening level assessments (59,60). Only the P*h*ATE and GREAT-ER models appear to have been used thus far, however, to generate robust datasets reporting pharmaceutical concentrations in surface waters.

Risk Characterization

This step involved comparing available data on exposure and effects in order to characterize the potential for EE2 to adversely affect aquatic communities at current environmental levels and at possible future environmental levels. The studies noted above, in "Effects Characterization", of effects downstream of WWTPs indicate that feminization or complete sex reversal of male fish is occurring in some locations and that estrogenic compounds are present in the same waters. As with most field studies, the presence of other chemical constituents in WWTP effluents, in this case other well-known estrogenic substances, such as E2 (the natural estradiol), E1 (estrone, an estrogen metabolite), industrial chemicals, phyto/mycoestrogens, and other substances with estrogenic properties, prevents accurate quantifying of the contribution of EE2 to the observed effects. Therefore, CDER conducted this risk assessment by first commissioning an ecological effect assessment, which resulted in a literature review-derived PNEC of 0.1 ng/L for EE2 aquatic chronic toxicity, and then obtained a distribution of PECs for EE2 based on limited EE2 surface water monitoring data along with surface water characteristics and other data as inputs to an exposure model.

As discussed in the "Modeling Approaches to Determining EE2 Concentrations", an RQ is the ratio of an environmental concentration, in this case PECs, to a PNEC. An RQ of < 1 generally indicates that the contaminant alone is unlikely to cause adverse effects. An RQ of 1 or higher indicates either that the contaminant is likely to cause adverse effects or, in the case of a screening assessment using worst-case or high-end assumptions and/or data, as in this case, that additional analysis is needed to obtain a more realistic assessment of risk.

The RQs obtained from this study are shown in Table IV. In the first row is the median mean flow RQ of 0.0064 (i.e., the predicted mean flow median concentration of 0.00064 ng/L divided by the PNEC of 0.1 ng/L). This RQ is more than 2 orders of magnitude below an RQ of 1, indicating that the predicted median EE2 concentration in US surface waters is highly unlikely to cause adverse effects. In the second row is

Table IV. Summary of EE2 Screening-Level Risk Quotients (RQs) Under Mean-Flow Assumptions Based on a PNEC of 0.1 ng/L

Measurement	PEC (ng/L)	RQ[a]
Median	0.00064	0.0064
90th percentile	0.0075	0.075
99th percentile (approx.)	0.1	1
Maximum	0.46	4.6

[a] RQ, or risk quotient, is the ratio of the predicted environmental concentration (PEC) to a predicted no effects concentration (PNEC) and is an indicator of the level of environmental risk. A risk quotient over 1 suggests that either additional analysis is needed (for screening level assessments) or that adverse ecological effects are likely (for robust, comprehensive assessments)

the 90th percentile RQ of 0.075, which also is substantially below an RQ of 1 and thus also indicative of a low likelihood of adverse effects even at the relatively high 90th percentile level. The third row in Table IV is the approximately 99th percentile RQ of 1, which indicates that predicted mean flow concentrations of EE2 in approximately 99% (or more) of the US stream and other water segments located below WWTPs (i.e., segments potentially impacted by EE2 in WWTP effluents) are less than a PNEC of 0.1 ng/L. The last row in Table IV is the maximum RQ of 4.6, which indicates that in some locations the maximum mean flow concentration of EE2 in US surface waters is expected to exceed the EE2 chronic toxicity PNEC of 0.1 ng/L by close to fivefold. Such exceedances may be important if they overlap with critical habitats, such as those containing endangered or threatened ("listed") species. As noted previously, conservative, screening-level assumptions were used in the exposure modeling, and thus additional refinements of this EE2 analysis likely would only reduce these RQs and result in PECs that are less than the 0.1 ng/L PNEC in more than 99% of the modeled effluent-affected stream segments.

Several caveats exist regarding these results: (1) the concentration data represent the stream segments downstream of WWTPs and thus likely overstate the impact upstream or nationally; (2) metabolic and environmental factors contributing to reduction or enhancement of EE2 or metabolite concentrations, such as the metabolism of the product in humans, the conjugation (biological inactivation) of EE2 or metabolites entering WWTPs, and the deconjugation (reactivation) within WWTPs, are not addressed; (3) screening-level simplifications were used in the exposure modeling, such as the use of zero-depletion assumptions and the focus only on waters downstream of WWTPs, which would tend to overestimate the true exposure; (4) extrapolating PNECs from the available toxicity data is inherently limited and could over- or underestimate the true effects; and (5) synergistic and antagonistic effects have not been assessed, which also could either over- or underestimate the true effects. Given the conservative assumptions used in this analysis, these results would appear to be a reasonable screening-level assessment that likely does not underestimate the risks from EE2 in the environment.

Cumulative (additive) effects from EE2 and other estrogenic substances in the environment were examined briefly. Anderson *et al.* (6) recently showed how total estrogenicity from human-derived estrogens (endogenous and pharmaceutical) compares to an overall PNEC for E2-eq. E1, E2, E3, and EE2 concentrations were modeled using P*h*ATE and by converting the estrogenicity of these compounds to E2-eq using potency factors of 0.3, 0.03, and 20 for E1, E3, and EE2, respectively, based on fish chronic reproductive toxicity and other data. These authors derived a long-term E2-eq PNEC of 2.0 ng/L, which is equivalent to the EE2 PNECs of 0.1 ng/L noted in Table III, assuming an E2-eq potency factor of 20 for EE2. The authors estimated that approximately 98.9% of stream segments have a mean flow E2-eq concentration from human endogenous and pharmaceutical estrogens that is lower than their derived long-term E2-eq PNEC. As would be expected because of the inclusion of these other, though less potent, estrogens, this percentage is slightly lower than the approximately 99% estimated above for EE2 alone.

While EE2's contribution to direct human sources of estrogenicity from excretion of endogenous and pharmaceutical estrogens has been studied in some detail, its contribution to total estrogenicity from other sources in the aquatic environment has received relatively little attention. These other sources of estrogenicity include agriculture (livestock excretions and pesticides), industry (chemicals), and natural sources (myco/phytoestrogens and wildlife excretions). Only a few studies could be found that have examined and compared these various other sources. Johnson *et al.* calculated that human excretion of estrogens in the UK is about 20% of farm animal excretion (61). In contrast, the human excretion of estrogens in the USA is about 3% of farm animal excretion, using an estimated total excreted estrogens from farm animals in the USA of 49,000 kg/year (62), and an estimated total excreted estrogens from humans of 4.4 kg/year/million inhabitants, which for the 2012 US population of about 313 million is about 1,400 kg/year of estrogens (63). The pathways through which excreted animal estrogens reach surface waters, however, and the extent to which estrogens instead remain or are degraded in the soil, contribute substantial uncertainty to these proportions and to estimates of farm animal estrogens reaching surface waters. Much of the farm waste enters waste collection and treatment systems or enters the soil directly or indirectly (e.g., as a soil amendment) and is degraded naturally. Farm waste also runs off into surface waters, enters surface water through groundwater pathways, or is discharged from collection systems with little treatment. Thus, the exact amounts or even broad ranges of farm waste that enter these various pathways are difficult to estimate.

Vajda *et al.* (28) (corrected per personal communication, January 9, 2013) estimated that EE2 as a fraction of a broad range of estrogenic substances in a WWTP effluent in Colorado ranges from 8 to 38%, based on E2-eqs for EE2 that ranged up to 20. These percentages would likely decrease as the effluent becomes diluted in the receiving water, which is expected to contain other estrogenic substances. For example, Kolodziej *et al.* (64) reviewed data on agriculture, aquaculture, and natural spawning fish as sources of estrogens and found levels from these sources that in some cases were comparable to estrogenic levels detected in municipal wastewater effluent. More recently, Kolpin *et al.* (65) conducted sampling in the upper reaches of the Potomac River watershed, where a high presence and severity of

intersex disorders in fish have been documented. This study indicated that agriculture (pesticides, veterinary drugs, and phyto/mycoestrogens) appears to be the primary cause of these effects.

As indicated by this small sample of studies on total estrogenicity, the contribution of EE2 to the total estrogenicity of surface waters from all sources, while still highly uncertain and variable, appears to be relatively low overall. The comparison of local, regional, or national estimates of total estrogenicity with national estimates of EE2, however, is only useful for providing a rough sense of the overall contribution of EE2 to total estrogenicity at this time and not for developing mitigation or response plans. Nevertheless, these comparisons do highlight the areas for additional study to better characterize estrogenicity of environmental factors.

DISCUSSION

Evidence indicates that there is a correlation between exposure to estrogenic substances in the environment—including human and animal endogenous and pharmaceutical estrogens, and other natural and anthropogenic substances—and disruption of the endocrine system in some aquatic biota. Regarding EE2 in particular, recent studies and data noted in this paper suggest that the mean flow long-term PECs for EE2 in approximately 99% or more of US surface waters downstream of WWTPs are less than a PNEC of 0.1 ng/L, and that the median PEC is more than two orders of magnitude less than this PNEC. The approximately 1% of exceedances are in localized, effluent-dominated stream segments. Similar results are found with pharmaceutical estrogens more broadly.

Some uncertainty–both model- and variable-based—is associated with the PECs, PNEC, and assessment of cumulative or additive effects of estrogenic mixtures that share the same or similar mode of action (66,67) or are synergistic with other substances (68,69). In addition, this analysis focuses on long-term, chronic risks and not the potential for acute toxicity from short-term episodic peak concentrations, such as from the release of untreated wastewater due to WWTP problems or "combined sewer overflows" (whereby storm water and untreated sewage are combined), or in highly effluent-dominated streams. This analysis also does not assess whether any of the known or predicted exceedances, short- or long-term, overlap with critical habitats, such as those containing endangered or threatened ("listed") species. These uncertainties and limitations are addressed to some extent via the use of worst-case assumptions and uncertainty factors in the risk assessment, but additional research and refinement are needed to more fully understand the environmental impact in a specific aquatic environment.

One scientific area undergoing significant discussion recently is dose–response mechanisms of estrogenic substances. The traditional paradigm of environmental toxicology assumes that environmental contaminants as evaluated by dose–response curves have effects that are monotonic in nature (i.e., the slope of the dose–response curve does not change sign). Estrogens, however, have been shown to exhibit nonmonotonic physiological responses at some doses (i.e., a nonlinear relationship between dose and effect, whereby the slope of the curve changes sign somewhere within the range of doses examined) (70–75). In response, EPA is investigating the implications of the nonmonotonic dose response to EPA testing and risk assessment procedures (76), recently concluding in a draft state-of-the-science paper that current testing strategies are unlikely to mischaracterize, as a consequence of nonmonotonic dose responses, a chemical that has the potential for adverse perturbations of the estrogen (or androgen or thyroid) pathways (72).

A related research area is the use of acute toxicity studies for estimating chronic environmental toxicity. As noted in CDER guidance (24), evaluation of the potential ecological toxicity of drugs with either sublethal effects at maximum environmental concentrations or that are lipophilic should include studies focusing on long-term effects at lower doses. This approach has recently been utilized by some non-US regulatory entities, which have begun including direct testing for long-term reproductive and endocrine-related toxicity in aquatic organisms in addition to extrapolating from short-term studies (77). New understanding and tools are also being developed in the USA (2,78–80). For example, EPA is currently implementing a two-tier EDSP (12). More broadly, the Tox21 program, which is a collaboration among multiple federal agencies—EPA's National Center for Computational Toxicology (NCCT), the National Institutes of Environmental Health Sciences (NIEHS)/National Toxicology Program (NTP), the National Institutes of Health (NIH) Chemical Genomics Center (NCGC), and FDA—is designed to accelerate the development of mechanism-based *in vitro* screens to ultimately reduce the use of low-throughput, high-cost conventional toxicity testing that uses animal models (79,81). The pilot-phase collection of approximately 3,000 compounds already have been screened against the estrogen nuclear receptor ERα, along with nine other receptors, with promising results (82).

The other key area within the pharmaceutical estrogen risk assessment paradigm that is undergoing improvement is the exposure assessment. A more comprehensive approach utilizing uniformity in both measuring technologies and longitudinal time points, in conjunction with an inclusive geographical distribution, will provide a more accurate representation of the current state of EE2 and other human and natural estrogenic substances in US WWTP effluents and surface waters. As data improve, it may be possible to quantify estrogenic substances in the environment and conduct a more comprehensive evaluation of total environmental estrogenicity, which in turn will support the development of any needed preventive and mitigative measures by municipalities and other responsible parties.

EE2's future impact on the environment is uncertain. Although population increase is a driver for increased EE2 use, other factors, such as increased contraception options, may lower use. In addition, EE2 dosing regimens have been decreasing over the years, and EE2 has been targeted by some for possible replacement by E2, which is more biodegradable (83). More broadly, CDER currently is involved with a number of activities aimed at assessing and minimizing potential impacts from pharmaceuticals, including continuing to evaluate how CDER's NEPA program is implemented. These efforts include partnerships and collaborations both internally with other FDA centers, and externally with other federal agencies and groups, including EPA, the US Geological Survey, the US Department of

Agriculture, and the White House Office of National Drug Control Policy (84,85). CDER also is collaborating with other government agencies on several projects, including prioritizing pharmaceuticals for research on environmental and human health exposure and effects (21,86), conducting additional analysis for drugs with hormonally active properties, developing methods for assessing the clinical endocrine disruption potential of drugs (87), and examining the results of recent efforts to identify and address the most critical questions remaining about drugs in the environment (88). New initiatives such as personalized medicine and "green pharma" are being studied by CDER, the American Medical Association, and others (83,85,89–97). Also, wastewater treatment systems continue to improve and be replaced (98–100), which in turn likely will improve the removal of estrogens and other pharmaceuticals from human waste.

CONCLUSION

Based on measured and modeled surface water concentration data and published chronic ecotoxicity data, the levels of EE2, the most potent of the human pharmaceutical or endogenous estrogens, in approximately 99% or more of surface waters downstream of WWTPs are expected to be less than a PNEC of 0.1 ng/L. Exceedances are expected to be primarily in localized, effluent-dominated stream segments. The median mean flow concentration of EE2 is estimated to be more than two orders of magnitude below this PNEC. Similar results are found for other pharmaceutical estrogens. The contribution of pharmaceutical estrogens more broadly to total estrogenic load in the environment, while still highly uncertain and variable at this time, appears to be low compared to other sources. While the current approaches for evaluating the environmental impact of pharmaceutical estrogens remain appropriate, it will be valuable to revisit the toxicity of and state of exposure to these substances, especially for sensitive environments, as analytical methods, monitoring strategies, models, and estrogenicity testing standards for acute and chronic studies used to predict aquatic population-level effects improve. These improvements will refine risk estimates and provide a basis for any next steps.

ACKNOWLEDGMENTS

The authors wish to thank Douglas Throckmorton, Deborah Livornese, Luis Valerio, and Roxane Modares from FDA/CDER for helpful discussions and review. The authors also wish to thank ICF International and IceTech, Inc. staff, in particular Margaret McVey, Arun Varghese, Isaac Warren, Christine Hartman, and Michael Smith, for providing much of the literature, data, and analysis used in this review (under contract no. HHSF223200910010I). Authors are listed according to first author (primary author) and last author (principal investigator) emphasis.

DISCLAIMER

The findings and conclusions in this article have not been formally disseminated by FDA and should not be construed to represent any FDA determination or policy. The mention of commercial products, their sources, or their use in connection with material reported herein is not to be construed as either an actual or implied endorsement of such products by the Department of Health and Human Services.

REFERENCES

1. Daughton CG. Published literature relevant to the issues surrounding PPCPs as environmental contaminants. In: EPA US (eds). http://www.epa.gov/ppcp/lit.html. 2012 .
2. Brooks BW, Berninger JP, Kristofco LA, Ramirez AJ, Stanley JK, Valenti TW. Pharmaceuticals in the environment: lessons learned for reducing uncertainties in environmental risk assessment. Prog Mol Biol Transcl Sci. 2012;112:231–58.
3. UNEP. State of the science of endocrine disrupting chemicals examined in landmark UN report. UNEP News Centre. 2013. http://www.unep.org/newscentre/Default.aspx?DocumentID=2704&ArticleID=9403&l=en; http://unep.org/pdf/9789241505031_eng.pdf.
4. USFDA. National Environmental Policy Act; Revision of Policies and Procedures; final rule. Federal Register; 1997.
5. Wise A, O'Brien K, Woodruff T. Are oral contraceptives a significant contributor to the estrogenicity of drinking water? Environ Sci Technol. 2011;45(1):51–60.
6. Anderson PD, Johnson AC, Pfeiffer D, Caldwell DJ, Hannah R, Mastrocco F, *et al*. Endocrine disruption due to estrogens derived from humans predicted to be low in the majority of U.S. surface waters. Environ Toxicol Chem. 2012;31(6):1407–15.
7. Caldwell DJ, Mastrocco F, Anderson PD, Lange R, Sumpter JP. Predicted-no-effect concentrations for the steroid estrogens estrone, 17 beta-estradiol, estriol, and 17 alpha-ethinylestradiol. Environ Toxicol Chem. 2012;31(6):1396–406.
8. Caldwell DJ, Mastrocco F, Nowak E, Johnston J, Yekel H, Pfeiffer D, *et al*. An assessment of potential exposure and risk from estrogens in drinking water. Environ Health Perspect. 2010;118(3):338–44.
9. Diamanti-Kandarakis E, Bourguignon JP, Giudice LC, Hauser R, Prins GS, Soto AM, *et al*. Endocrine-disrupting chemicals: an endocrine society scientific statement. Endocr Rev. 2009;30(4):293–342.
10. USEPA. Revisions to the Unregulated Contaminant Monitoring Regulation (UCMR 3) for Public Water Systems. 77 Federal Register 26071; 2012.
11. European Parliament. Surface waters: 12 new controlled chemicals, three pharmaceuticals on watch list. 2013. http://www.europarl.europa.eu/news/en/pressroom/content/20130701IPR14760/.
12. Endocrine Screening Program (EDSP). Washington (DC): Office of Chemical Safety and Pollution Prevention, US Environmental Protection Agency 2012. http://www.epa.gov/endo/. Accessed 1 Aug 2012
13. USEPA. Amendment to the universal waste rule: addition of pharmaceuticals. In: USEPA, editor. Federal register, 73(232). Washington, DC: US Environmental Protection Agency; 2008. p. 26.
14. Ankley G, Erickson R, Hoff D, Mount D, Lazorchak J, Beaman J, *et al*. Draft white paper: aquatic life criteria for contaminants of emerging concern, part i, general challenges and recommendations. Prepared by the Office of Water and Office of Research and Development Emerging Contaminants Workgroup. Washington, DC: U.S. Environmental Protection Agency; 2008.
15. USEPA. Science advisory board: aquatic life water quality criteria for contaminants of emerging concern. Washington, DC: U.S. Environmental Protection Agency; 2008.
16. Kostich MS, Lazorchak JM. Risks to aquatic organisms posed by human pharmaceutical use. Sci Total Environ. 2008;389(2–3):329–39.
17. Snyder SA. Occurrence, treatment, and toxicological relevance of EDCs and pharmaceuticals in water. Ozone Sci Eng. 2008;30(1):65–9.
18. Debroux J-F, Soller JA, Plumlee MH, Kennedy LJ. Human health risk assessment of non-regulated xenobiotics in

recycled water: a review. Hum Ecol Risk Assess Int J. 2012;18(3):517–46.
19. Grzybowski W. Comment on "Are oral contraceptives a significant contributor to the estrogenicity of drinking water?". Environ Sci Technol. 2011;45(17):7605.
20. Daughton C. Pharmaceutical ingredients in drinking water: overview of occurrence and significance of human exposure. Washington DC: American Chemical Society; 2010.
21. USFDA. Response to citizen petition to the FDA commissioner under the national environmental policy act and administrative procedure act requesting an amendment to a FDA rule regarding human drugs and biologics. 2013. p. 16.
22. Committee on the Assessment of Water Reuse as an Approach to Meeting Future Water Supply Needs; National Research Council. Water reuse: potential for expanding the nation's water supply through reuse of municipal wastewater. Washington, DC: The National Academies Press; 2012.
23. Jelić A, Petrović M, Barceló D. Pharmaceuticals in drinking water. In: Barceló D, editor. Emerging organic contaminants and human health. Berlin: Springer; 2012. p. 47–70.
24. USFDA. In: Center for Biologics Evaluation and Research, editor. Guidance for industry: environmental assessment of human drug and biologics application. Rockville: US Food and Drug Administration; 1998. p. 39.
25. USEPA. Guidelines for ecological risk assessment. Fed Regist. 1998;63:26846–924.
26. Stephan CE, Mount DI, Hansen DJ, Gentile JH, Chapman GA, Brungs WA. Guidelines for deriving numerical national water quality criteria for the protection of aquatic organisms and their uses. Washington, D.C.: U.S. EPA; 1985.
27. Jobling S, Nolan M, Tyler CR, Brighty G, Sumpter JP. Widespread sexual disruption in wild fish. Environ Sci Technol. 1998;32(17):2498–506.
28. Vajda AM, Barber LB, Gray JL, Lopez EM, Woodling JD, Norris DO. Reproductive disruption in fish downstream from an estrogenic wastewater effluent. Environ Sci Technol. 2008;42(9):3407–14.
29. USFDA. Drugs@FDA. 2011. http://www.accessdata.fda.gov/scripts/cder/drugsatfda/.
30. Mosher W, Jones J. Use of contraception in the United States: 1982–2008. National Center for Health Statistics. Vital Health Stat. 2010;23:29.
31. IMS. IMS national sales perspectives, year 2007–2011. Parsippany: IMS Health; 2012. Extracted April 2012.
32. Chlebowski RT, Kuller LH, Prentice RL, Stefanick ML, Manson JE, Gass M, *et al*. Breast cancer after use of estrogen plus progestin in postmenopausal women. New Engl J Med. 2009;360(6):573–87.
33. de Mes TZD, Zeeman G, Lettinga G. Occurrence and fate of estrone, 17β-estradiol and 17α-ethynylestradiol in STPs for domestic wastewater. Rev Environ Sci Biotechnol. 2005;4(4):275–311.
34. Gutendorf B, Westendorf J. Comparison of an array of in vitro assays for the assessment of the estrogenic potential of natural and synthetic estrogens, phytoestrogens and xenoestrogens. Toxicology. 2001;166(1–2):79–89.
35. Céspedes R, Petrovic M, Raldúa D, Saura Ú, Piña B, Lacorte S, *et al*. Integrated procedure for determination of endocrine-disrupting activity in surface waters and sediments by use of the biological technique recombinant yeast assay and chemical analysis by LC–ESI-MS. Anal Bioanal Chem. 2004;378(3):697–708.
36. Endocrine Disruptors Research. U.S. Environmental Protection Agency; 2012. http://www.epa.gov/endocrine/. Accessed 7 Aug 2012.
37. Hyder SM, Chiappetta C, Stancel GM. Synthetic estrogen 17 alpha-ethinyl estradiol induces pattern of uterine gene expression similar to endogenous estrogen 17 beta-estradiol. J Pharmacol Exp Ther. 1999;290(2):740–7.
38. Islinger M, Willimski D, Volkl A, Braunbeck T. Effects of 17a-ethinylestradiol on the expression of three estrogen-responsive genes and cellular ultrastructure of liver and testes in male zebrafish. Aquat Toxicol. 2003;62(2):85–103.
39. Urbatzka R, Rocha E, Reis B, Cruzeiro C, Monteiro RAF, Rocha MJ. Effects of ethinylestradiol and of an environmentally relevant mixture of xenoestrogens on steroidogenic gene expression and specific transcription factors in zebrafish. Environ Pollut. 2012;164:28–35.
40. Albero B, Sánchez-Brunete C, Miguel E, Pérez RA, Tadeo JL. Analysis of natural-occurring and synthetic sexual hormones in sludge-amended soils by matrix solid-phase dispersion and isotope dilution gas chromatography-tandem mass spectrometry. J Chromatogr. 2013;1283:39–45.
41. ECOTOXicology Database. 2011. http://cfpub.epa.gov/ecotox/. Accessed 29 March 2011
42. Nagpal NK, Meays CL. Water quality guidelines for pharmaceutically-active compounds (PhACs): 17α-ethinylestradiol (EE2), technical appendix. Province of British Columbia: Ministry of Environment; 2009. p. 27.
43. Caldwell DJ, Mastrocco F, Hutchinson TH, Lange R, Heijerick D, Janssen C, *et al*. Derivation of an aquatic predicted no-effect concentration for the synthetic hormone, 17 alpha-ethinyl estradiol. Environ Sci Technol. 2008;42(19):7046–54.
44. Soares J, Coimbra AM, Reis-Henriques MA, Monteiro NM, Vieira MN, Oliveira JMA, *et al*. Disruption of zebrafish (*Danio rerio*) embryonic development after full life-cycle parental exposure to low levels of ethinylestradiol. Aquat Toxicol. 2009;95(4):330–8.
45. Chemicals and the Water Framework Directive: Draft Environmental Quality Standards, Ethinylestradiol (EE2). European Commission, Scientific Committee on Health and Environmental Risks; 2011.
46. Nagpal NK, Meays CL. Water quality guidelines for pharmaceutically-active compounds (PhACs): 17α-ethinylestradiol (EE2). Province of British Columbia: Ministry of Environment; 2009. p. 8.
47. Surface waters: new chemicals added to EU risk list (press release). European Parliment; 2012.
48. Hannah R, D'Aco VJ, Anderson PD, Buzby ME, Caldwell DJ, Cunningham VL, *et al*. Exposure assessment of 17 alpha-ethinylestradiol in surface waters of the United States and Europe. Environ Toxicol Chem. 2009;28(12):2725–32.
49. Barber LB, Furlong ET, Keefe SH, Brown GK, Cahill J. Natural and contaminant organic compounds in the Boulder Creek watershed, Colorado, during high-flow and low-flow conditions, 2000. Chapter 5 in Murphy, S.F., Verplanck, P.L., and Barber, L.B., eds., Comprehensive water quality of the Boulder Creek Watershed, Colorado, during high-flow and low-flow conditions. 2000. U.S. Geological Survey Water-Resources Investigations Report 03-4045, p. 103–44.
50. Huang CH, Sedlak DL. Analysis of estrogenic hormones in municipal wastewater effluent and surface water using enzyme-linked immunosorbent assay and gas chromatography/tandem mass spectrometry. Environ Toxicol Chem. 2001;20(1):133–9.
51. Benotti MJ, Trenholm RA, Vanderford BJ, Holady JC, Stanford BD, Snyder SA. Pharmaceuticals and endocrine disrupting compounds in US drinking water. Environ Sci Technol. 2009;43(3):597–603.
52. Survey of Endocrine Disruptors in King County Surface Waters. Washington State Department of Natural Resources and Parks: Prepared by R. Jack and D. Lester, DNRP Water and Land Resources Division, Seattle, WA, 2007.
53. STOrage and RETrieval (STORET). Repository for water quality, biological, and physical data. Washington, D.C: U.S. Environmental Protection Agency; 2011.
54. National Water Information System (NWIS). U.S. Geographical Survey; 2011. http://waterdata.usgs.gov/nwis.
55. Cunningham VL, Buzby M, Hutchinson T, Mastrocco F, Parke N, Roden N. Effects of human pharmaceuticals on aquatic life: next steps. Environ Sci Technol. 2006;40(11):3456–62.
56. Anderson PD, D'Aco VJ, Shanahan P, Chapra SC, Buzby ME, Cunningham VL, *et al*. Screening analysis of human pharmaceutical compounds in US surface waters. Environ Sci Technol. 2004;38(3):838–49.
57. Kostich M, Flick R, Martinson J. Comparing predicted estrogen concentrations with measurements in US waters. Environ Pollut. 2013;178:271–7.
58. GREAT-ER. European Chemical Industry (CEFIC); 2012. http://www.great-er.org/pages/GenericSubPage.cfm?pageId=31&parentPgId=2.
59. Aronson D, Weeks J, Meylan B, Guiney PD, Howard PH. Environmental release, environmental concentrations, and

ecological risk of *N*,*N*-diethyl-*m*-toluamide (DEET). Integr Environ Assess Manag. 2012;8(1):135–66.
60. Exposure and fate assessment screening tool (E-FAST), Version 2.0, Documentation manual. Washington (DC): Exposure Assessment Branch, Office of Pollution Prevention and Treatment: U.S. Environmental Protection Agency; 2012. http://onlinelibrary.wiley.com/doi/10.1002/ieam.271/pdf. Accessed 31 July 2012
61. Johnson AC, Williams RJ, Matthiessen P. The potential steroid hormone contribution of farm animals to freshwaters, the United Kingdom as a case study. Sci Total Environ. 2006;362 (1–3):166–78.
62. Lange IG, Daxenberger A, Schiffer B, Witters H, Ibarreta D, Meyer HHD. Sex hormones originating from different livestock production systems: fate and potential disrupting activity in the environment. Anal Chim Acta. 2002;473(1–2):27–37.
63. Combalbert S, Hernandez-Raquet G. Occurrence, fate, and biodegradation of estrogens in sewage and manure. Appl Microbiol Biotechnol. 2010;86(6):1671–92.
64. Kolodziej EP, Harter T, Sedlak DL. Dairy wastewater, aquaculture, and spawning fish as sources of steroid hormones in the aquatic environment. Environ Sci Technol. 2004;38(23):6377–84.
65. Rodriguez-Rojas A, Rodriguez-Beltran J, Couce A, Blazquez J. Antibiotics and antibiotic resistance: a bitter fight against evolution. Int J Med Microbiol. 2013;303(6–7):293–7.
66. Rajapakse N, Silva E, Kortenkamp A. Combining xenoestrogens at levels below individual no-observed-effect concentrations dramatically enhances steroid hormone action. Environ Health Perspect. 2002;110(9):917–21.
67. Silva E, Rajapakse N, Kortenkamp A. Something from "Nothing"—eight weak estrogenic chemicals combined at concentrations below NOECs produce significant mixture effects. Environ Sci Technol. 2002;36(8):1751–6.
68. Carpenter DO, Arcaro K, Spink DC. Understanding the human health effects of chemical mixtures. Environ Health Perspect. 2002;110(S1):25–42.
69. Park JY, Lee BC, Ra JS, Lee J, Kim SD. Effect of copper complexation on the estrogenic activities of endocrine-disrupting compounds using e-screen bioassay. Environ Toxicol Chem. 2008;27(3):535–41.
70. Birnbaum LS. Environmental chemicals: evaluating low-dose effects. Environ Health Perspect. 2012;120(4):A143–4.
71. Vandenberg LN, Colborn T, Hayes TB, Heindel JJ, Jacobs Jr DR, Lee D-H, *et al*. Regulatory decisions on endocrine disrupting chemicals should be based on the principles of endocrinology. Reprod Toxicol. 2013;38:1–15.
72. USEPA. State of the science evaluation: nonmonotonic dose responses as they apply to estrogen, androgen, and thyroid pathways and EPA testing and assessment procedures. Washington, D.C.: USEPA; 2013.
73. Rhomberg LR, Goodman JE. Low-dose effects and nonmonotonic dose–responses of endocrine disrupting chemicals: has the case been made? Regul Toxicol Pharmacol. 2012;64(1):130–3.
74. Dekant W, Colnot T. Endocrine effects of chemicals: aspects of hazard identification and human health risk assessment. Toxicol Lett. 2013;223(3):280–6.
75. Zoeller RT, Brown TR, Doan LL, Gore AC, Skakkebaek NE, Soto AM, *et al*. Endocrine-disrupting chemicals and public health protection: a statement of principles from the endocrine society. Endocrinology. 2012;153(9):4097–110.
76. U.S. Environmental Protection Agency. Non-monotonic dose response curves research. 2012. http://epa.gov/ncct/edr/nonmonotonic.html.
77. EMEA. Guidelines on the environmental risk assessment of medicinal products for human use. London: European Medicine Agency (EMEA); 2006. Contract no.: EMEA/CHMP/SWP/4447/00.
78. Connon RE, Geist J, Werner I. Effect-based tools for monitoring and predicting the ecotoxicological effects of chemicals in the aquatic environment. Sensors. 2012;12(9):12741–71.
79. Sun H, Xia M, Austin C, Huang R. Paradigm shift in toxicity testing and modeling. AAPS J. 2012;14(3):473–80.
80. Brausch JM, Connors KA, Brooks BW, Rand GM. Human pharmaceuticals in the aquatic environment: a review of recent toxicological studies and considerations for toxicity testing. Rev Environ Contam Toxicol. 2012;218:1–99.
81. Schmidt CW. TOX 21: new dimensions of toxicity testing. Environ Health Perspect. 2009;117(8):A348–53.
82. Huang R, Xia M, Cho M-H, Sakamuru S, Shinn P, Houck KA, *et al*. Chemical genomics profiling of environmental chemical modulation of human nuclear receptors. Environ Health Perspect. 2011;119(8):1142–8.
83. Stanczyk FZ, Archer DF, Bhavnani BR. Ethinyl estradiol and 17β-estradiol in combined oral contraceptives: pharmacokinetics, pharmacodynamics and risk assessment. Contraception. 2013;87:706–27.
84. USEPA, USDA, USHHS, USDOI. Memorandum of understanding on Sustainability of Federal Collaboration on Pharmaceuticals in Drinking Water. U.S. Environmental Protection Agency, U.S. Department of Agriculture, U.S. Department of Health and Human Services, and U.S. Department of Interior, 2012.
85. USFDA. In: Consumer Health Information, editor. How to dispose of unused medicines. Rockville: US Food and Drug Administration; 2012. p. 2.
86. Kostich MS, Batt AL, Lazorchak JM. Concentrations of prioritized pharmaceuticals in effluents from 50 large wastewater treatment plants in the US and implications for risk estimation. Environ Pollut. 2014;184:354–9.
87. USFDA. Endocrine disruption potential of drugs: nonclinical evaluation (draft). Rockville: US Food and Drug Administration; 2013.
88. Boxall ABA, Rudd MA, Brooks BW, Caldwell DJ, Choi K, Hickmann S, *et al*. Pharmaceuticals and personal care products in the environment: what are the big questions? Environ Health Perspect. 2012;120(9):1221–9.
89. Gao P, Shi Y. Characterization of supersaturatable formulations for improved absorption of poorly soluble drugs. AAPS J. 2012;14(4):703–13.
90. Daughton CG, Ruhoy IS. Lower-dose prescribing: minimizing "side effects" of pharmaceuticals on society and the environment. Sci Total Environ. 2013;443:324–37.
91. Keil F. Pharmaceuticals for human use: an integrated strategy for reducing the contamination of water bodies. In: Kümmerer K, Hempel M, editors. Green and sustainable pharmacy. Berlin: Springer; 2010. p. 225–41.
92. Hamburg MA, Collins FS. The path to personalized medicine. New Engl J Med. 2010;363(4):301–4.
93. Woodcock J. The prospects for personalized medicine in drug development and drug therapy. Clin Pharmacol Ther. 2007;81 (2):164–9.
94. Daughton CG, Ruhoy IS. Green pharmacy and pharmecovigilance: prescribing and the planet. Expert Rev Clin Pharmacol. 2011;4(2):211–32.
95. Daughton CG, Ruhoy IS. Reducing the ecological footprint of pharmaceutical usage: linkages between healthcare practices and the environment. In: Kümmerer K, Hempel M, editors. Green and sustainable pharmacy. Berlin: Springer; 2010. p. 77–102.
96. Diab R, Jaafar-Maalej C, Fessi H, Maincent P. Engineered nanoparticulate drug delivery systems: the next frontier for oral administration? AAPS J. 2012;14(4):688–702.
97. Moore K, Townsend J, Spieler J, Coffey PS, Blithe D, Arndorfer E, *et al*. A greenprint for sustainable contraceptive research and development. Contraception. 2013;98 (3):347–51.
98. Barber LB, Vajda AM, Douville C, Norris DO, Writer JH. Fish endocrine disruption responses to a major wastewater treatment facility upgrade. Environ Sci Technol. 2012;46(4):2121–31.
99. Marfil-Vega R, Suidan MT, Mills MA. Assessment of the abiotic transformation of 17β-estradiol in the presence of vegetable matter–II: the role of molecular oxygen. Chemosphere. 2012;87(5):521–6.
100. Marfil-Vega R, Suidan MT, Mills MA. Abiotic transformation of estrogens in synthetic municipal wastewater: an alternative for treatment? Environ Pollut. 2010;158(11):3372–7.