Monitoring of emerging contaminants of concern in the aquatic environment: a review of studies showing the application of effect-based measures

Azeez Yusuf, Dylan O’Flynn, Blanaid White, Linda Holland, Anne Parle-McDermott, Jenny Lawler, Thomas McCloughlin, Denise Harold, Belinda Huerta and Fiona Regan

Water scarcity is increasingly a global cause of concern mainly due to widespread changes in climate conditions and increased consumptive water use driven by the exponential increase in population growth. In addition, increased pollution of fresh water sources due to rising production and consumption of pharmaceuticals and organic chemicals will further exacerbate this concern. Although surface water contamination by individual chemicals is often at very low concentration, pharmaceuticals for instance are designed to be efficacious at low concentrations, creating genuine concern for their presence in freshwater sources. Furthermore, the additive impact of multiple compounds may result in toxic or other biological effects that otherwise will not be induced by individual chemicals. Globally, different legislative frameworks have led to pre-emptive efforts which aim to ensure good water ecological status. Reports detailing the use and types of effect-based measures covering specific bioassay batteries that can identify specific mode of actions of chemical pollutants in the aquatic ecosystem to evaluate the real threat of pollutants to aquatic lives and ultimately human lives have recently emerged from monitoring networks such as the NORMAN network. In this review, we critically evaluate some studies within the last decade that have implemented effect-based monitoring of pharmaceuticals and organic chemicals in aquatic fauna, evaluating the occurrence of different chemical pollutants and the impact of these pollutants on aquatic fauna with special focus on pollutants that are contaminants of emerging concern (CEC) in urban wastewater. A critical discussion on studies that have used effect-based measures to assess biological impact of pharmaceutical/organic compound in the aquatic ecosystem and the endpoints measurements employed is presented. The application of effect-based monitoring of chemicals other than assessment of water quality status is also discussed.

Introduction

A large proportion of fresh water sources in Europe are heavily polluted mainly due to anthropogenic influences in the form of point or diffuse pollution sources from industrial waste, household/sewage and most importantly agricultural sources. The European Environment Agency (EEA) reports that only 40% of European water bodies including lakes, rivers and coastal waters are of good ecological status, which may further reduce due to uncontrolled human activities.

One of the major anthropogenic factors significantly contributing to surface and underground pollution in Europe is pharmaceutical in origin. According to the European Federation Pharmaceutical Industry and Associations (EFPIA), about €200 billion worth of active pharmaceutical ingredients (APIs) were produced in Europe in 2016 alone. Besides considering local consumption, production waste from pharmaceutical industries can will diffuse into surface and ground waters. In fact, APIs are one of the few known pollutants known to permeate through into ground water sources. Unfortunately, while many of the APIs might be present in water sources in very small concentrations, the design of pharmaceuticals to be efficacious at very low concentration creates concern about possible biological consequences on both aquatic and human lives. Pre-emptive efforts have been made globally by different governmental bodies attempting to ensure recovery of surface and ground waters.
Table 1: Major pharmaceuticals in the CEC list and their reported mode of action

<table>
<thead>
<tr>
<th>Pharmaceutical</th>
<th>log Kow</th>
<th>MW (g mol⁻¹)</th>
<th>Mode of action (MOA)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Azithromycin</td>
<td>4.32</td>
<td>785.0</td>
<td>Binds and inhibits assembly of large ribosome subunit 50S to prevent bacteria protein synthesis</td>
<td>12</td>
</tr>
<tr>
<td>Diclofenac</td>
<td>4.51</td>
<td>334.23</td>
<td>Inhibits Cox1 and 2 to prevent prostaglandin synthesis, blocking pain signalling</td>
<td>13</td>
</tr>
<tr>
<td>Clarithromycin</td>
<td>3.16</td>
<td>747.953</td>
<td>Prevents bacteria growth and division by binding to 23S rRNA, a component of the 50S subunit of bacterial ribosome, inhibiting peptides translation</td>
<td>14</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>3.06</td>
<td>733.93</td>
<td>Binds and inhibits assembly of large ribosome subunit 50S to prevent bacteria protein synthesis</td>
<td>15</td>
</tr>
<tr>
<td>Amoxicillin</td>
<td>0.87</td>
<td>365.4</td>
<td>Binds to penicillin-binding proteins that inhibit transpeptidation, leading to activation of autolytic enzymes e.g. autolysins in the bacterial cell wall. This leads to lysis of the cell wall, and thus, the destruction of the bacterial cell</td>
<td>16</td>
</tr>
</tbody>
</table>
### Table 1 (Contd.)

<table>
<thead>
<tr>
<th>Pharmaceutical</th>
<th>log $K_{ow}$</th>
<th>$MW$ (g mol$^{-1}$)</th>
<th>Mode of action (MOA)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ciprofloxacin</td>
<td>0.28</td>
<td>331.4</td>
<td>Inhibits DNA replication by inhibiting activity of DNA gyrase and topoisomerase IV preventing unwinding of DNA in bacteria</td>
<td>17</td>
</tr>
<tr>
<td>Trimethoprim</td>
<td>0.65</td>
<td>290.32</td>
<td>Inhibit dihydrofolate reductase synthesis of tetrahydrofolate which is required for thymidine biosynthesis and thus inhibits DNA synthesis</td>
<td>18</td>
</tr>
<tr>
<td>Sulfamethoxazole</td>
<td>0.48</td>
<td>253.28</td>
<td>Competitively inhibits dihydropteroate synthase preventing the formation of dihydropteroic acid, a precursor of folic acid which is required for bacterial growth</td>
<td>19</td>
</tr>
<tr>
<td>Gemfibrozil</td>
<td>2.47</td>
<td>236.3</td>
<td>Sodium channel blocker. It binds preferentially to voltage-gated sodium channels in their inactive conformation, which prevents repetitive and sustained firing of an action potential</td>
<td>20</td>
</tr>
<tr>
<td>Venlafaxine</td>
<td>3.2</td>
<td>277.4</td>
<td>Activates PPAR-α to stimulate peroxisomal β-oxidation by up-regulating the expression of all three important peroxisomal β-oxidation enzymes (acyl-coa oxidase, 2-trans-enoyl-coa hydratase, and thiolase)</td>
<td>21</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Increases serotonin, norepinephrine, and dopamine levels in the brain by blocking transport proteins that facilitate its reuptake at the presynaptic terminal</td>
<td>22</td>
</tr>
</tbody>
</table>

$log K_{ow}$: n-octanol/water partition coefficient.
Effect-based measures in assessing water quality status

More than 100,000 chemicals are registered with the European Chemical Agency (ECHA) with known safety data sheets and about 30,000 of these chemicals are sold and highly consumed annually across Europe. This chemicals eventually end up in thousands of surface waters across Europe. Monitoring this high volume of pollutants is an arduous task, although high resolution mass spectrometry (HRMS) in conjunction with gas or liquid chromatography has allowed high throughput monitoring of hundreds of chemicals at a time. This has resulted in an update and revision of existing priority lists or watch list, developed to monitor water quality, with the inclusion of new chemicals that are derived from the contaminants of emerging concerns (CECs) list (Table 1).

Legislation leading to effect-based monitoring of water quality status

The European Union Water Framework Directive (WFD) was established with the aim of encouraging stakeholders to set targets and meet them so as to achieve good water ecological status. This directive was also promulgated to ensure protection and sustainability of fresh water resources in Member States. However, one of the major challenges in implementing the European Union WFD is achieving a reduced high volume of pollutants is an arduous task, although high resolution mass spectrometry (HRMS) in conjunction with gas or liquid chromatography has allowed high throughput monitoring of hundreds of chemicals at a time. This has resulted in an update and revision of existing priority lists or watch list, developed to monitor water quality, with the inclusion of new chemicals that are derived from the contaminants of emerging concerns (CECs) list (Table 1).

Policy transition and formation of standards for effect-based monitoring

Although the chemical status of surface waters in Europe is constantly being updated with new pollutants added to the CEC list, there is an increasing need to evaluate the biological implication of the chemical mixtures on aquatic ecosystems and human health. The European subgroup Chemical Monitoring and Emerging Pollutants (CMEP) legislated within the Common Implementation Strategy (CIS) was tasked with developing a technical report that identifies effect-based measures (EBMs) for chemical pollution in the aquatic biota. The directive from the CMEP indicated that the report was to identify EBM tools such as bioassays and biomarkers that would be applicable in surveillance, operational and investigative programmes that assesses ecological status, to provide useful data that drives hypothesis generation and correlation with ecological observations. Based on the result of EBM-based studies, the toxicity index of a water body can be understood in the context of a dynamic monitoring outcome, complemented with a battery of in vitro and in vivo assays that can evaluate short- and long-term effects on aquatic organisms like algae, invertebrates and fish which are the major representatives of WFD Biological Quality Elements (BQE). A consequence of this was the establishment of novel research activities leading to the funding of a large EU-wide Project SOLUTIONS. This project drafted a common position on the application of EBMs in diagnosis and water quality monitoring, such as the use of effect-based trigger values to determine water quality. In addition, the activities of the European monitoring network (NORMAN) led to the agreement and establishment of a unified battery of bioassays that will cover modes of actions of all chemicals that pose harm to aquatic ecosystems and human health. Standard operating procedures for in vitro and in vivo bioassay batteries were published for different mode of actions (MOAs) such as chemicals’ estrogenicity, androgenicity and ability to modulate immune response via activation of aryl hydrocarbon receptor or activation of glucocorticoid receptor and the peroxisome activated receptor, for instance.

This review is an assessment of some studies using effect-based monitoring tools as a snapshot to evaluate the biological impact of pharmaceutical pollutants on aquatic fauna with special highlight of pharmaceutical contaminants that are CEC in urban wastewater. A brief discussion on the findings of studies that implemented effect-based measures in the assessment of the biological effect of pharmaceutical and organic compound contaminants in the aquatic ecosystem as well as the types of endpoints investigated are presented. The application of effect-based monitoring of chemicals other than for the assessment of water quality status is also discussed.
Literature search strategy and selection criteria

The published literature evaluated in this study were systematically retrieved from Scopus® (Elsevier, Netherlands) and PubMed databases in December of 2020. Published studies between 2011 and 2020 that investigated some form of effect-based monitoring of pharmaceutical and organic chemical pollution in different water bodies were selected based on the search criteria below. Several keywords were used to search for effect-based monitoring of pharmaceutical: “bioassay” or “biomonitoring” or “effect-based monitoring” and “pharmaceutical(s)” or “drug(s)” or “chemical” and “water” or “freshwater” or “surface water” or “aquatic” and “pollution” or “contamination”. These terms were searched across keywords, titles and abstracts and only research articles were included.

Workflow for MOA determination

The general workflow for the determination of the MOA of chemicals, based on the studies reviewed here, generally follow four predominant stages, which involves (1) identification and extraction of target chemical(s) from matrix, (2) pre-concentration of target chemical(s) and processing of samples, (3) bioassay set up for hypothesised MOA and (4) data processing and interpretation of study findings. The sample extraction entails all processes involving sample collection from the matrix which were mainly wastewater treatment plants (WWTP). Other sources of sample collection include river sediments or water, freshwater sources and sewage treatment.

Fig. 2 Illustration of the types of effect-based bioassays and their reported endpoint application: Selecting bioassay for effect-based monitoring often start with identification of appropriate matrix for sample collection. Collected samples by either passive or grab sampling are prepared for assay either through water reconstitution with pure chemical samples or spiking of collected water samples. This is then followed by endpoint selection and bioassay matching.
Major mode of actions and endpoints

The importance of the need for effect based monitoring as an alternative to generic assessment of risks for individual chemicals, has been previously suggested. However, there has been significant change in knowledge since then. The use of bioassays as a measure of water quality status is inherently dependent on the biological effects of pharmaceuticals and other organic chemicals that can be induced in biological faunas. Respectively, this design is also dependent on the chemistry of each individual chemical. There are myriads of bioassays that have been recommended by, for instance, the NORMAN network or the SOLUTIONS project and the different types of MOAs investigated in some of the studies evaluated here are shown in Tables 2–4.

Major primary endpoints assessment from samples at WWTPs and rivers were estrogenicity and androgenicity and analyses of such samples containing different chemical mixtures (Fig. 2). For example, diclofenac, carbamazepine, ciprofloxacin and venlafaxine are some of the commonly analysed compounds for estrogenic and/or androgenic activities. Most of these chemicals with the exception of diclofenac have log Kow of <4.5 indicating a relatively water soluble nature (Table 1). On the other hand, diclofenac has a log Kow of 4.51 indicating a lipophilic nature, which explain the estrogenicity exhibited by diclofenac as demonstrated by Klopcic et al. Interestingly, diclofenac log Kow lies in the range of log Kow that has been demonstrated to belong to estrogenic compounds. Some other studies investigated mutagenicity, aryl hydrocarbon receptor activation in detecting dioxin-like activities, glucocorticogenicity and genotoxicity. While these are specific endpoints that have been somewhat legislated for in biomonitoring of water quality, the list of endpoints that are measurable is open-ended. Chemicals modifying biological processes do so at the molecular level through activation of specific proteins that may be modified by perturbed gene expression. Such outcome measures are often investigated using in vitro tests although some instances of in vivo gene expression assays exists. For instance, Zhang et al. (2017) measured the alteration of glucose metabolism and gene expression in zebrafish embryos and were able to detect significant alteration in expression of genes involved in lipid synthesis upon exposure to environmentally relevant concentration of target analytes including estradiol, mifepristone and pregnanediol in river water samples. Aramboureou et al. (2019) monitored whole organism response of Chironomus riparius to river sediments contaminated with heavy metals and polyaromatic hydrocarbons (PAHs) in addition to evaluation of gene expression changes such as genes involved in stress gene response, those modulated by insulin receptor activities and edysone receptor activities. Another major endpoint often measured is the detection of specific established biomarkers, and these tend to vary from study to study. Interestingly, the main set of biomarkers often investigated are antioxidant biomarkers or biomarkers of metabolic pathways. Although these in vivo tests demonstrate a quantifiable biological response, it must be noted that such systems are not easy to set-up and may be time consuming compared to bioassays like the luciferase based assays such as CALUX and ER/DR-LUC bioassays.

Determining the endpoint measure may be influenced by the type of analytes expected to be present within the sample mixtures, i.e. only determining endpoint measures such as estrogenicity or androgenicity for suspected endocrine disrupting chemicals (EDCs). Predominantly, studies looking into possible MOAs of compounds within the CEC list such as diclofenac, carbamazepine, venlafaxine and trimethoprim primarily investigated estrogenicity and androgenicity of the sample mixtures wherein they are present. While some studies have not clearly stated reason for the choice of bioassay, this may also be influenced by the regulations in the WFD, listing possible bioassays that should be tested rather than careful analysis of the chemistry of the analytes. The implication of this may be the observations of non-estrogenicity or non-androgenicity of samples while the sample may induce activation of other biological pathways. In a similar fashion, MOAs for organic chemicals in pesticides were mainly evaluated through genotoxicity, thyroxine displacement from transeurythrin and glucocorticogenicity in addition to estrogenicity and androgenicity.
Table 2 Summary data from studies investigating MOA of sample mixtures from WWTP using grab sampling

<table>
<thead>
<tr>
<th>Sample type</th>
<th>Sampling method</th>
<th>Bioassay</th>
<th>MOA</th>
<th>BEQ/EEQ$^a$</th>
<th>Major analytes (total target analytes)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>WWTP</td>
<td>Grab</td>
<td>E-screen</td>
<td>Estrogenicity</td>
<td>WWTP1 = 28.5 ± 10.6 ng E2 L$^{-1}$</td>
<td>Diclofenac, naproxen, bisphenol A (15)</td>
<td>40</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>WWTP2 = 6.0 ± 3.2 ng E2 L$^{-1}$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MELN</td>
<td></td>
<td></td>
<td>Estrogenicity</td>
<td>WWTP1 = 21.7 ± 4.2 ng E2 L$^{-1}$</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>WWTP2 = 6.7 ± 1.7 ng E2 L$^{-1}$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>WWTP</td>
<td>Grab</td>
<td>H4IIE-luc</td>
<td>Dioxin-like activity</td>
<td>0.1 to 0.7 ng TCDD L$^{-1}$</td>
<td>Diclofenac, ketoprofen, acetaminophen, carbamazepine, atenolol, clarnaﬁmycin, amoxicillin, doxycycline</td>
<td>76</td>
</tr>
<tr>
<td></td>
<td></td>
<td>MVLN</td>
<td>Estrogenicity</td>
<td>0.1 to 5.1 ng E2 L$^{-1}$</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>MDA-kb2 cells with endogenous AR</td>
<td>Androgenicity</td>
<td>1–4 ng testosterone L$^{-1}$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>WWTP</td>
<td>Grab</td>
<td>YES</td>
<td>Estrogenicity</td>
<td>0.83 ng E2 L$^{-1}$</td>
<td>Atrazine, bisphenol A, nonylphenol</td>
<td>30</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Algae growth</td>
<td>—</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Acetylcholinesterase (AChE) activity</td>
<td>—</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WWTP</td>
<td>Grab</td>
<td>ERE-CALUX</td>
<td>Estrogenicity</td>
<td>1 ng E2 L$^{-1}$</td>
<td>Terbutylazine, prometryn, caffeine, clarnaﬁmycin, levomefotmeprazin (25)</td>
<td>47</td>
</tr>
<tr>
<td>WWTP</td>
<td>Grant and passive (DGT) sampling</td>
<td>ERE-CALUX</td>
<td>Estrogenicity</td>
<td>0.05 ± 0.01 ng E2 L$^{-1}$ to 29 ± 4 ng E2 L$^{-1}$</td>
<td>—</td>
<td>77</td>
</tr>
<tr>
<td>WWTP</td>
<td>Grab</td>
<td>Algal growth inhibition</td>
<td>—</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WWTP</td>
<td>Fish collection from sites</td>
<td>VTG assay</td>
<td>Endocrine disruption</td>
<td>—</td>
<td>—</td>
<td>78</td>
</tr>
<tr>
<td>WWTP</td>
<td>Grab</td>
<td>Yeast bio-reporter</td>
<td>Estrogenicity</td>
<td>0.7–14.0 ng E2 L$^{-1}$</td>
<td>—</td>
<td>79</td>
</tr>
<tr>
<td>WWTP</td>
<td>Grab</td>
<td>DR-LUC (H4L1.1c4)</td>
<td>Dioxin-like activity</td>
<td>5.7–10.2$^a$</td>
<td>Benz[a]pyrene, ﬂuoranthene, and tributyltin (205)</td>
<td>41</td>
</tr>
<tr>
<td>WWTP</td>
<td>Grab</td>
<td>ER-LUC (VM7Luc4E2)</td>
<td>Estrogenicity</td>
<td>0.11–7.1$^a$</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>AR-EcoScreen</td>
<td>Androgenicity/Androgenicity</td>
<td>0.38–3.9$^a$</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>TTR-binding</td>
<td>Thyroxine displacement from TTR protein</td>
<td>2–200 ng of thyroid hormone (T4) L$^{-1}$</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>WWTP</td>
<td>Grab</td>
<td>Ames II</td>
<td>Mutagenicity</td>
<td>—</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Thamnatoxkit F™</td>
<td>Juvenile mortality of thamnocephalus platyurus</td>
<td>—</td>
<td></td>
<td></td>
</tr>
<tr>
<td>WWTP</td>
<td>Grab</td>
<td>YES</td>
<td>Estrogenicity</td>
<td>Reported as percentage receptor activation or inhibition</td>
<td>—</td>
<td>80</td>
</tr>
<tr>
<td></td>
<td></td>
<td>YAS</td>
<td>Androgenicity</td>
<td>—</td>
<td></td>
<td></td>
</tr>
<tr>
<td>WWTP</td>
<td>Grab</td>
<td>Ames</td>
<td>Mutagenicity</td>
<td>—</td>
<td>—</td>
<td>81</td>
</tr>
<tr>
<td>WWTP</td>
<td>Grab</td>
<td>ER-CALUX</td>
<td>Estrogenicity</td>
<td>0.925–23.8</td>
<td>Bisphenol A, E1, E2, EE2 (6)</td>
<td>81</td>
</tr>
<tr>
<td>WWTP sediments and river basin</td>
<td>Grab</td>
<td>Antioxidant biomarkers</td>
<td>Superoxide dismutase (SOD), glutathione S-transferase activity (GST)</td>
<td>—</td>
<td>Salicylic acid, 4-tert-octylphenol, Pp′-dichlorodiphenyldichloroethene (168)</td>
<td>42</td>
</tr>
<tr>
<td></td>
<td></td>
<td>H4IIE-luc</td>
<td>Dioxin-like activity</td>
<td>0.1–0.8 ng of sediment per mL</td>
<td></td>
<td></td>
</tr>
<tr>
<td>WWTP</td>
<td>Grab</td>
<td>—</td>
<td>Genotoxicity</td>
<td>0.9–3.6 ng of sediment/mL</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>—</td>
<td>Estrogenicity</td>
<td>0.53 to 17.9 ng of E2 L$^{-1}$</td>
<td>(75)</td>
<td>43</td>
</tr>
</tbody>
</table>
Table 2 (Contd.)

<table>
<thead>
<tr>
<th>Sample type</th>
<th>Bioassay</th>
<th>MOA</th>
<th>BEQ/EEQa</th>
</tr>
</thead>
<tbody>
<tr>
<td>WWTP</td>
<td>Grab</td>
<td>Estrogenticity</td>
<td>0.05 to 0.35 ng of E2 L⁻¹</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Dioxin-like activity</td>
<td>0.003–0.56 ng E2 L⁻¹</td>
</tr>
<tr>
<td>WWTP</td>
<td>Grab</td>
<td>Genotoxicity</td>
<td>2.13 ± 0.48 ng E2 L⁻¹</td>
</tr>
<tr>
<td>WWTP</td>
<td>Grab</td>
<td>Estrogenicity</td>
<td>0.73 ± 0.08 ng E2 L⁻¹</td>
</tr>
<tr>
<td>WWTP</td>
<td>Grab</td>
<td>Androgenicity</td>
<td>0.034–2.4 ng E2 L⁻¹</td>
</tr>
<tr>
<td>WWTP</td>
<td>Grab</td>
<td>Genotoxicity</td>
<td>0.035–0.026 ng E2 L⁻¹</td>
</tr>
<tr>
<td>WWTP</td>
<td>Grab</td>
<td>Oxicative stress</td>
<td>0.012–0.26 ng E2 L⁻¹</td>
</tr>
<tr>
<td>WWTP</td>
<td>Grab</td>
<td>Oxidative stress</td>
<td>0.005–0.003 ng R1881 L⁻¹</td>
</tr>
<tr>
<td>WWTP</td>
<td>Grab and passive</td>
<td>Estrogenticity</td>
<td>0.01 to 0.11 ng E2</td>
</tr>
<tr>
<td>WWTP</td>
<td>Grab and passive</td>
<td>Androgenicity</td>
<td>0.051–5.8 ng R1881 L⁻¹</td>
</tr>
<tr>
<td>WWTP secondary site</td>
<td>Grab</td>
<td>Estrogenticity</td>
<td>0.45–42 ng E2 L⁻¹</td>
</tr>
<tr>
<td>WWTP</td>
<td>Grab</td>
<td>Androgenicity</td>
<td>0.45–42 ng E2 L⁻¹</td>
</tr>
<tr>
<td>WWTP</td>
<td>Grab</td>
<td>Dioxin-like activity</td>
<td>0.45–42 ng E2 L⁻¹</td>
</tr>
<tr>
<td>STP</td>
<td>Grab</td>
<td>Estrogenicity</td>
<td>1.2–35 ng E2 L⁻¹</td>
</tr>
<tr>
<td>STP</td>
<td>Grab</td>
<td>Androgenicity</td>
<td>1.5–50 ng E2 L⁻¹</td>
</tr>
<tr>
<td>STP</td>
<td>Grab</td>
<td>Dioxin-like activity</td>
<td>1.4–8 ng TCDD L⁻¹</td>
</tr>
</tbody>
</table>

a BEQ/EEQ are bioequivalence/estrogenic equivalence, * values are Toxic Mode of Action (TMoA) calculated as no-observed-effect concentration transformed to bioanalytical equivalent of reference compounds, DGT = diffusive gradient in thin film, E2 = estradiol.
Bioassays utilised to measure endpoints

Directives such as the WFD or recommendations from different studies have aggregated into listing series of endpoints that can be measured by a battery of bioassays. It is suggested that these should be implemented in guaranteeing accurate determination of water quality status through biomonitoring. However, there are no details on what type of bioassays must be used to determine such endpoints. In general, bioassays are reporter gene based as seen in the CALUX system, DR/ER-LUC, MDA-kb2, MELN and MVLN or H4IIE-luc systems, where genes specific to the endpoint such as estrogen receptor for estrogenicity, and a response element for that receptor are engineered in a plasmid vector located upstream of a luciferase enzyme. Activation of the receptor by the ligand, which in this case is the chemical contaminant, results in activation of the receptor and binding with the response element. This facilitates transcription of the luciferase enzyme that can catalyse a non-fluorescent substrate to a luminescent product. AR-CALUX and AR-GeneBLazer were also used in monitoring sample androgenicity. One main reason for the popularity of YAS assay amongst these studies may be due to the low cost compared to the other androgenicity assays such as AR-CALUX. Of all the systems used for endpoint measurement, the MDA-kb2 reporter cell line seem to be the most versatile system as it contains reporter genes for the androgen and glucocorticoid receptors that can detect androgenicity and glucocorticogenicity respectively. This type of system may help circumvent the costly nature of most bioassays if more reporter genes can be incorporated.

Table 3  Some studies investigating MOA of sample mixtures from WWTP using passive sampling technique

<table>
<thead>
<tr>
<th>Sample type</th>
<th>Sampling method</th>
<th>Bioassay</th>
<th>MOA</th>
<th>BEQ/EEQ</th>
<th>Major analytes (total target analytes)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>WWTP Passive/POCIS</td>
<td>Nrf2 reporter</td>
<td>Oxidative stress, Inflammation</td>
<td>—</td>
<td>Sulfamethoxazole, trimethoprim, metronidazole, erythromycin, clarithromycin, venlafaxine, carbamazepine</td>
<td>31</td>
<td></td>
</tr>
<tr>
<td>WWTP Passive (large volume solid phase extraction)</td>
<td>Transcriptional changes of Danio rerio</td>
<td>Androgenicity</td>
<td>1.3 ng E2 L⁻¹</td>
<td>Estrone, 17β-estradiol, mifepristone, pregnanediol, androstenedione, androsterone</td>
<td>60</td>
<td></td>
</tr>
<tr>
<td>WWTP Passive (POCIS)</td>
<td>Comet assay</td>
<td>Genotoxicity</td>
<td>—</td>
<td>Acetobutol, bisoprolol, venlafaxine, carbamazepine</td>
<td>61</td>
<td></td>
</tr>
<tr>
<td>WWTP Passive (SPMD and POCIS)</td>
<td>H4IIE-luc</td>
<td>Dioxin-like activity</td>
<td>0.008 to 2.0 ng TCDD L⁻¹</td>
<td>Ciprofloxacin, erythromycin, trimethoprim, carbamazepine, diclofenac</td>
<td>45</td>
<td></td>
</tr>
<tr>
<td>WWTP Passive (POCIS) (compared POCIS pest with POCIS 20 d)</td>
<td>MVLN</td>
<td>Estrogenicity</td>
<td>0.1 to 5.4 ng E2 L⁻¹</td>
<td>Carbamazepine, ciprofloxacin, diclofenac, erythromycin, sulfamethoxazole, trimethoprim</td>
<td>88</td>
<td></td>
</tr>
<tr>
<td>WWTP Passive</td>
<td>Ames YES</td>
<td>Genotoxicity</td>
<td>—</td>
<td>Ciprofloxacin, Azithromycin</td>
<td>46</td>
<td></td>
</tr>
<tr>
<td>WWTP Passive</td>
<td>—</td>
<td>Estrogenicity</td>
<td>0.3–10.8 ng E2 L⁻¹</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

TCDD = 2,3,7,8-tetrachlorodibenzo-p-dioxin, DHT = dihydrotestosterone.
<table>
<thead>
<tr>
<th>Sample type</th>
<th>Sampling method</th>
<th>Bioassay</th>
<th>MOA</th>
<th>BEQ/EEQ</th>
<th>Major analytes (total target analytes)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>River</td>
<td>Passive/POCIS and SPMD</td>
<td>CAFLUX assay</td>
<td>Dioxin-like activity</td>
<td>SPMD = 0.0029–0.0073 ng TCDD L(^{-1})</td>
<td>Carbamazepine, citalopram, codeine, diclofenac, diltiazem, irbesartan, trimethoprim and venlafaxine (77)</td>
<td>137</td>
</tr>
<tr>
<td></td>
<td></td>
<td>MVLN</td>
<td>Estrogenicity</td>
<td>SPMD = 0.37–0.78 pg E2 L(^{-1})</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>MDA-kb2 cells with endogenous AR</td>
<td>Androgenicity</td>
<td>SPMD = 1 × 10(^{-4})-0.0035 ng DHT L(^{-1}) POCIS = 0.59–55 pg DHT L(^{-1})</td>
<td></td>
<td></td>
</tr>
<tr>
<td>River sediment</td>
<td>Passive/POCIS and chemcather</td>
<td>Planar-YES</td>
<td>Estrogenicity</td>
<td></td>
<td></td>
<td>157</td>
</tr>
<tr>
<td></td>
<td></td>
<td>YES</td>
<td>—</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>River sediment</td>
<td>Grab</td>
<td>Gene expression analysis targeting</td>
<td>EcR</td>
<td></td>
<td></td>
<td>68</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>InR Heat shock proteins</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Cyp450, SOD, Biotransformation reactions</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>River water and</td>
<td>Grab</td>
<td>ERE-CALUX</td>
<td>Estrogenicity</td>
<td>200–937 pg E2 L(^{-1})</td>
<td></td>
<td>97</td>
</tr>
<tr>
<td>sediment</td>
<td></td>
<td>DRE-CALUX</td>
<td>Dioxin-like activity</td>
<td>0.6–42 pg TCDD g(^{-1})</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Endocrine disruption</td>
<td>0.021–0.13 ng E2 L(^{-1})</td>
<td></td>
<td>133</td>
</tr>
<tr>
<td>River</td>
<td>Grab</td>
<td>YES</td>
<td>Estrogenicity</td>
<td>0.09 ng L(^{-1}) (antiestrogenic)</td>
<td></td>
<td>120</td>
</tr>
<tr>
<td></td>
<td></td>
<td>VTG assay</td>
<td>Endocrine disruption</td>
<td>1.96–3.13 ng DHT L(^{-1})</td>
<td></td>
<td></td>
</tr>
<tr>
<td>River</td>
<td>Passive/SPMD and POCIS</td>
<td>POCIS YES</td>
<td>Estrogenicity</td>
<td>0.03–0.28 pm E2 L(^{-1})</td>
<td></td>
<td>107</td>
</tr>
<tr>
<td></td>
<td></td>
<td>SPMD YES</td>
<td>Estrogenicity</td>
<td>—</td>
<td></td>
<td></td>
</tr>
<tr>
<td>River</td>
<td>Grab</td>
<td>ER-LUC</td>
<td>Estrogenicity</td>
<td>0.01–0.84 ng E2-EQ g(^{-1})</td>
<td>Naphthalene, phenanthrene, Anthracene, fluoranthene, pyrene</td>
<td>158</td>
</tr>
<tr>
<td></td>
<td></td>
<td>DR-LUC/TTR-binding</td>
<td>Dioxin-like activity</td>
<td>0.05–0.56 ng TCDD-EQ g(^{-1})</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Thyroxine displacement from TTR protein</td>
<td>0.4–36.7 μg t4-EQ g(^{-1})</td>
<td></td>
<td></td>
</tr>
<tr>
<td>River</td>
<td>Passive/POCIS</td>
<td>MVLN</td>
<td>Estrogenicity</td>
<td>≤0.2211 g(^{-1})</td>
<td>Bromosynil (31)</td>
<td>48</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Hepa1.12cR reporter</td>
<td>Mutagenicity</td>
<td>0.014–0.791 ng E2 L(^{-1})</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Transiently transfected CHO-K1</td>
<td>Androgenicity</td>
<td>0.01–0.064 ng R1881 L(^{-1})</td>
<td></td>
<td></td>
</tr>
<tr>
<td>River</td>
<td>Grab and passive/POCIS</td>
<td>Grab YAS</td>
<td>Androgenicity</td>
<td>0.154–1.089 ng TCDD L(^{-1})</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>POCIS</td>
<td>—</td>
<td>50 μg flutamide L(^{-1})</td>
<td></td>
<td></td>
</tr>
<tr>
<td>River sediment</td>
<td>Passive/POCIS</td>
<td>MELN</td>
<td>Estrogenicity</td>
<td>1.0–3.2 ng E2 g(^{-1})</td>
<td>Carbamazepine, gemfibrozil, pregnenolone, prednisone, prednisolone</td>
<td>150</td>
</tr>
<tr>
<td></td>
<td></td>
<td>MDA-kb2</td>
<td>Glucocorticigenicity</td>
<td>11.1–180.3 μg Dex g(^{-1})</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Surface water</td>
<td>Grab</td>
<td>GR-CALUX</td>
<td>Glucocorticigenicity</td>
<td>&gt;0.4–&lt;3.0 ng Dex L(^{-1})</td>
<td></td>
<td>59</td>
</tr>
</tbody>
</table>

\(\text{nd} = \text{no detection.}\)
Detected estrogenic activity in all matrices

For most relevant endpoints, there are a multitude of bioassays that are employed in evaluating such endpoints. For example, estrogenicity is a measure of the activation of the ER and this endpoint can be evaluated using YES, ER-CALUX, MELN or MVLN bioassays. Some of the EEQ values reported for estrogenicity for individual bioassays were in line or well below the calculated effect-based trigger values (EBT) values of estrogenicity while some were considerably higher according to previously reported data.\textsuperscript{103} For example, the EBT value reported for MELN assay by \textit{Escher et al.} (2018) was 0.68 ng E2 L\textsuperscript{-1} while many studies reported EEQ values for estrogenicity using MELN bioassay above 1.0 ng E2 L\textsuperscript{-1}.\textsuperscript{40,103–106} Thus, the problem of the acceptability of these values from experimental data arises when considering wider applications in water quality assessment. Unfortunately, without a universally acceptable EBT values that can be referenced for a particular endpoint, correlating data from different bioassays on the same endpoint may become cumbersome, requiring caution during data interpretation. This phenomenon was observed in some of the studies reviewed here, in which different bioassays were used to evaluate the same endpoint with some differences in the EEQ values obtained.\textsuperscript{30,40,44,87} The difference between the values recorded by the different bioassays for the same endpoint in a particular study may not be large enough to be significant, in which case such differences may be ignored as observed, for instance, in the data reported by \textit{Konig et al.} (2017) and \textit{Kienle et al.} (2019) (< 0.2 ng E2 L\textsuperscript{-1}).\textsuperscript{30,44} This becomes problematic in instances where such difference is considerably large as in the studies by \textit{Schiliro et al.} (2012) and \textit{Spina et al.} 2020 (1.7 ng E2 L\textsuperscript{-1} and 6.8 ng E2 L\textsuperscript{-1} respectively between E-Screen and MELN assays).\textsuperscript{90,83} Therefore, it becomes pertinent to develop ways of deriving EBT values for individual bioassays for a given endpoint. According to \textit{Escher et al.} (2018), this should be an average generic EBT value per endpoint considering all possible bioassay data and such value should be adjusted with a bioassay-specific sensitivity factor that makes it relevant across the board.\textsuperscript{103}

As stated earlier, a high proportion of studies that sampled at WWTP, rivers and other water sources (Tables 2–4), investigated estrogenicity. The limit of detection of estrogenicity varies between all these studies and even at this, estrogenic activities is more likely to be detected than any other mode of actions, although some studies reported no detection of estrogenic activities is some of the sampled locations.\textsuperscript{93,107} This may be attributed to the chemical properties of the analytes in the collected samples. For short and long term exposure, EEQ values <0.5 ng of E2 L\textsuperscript{-1} is considered to be the safe level.\textsuperscript{108} Interestingly, while some studies reported estrogenic activities at values below 0.5 ng of E2 L\textsuperscript{-1},\textsuperscript{111,118} many others reported a range of EEQ values above 0.5 ng of E2 L\textsuperscript{-1}.\textsuperscript{30,100,106,110,112–118} Overall, a wide range of estrogenic activity were detected from WWTP, both influents and effluents. Lower values of estrogenic activities were generally recorded in WWTP effluents for studies applying estrogenicity assessment to evaluate water quality and waste treatment efficiency.\textsuperscript{80,109,110,119} In these studies, the low estrogenic activities were linked to elimination of chemical pollutants in the treatment processes. Conversely, high estrogenicity values well above 100 ng E2 L\textsuperscript{-1}
were also recorded in other studies while some others reported
anti-estrogenic activities. Quantitating anti-
estrogenicity in these studies corresponds to measurement of the
decrease in detected fluorescence, luminescence or absorb-
ance signal given by a specified amount of competing standard
ligand for the estrogen receptor, i.e. as EEQ of the antagonist in
ng of reference antagonist L⁻¹. These findings can potentially
impact decision making supporting the need for effect-based
monitoring of pollutants or contaminants as a standard
assessment of water quality at WWTPs or some other water
bodies. However, recorded estrogenicity in samples collected
upstream from some WWTP discharge points may be found to
exceeded some calculated risk trigger values or values obtained
at the WWTP by same assay as noted by Archer et al. (2020). This
This highlights the significance of how alternative pollution
sources may be contributing towards accumulation of estro-
getic or xenogenic contaminants in the environment and
ultimately the interpretation of results from assays at specific
locations.

In general, the estrogenic activities recorded downstream of
WWTPs were lower compared to those measured at the WWTP,
for obvious reasons. WWTP, especially the influents, are hot-
spots containing higher concentration of chemicals. Even
though the effluents discharged into other surface water bodies
such as rivers and streams typically have lower CEC concen-
trations than influent samples, effluent CEC concentrations are
generally higher than in the secondary sites due to dilution that
occurs when effluents mix with surface waters. WWTPs serve
residential, hospital and industrial buildings where direct
human activities impact the level of estrogens and xeno-
estrogens in household or commercial wastes. For instance,
excreted estrogens and unused estrogenic drugs as well as other
xenogenic drugs are disposed through the sewage
systems, and this leads to an increase in the pressure of these
chemicals on the STPs. Although YES assay recorded by higher
estrogenicity compared to MELN assay, this is likely dependent
on the sensitivity of the assays as explained earlier.

In situ quantification of VTG is an efficient way to detect
induction of estrogenic pathways and to assess the real life
impact of xenoestrogens. VTG secretion, unique to female fish
species, has been widely used in different studies as a marker of
feminisation in different male fish species due to estrogenic
influence of contaminants in water bodies. Assessment of VTG
expression is often used in conjunction with other
estrogenicity bioassays as a complementary method for
assessment of endocrine disruption within aquatic fauna. Such
application was employed by Geraudie et al. (2017) and Ganser
et al. (2019) in their studies where YES assay was used to eval-
uate estrogenicity of water samples in addition to the VTG
assay. Unexpectedly, while Ganser et al. found significant
induction of estrogenic signals from the YES assay, VTG level
was not significantly induced in the male fishes sampled. On
the contrary, Geraudie et al. discovered significant increase in
VTG levels complemented by positive estrogenic activity as
detected by the YES assay even though both studies reported
similar ranges of E2 equivalence in the sample mixtures (Tables
2 and 3). Many factors could be responsible for this disparity in
the two studies, all of which are required to be considered when
correlating findings of such complementary assays. Some of
these include length of exposure of sampled fish to estrogens
and xenoestrogens within the aquatic environment and species-
specific response of fishes to chemicals, as different sampled
species are bound to have different responses to xenoestro-
gens, especially when the concentration of contaminants is
high enough to induce response in the bioassay but not enough
to cause VTG secretion.

Grab vs. passive sampling and
estrogenicity in context of WWTP

Of the major sampling methods available, composite and
passive sampling were the most common in the studies
reviewed. Interestingly, “grab sampling” where one sample is
taken at a location for one specific time point time was often
synonymously used for composite sampling in which case
samples are grabbed from same location but over a period of
time (Tables 2 and 3). The choice of employing passive
sampling or grab sampling seem to be dictated by the type
and nature of the contaminants that are expected to be present in
the water body that is sampled. For instance, most persistent
organic contaminants within the aquatic ecosystem are hydro-
phobic in nature, while other commonly used pharmaceuticals
or pesticides are hydrophilic. These types of chemicals can be
possibly sampled using various passive samplers based on their
hydrophobic or hydrophilic properties. Based on this applica-
tion of passive sampling requires some level of prior knowl-
edge of the chemicals that are present in the sampling location,
to allow for compatibility between the passive sampler and
the contaminants to be sampled. On the other hand, application
of grab/composite sampling does not require prior information.
This may have reflected in the fact that more studies in general
employed grab over passive sampling. On average, from a short
survey of selected studies (Tables 2 and 3), estrogenicity recor-
ded from passive sampling (range = 0.06–10.8 ng E2 L⁻¹) was
not markedly different from those from grab samples (range = 0.05–42 ng E2 L⁻¹), indicating likelihood of no difference in the
sensitivity of both passive and grab sampling. This notion is
supported by findings of Guo et al. (2019), where samples from
passive and grab samples provided not only similar estrogenic
activities, but similar results in other endpoints investigated.
This was also similar to the findings of Ganser et al. (2019),
where grab samples were taken on two separate days, while
passive sampling using solid phase extraction, was used to obtain
samples in the time period between those two days. Although
the passive sampler could not detect estrogenicity at the initial
time point, the detection of estrogenic activity in the grab
sample may be as a result of possible high concentration of
analyte in the grab sample, which needs time to reach the same
levels in a passive sampler. In addition, the WWTP influents
are hotspots that contain high concentrations of chemical
pollutants which can be easily detected without the need of
passive sampling for extended periods. Extreme conditions in
the WWTP influents can also make application of passive
sampling difficult, thus favouring the use of grab sampling over passive sampling.

Variation in results from samples collected by different methods was also a phenomenon that was observed. Jalova et al. (2013) used SPMD and POCIS samplers in sample collection.45 POCIS sample showed estrogenic activities which were not detected in samples collected by SPMD. A similar finding was reported by Tousova et al. (2019), where the highest estrogenicity detected for POCIS was more than a thousand fold what was detected for SPMD.137 This can be explained by the difference in the pollutants chemistry that adsorb to the surface of the samplers. POCIS was developed to adsorb polar organic chemicals while SPMD adsorbs hydrophobic chemicals. As such, the polar solvent collected by the POCIS is likely more estrogenic. It is known that a polar group that is capable of donating hydrogen bonds on an aromatic system such as in the hydroxyl group is the most prominent structural feature that is crucial for estrogenic activity.138 This is not likely to be present on a hydrophobic compound.

**Other measured endpoints in all matrices**

Another common mode of actions is androgenicity, which is mainly evaluated by YAS yeast-based assay and AR-based reported assays like AR-GeneBLAzer, AR-EcoSCREEN and AR-CALUX. Unlike estrogenicity, where the highest reference chemical was E2, different reference chemicals like DHT and metribolone (R1881) were used by different studies making comparative analysis difficult. Nonetheless, the androgenic activity detected varied across all studies that used DHT as reference chemical. The lowest recorded values were often recorded in river samples as observed for estrogenicity, which was also observed for other endpoints like dioxin-like activities. This is expected because most water from different point sources entering the river would have been treated at a WWTP to reduce the level of contaminants. The same variability phenomenon for studies using different sample collection types such as SPMD vs. POCIS was observed and this is due to the difference in the chemistry of the collected chemicals regarding the polarity and hydrophobicity of the target analytes. Although most studies detected some level of androgenic activities, some studies recorded no androgenic activities. This lack of activity may be due to the pre-treatment of the water samples as found in the study by Wildhaber et al. (2015) where effluent samples that had been ozonated were tested.44 This is especially as ozonation is a widely used method for decontaminating wastewater, proven to remove steroids from waste water.66,139 This lack of activities not only indicate the application of effect monitoring in assessing the effectiveness of waste water treatment but also reiterate the importance of incorporating such bioassay in water treatment protocols for routine assessment.

Aryl hydrocarbon receptor activation (dioxin-like activities) was another common endpoint tested due to the implication of aryl hydrocarbon receptor activation in cancer development and progression.140 Like estrogenicity, dioxin-like activities were evaluated by numerous bioassays. Most of the studies interestingly used TCDD as the reference chemical but the difference in the sensitivities of these bioassays will also still require some caution in comparing the data. One study did not use TCDD, but benzo[a]pyrene (B[a]P) as the reference compound.69 This is because polycyclic aromatic hydrocarbons (PAH) are known activators of the AhR, and while they do not exhibit the toxic effect of dioxin-like chemicals, their application as a monitoring tool to activate AhR receptor is the main objective.

Assessment of changes to the genetic make-up of aquatic organisms is one of the debilitating effect of chemical contaminants, which is why genotoxicity assessment has been widely investigated globally.66,71,141-149 Genotoxicity was mainly evaluated by Ames, comet and p53-based reporter assay. However, whole organism assessments for DNA damage were also common. Genotoxocity was not detected in some studies and those that detected some genotoxic activities did not use the same data report format. This was based on the variation in the types of assays and the application, making the genotoxicity measurement not uniform across all studies as seen for bioassays like estrogenicity or androgenicity. As a result, the findings of these studies are open to different interpretations. For instance, mitomycin and cyclophosphamide were the reference chemicals used by Konig et al. (2017) and Valitalo et al. (2017), respectively.48, 50 Maier et al. (2015) reported the quantity of WWTP sediment that induced the LOD signal.42 The main set back to this disparity is the inability to make comparative analysis or generalised inference that can inform decision making.

Less specific endpoints such as glucocorticogenicity and gene expression profiling were observed.44, 59, 68, 69, 150-156 Glucocorticogenicity was mainly evaluated by reporter based bioassay and all three samples used dexamethasone as the reference compound. Some level of glucocorticogenic activities were recorded in all studies but as with other studies, the problem encountered is the quantification of BEQ in sediment samples as compared with water samples, which are not easily comparable and requires taking the matrix into consideration. For studies looking at gene expression, this bioassay is tailored to specific research questions, and as such are not generally relatable. However, the possible deduction from the findings of these two studies is the significant changes in transcriptional expression of key genes that affect molecular pathways can be successfully detected in a carefully designed bioassay.

**Endpoints on individually targeted analytes**

Besides evaluating the biological effect of a mixture of analytes in different water and sediment samples, there are also studies that investigated the biological effect of single pharmaceuticals using a myriad of bioassays (Table 5). These are usually focused on understanding the biological effect of single compounds (independent action) or mixture of chemicals (concentration addition) through modelling toxicities of individual
Table 5  Common endpoints evaluated for selected important pharmaceuticals

<table>
<thead>
<tr>
<th>Pharmaceutical</th>
<th>Endpoint</th>
<th>Organism/bioassay</th>
<th>Effective concentration</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diclofenac</td>
<td>Loss of shoot</td>
<td>Solanum lycopersicum</td>
<td>5 mg L(^{-1}) (EC50)</td>
<td>32</td>
</tr>
<tr>
<td>Diclofenac</td>
<td>Mobility</td>
<td>Ceriodaphnia silvestri</td>
<td>37.9 mg L(^{-1}) (EC50)</td>
<td>33</td>
</tr>
<tr>
<td>Diclofenac</td>
<td>Estrogenicity</td>
<td>YES</td>
<td>Anti-estrogenicity = 3.8 × 10(^{-5}) M (IC50)</td>
<td>34</td>
</tr>
<tr>
<td></td>
<td>Androgenicity</td>
<td>YES</td>
<td>Androgenicity = 5.2 × 10(^{-5}) M (EC50)</td>
<td>34</td>
</tr>
<tr>
<td></td>
<td>Glucocorticogenicity</td>
<td>MDA-kb2 cell line</td>
<td>Anti-androgenicity = 6.3 × 10(^{-5}) M (IC50)</td>
<td>34</td>
</tr>
<tr>
<td>Diclofenac</td>
<td>Genotoxicity (DNA strand breaks)</td>
<td>M. galloprovincialis</td>
<td>25 µg L(^{-1})</td>
<td>35</td>
</tr>
<tr>
<td>Gemfibrozil</td>
<td>Mortality, embryo development</td>
<td>Danio rerio</td>
<td>Mortality = 0.5 and 10 µg L(^{-1})</td>
<td>36</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Developmental abnormality = reduced 0.5 µg L(^{-1})</td>
<td></td>
</tr>
<tr>
<td>Venlafaxine</td>
<td></td>
<td></td>
<td>Mortality = 0.5 µg L(^{-1})</td>
<td>16a</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Developmental abnormality = reduced 0.5 and 10 µg L(^{-1})</td>
<td></td>
</tr>
<tr>
<td>Carbamazepine</td>
<td>Proteomics of DNA strand breaks, energy metabolism, oxidative stress response and biomarkers of lipid peroxidation, glutathione transferase (GST) activity</td>
<td>Blue mussels (Mytilus spp)</td>
<td>No effect up to 25 mg L(^{-1})</td>
<td>163</td>
</tr>
<tr>
<td></td>
<td>Ecotoxicity by means of in vitro bioluminescence and respirometry assays</td>
<td>Vibrio Fischeri</td>
<td>—</td>
<td>151</td>
</tr>
<tr>
<td></td>
<td>AChE activity</td>
<td>S. vittatum</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Carcinostatic mixture of 26 PCP including acetaminophen, ciprofloxacin, clarithromycin, clodribate, ibuprofen, erythromycin</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Venlafaxine</td>
<td>Circadian rhythm</td>
<td>Gambusia holbrooki</td>
<td>100 µg L(^{-1})</td>
<td>164</td>
</tr>
<tr>
<td>Carbamazepine</td>
<td>Estrogenicity</td>
<td>YES</td>
<td></td>
<td>165</td>
</tr>
</tbody>
</table>

* High concentration did not cause mortality. b CBZ concentration did not correspond with the estrogenticity of water sample.

contaminants.\(^{159}\) Pure samples (analytical grade) of these pharmaceuticals was reconstituted in different solvents such as DMSO, water or methanol rather than extraction from collected water samples. Diclofenac, gemfibrozil, venlafaxine, and carbamazepine were the assayed pharmaceuticals all of which are contained in the CEC list.\(^{7,14}\) Diclofenac is one of the most common pharmaceuticals often studied for its independent action. Investigation of its anti-estrogenic, androgenic and anti-androgenic effects, toxic effects of plant growth, mortality and DNA damaging effect on different aquatic organisms have been carried out.\(^{32-34,37,168}\) Interestingly, there are some pharmaceuticals found to have beneficial effects, such as venlafaxine and carbamazepine that were found to reduce abnormal embryo development in zebrafish at an environmentally-relevant concentration of 0.5 µg L\(^{-1}\).\(^{36}\) This is in line with the report of a previous study that documented the highest carbamazepine concentration of 5 µg L\(^{-1}\) in a German wastewater.\(^{164}\) As such, extrapolation of this result to what was observed in the study by Galus et al. (2013) would present the possible danger of such concentration to zebrafish in aquatic environments.\(^{36}\) The applicability of the results from the examples of studies in Table 5, is however questionable as most of the effective and inhibitory concentrations reported are quite above environmentally relevant concentration. For instance, the highest concentration of diclofenac that has been reported in a WWTP was below 2 µg L\(^{-1}\),\(^{162}\) which is more than 1000 folds lower than most EC50 or IC50 reported here.

Interpretation of the results from these studies portend a species-, pathway- and concentration-specific implication. As shown in Table 5, Sousa et al.,\(^{13}\) Damasceno et al.,\(^{11}\) and Klopcic et al.\(^{14}\) investigated different endpoints for diclofenac in different species or in vitro bioassay (evaluating estrogenicity, androgenicity and glucocorticogenicity) as in the case with Klopcic et al.\(^{14}\) reporting different effective or inhibitory concentrations. Galus et al.\(^{36}\) on other hand evaluated the bio-effect gemfibrozil, venlafaxine and carbamazepine on the same endpoint. Pestana et al.\(^{163}\) showed no effect of carbamazepine exposure in S. vittatum.
Application of effect-based monitoring assays in assessing chemical pollution and water quality

There is increasing argument for the incorporation of effect-based monitoring of chemical contaminants in assessment of water quality. As evidenced in some of the studies that have been reviewed here (Tables 2–5), these arguments highlight the importance of using effect monitoring to identify possible biological impact of chemical contaminants at environmentally relevant concentration. However, effect based methods have potential applicability that is beyond just detecting pollutants in water samples. A sophisticated application of estrogenicity measurement was observed in the studies by Spina et al. and Omoruyi and Pohjanvirta. Spina et al. employed measurement of estrogenic activity level to assess the efficiency of wastewater treatment by an eco-friendly method involving the use of mutant laccase enzyme from Trametes pubescens. In the same way Omoruyi and Pohjanvirta used a yeast bio-reporter assay similar to YES assay to measure the effectiveness of the Helsinki WWTP in water treatment by evaluating estrogenic activities from WWTP influent and effluent samples as well as tap water in residential residences and commercial bottled still and mineral water. By quantifying estrogenicity of WWTP samples before and after the treatment, it was possible to evaluate the water quality status after treatment which translated to the effectiveness of the novel eco-friendly method and the WWTP respectively in wastewater treatment. In the same way, Giebner et al. also used the effect measures at different stages of wastewater treatment to determine the percentage removal of chemical waste by quantifying the estrogenicity, anti-estrogenicity, androgenicity and anti-androgenicity at each stage. For instance, anti-estrogenicity of the wastewater was found to reduce from 65% inhibition of the ER from primary treatment of the influent to 39% inhibition of the ER after the second clarifier stage. De Baat et al. used a battery of in vitro and in vivo bioassays to test extracts from passive sampling to deduce the associated ecotoxological risks of metal and organic polar compounds as a ratio the bioanalytical responses observed to the published effect based trigger values to determine location specific contamination. By this, the researchers were able to identify pollution sources in different water bodies, making this strategy a potentially useful application to assess water quality status.

While studies like those of Geraudie et al. (2017) and Ganser et al. (2019) used VTG secretion only to assess feminisation of male fish species or endocrine disruption activities of contaminants, quantification of VTG level in fish has been applied in the assessment of anthropogenic influence on changes in chemical pollution profile in water bodies. Similar to the application of Spina et al. (2020) as discussed above, Morthorst et al. quantified VTG levels in juvenile brown trout over two periods, between 2000 through 2004 and 2010 through 2016 to determine trend of chemical pollution in the river body. It was found that average VTG levels in the fishes between 2000 and 2004 was significantly higher than the quantified level between 2010 and 2016. This was attributed to the reduced estrogenicity of the wastewater as a consequence of the improved wastewater treatment commitment of Danish government. One of the major reasons for advocating for the implementation of effect-based monitoring of water quality assessment was to address the setbacks of chemical analytical strategies. Chemical analytical techniques are considered to be inadequate to quantify the ever decreasing concentrations of chemical pollutants in the aquatic ecosystem, and some chemicals alone or in mixtures may induce biological effect even at very minimal concentrations that are undetectable by chemical analytical tools. With this in mind, it is advised that a more complete and effective strategy for measuring water quality status is to couple chemical analyses with effect based monitoring. While EBM studies often use both chemical analytical tools of different kinds to determine the pollutants within the matrix they investigated in addition to biomonitoring of the pollutants, concentrations of individual chemicals within a mixture are sometimes not reported. This results in a recurring problem: failure to measure individual concentrations of the chemical pollutants will not allow for correlation of the concentrations of these compounds within the mixture with the effects observed. Even when the concentrations are measured, this in addition to the documentation of BEQ or EEQ of the sample extracts as evaluated for specific MOAs (Tables 2–4), does not do justice to identify which of the chemicals within the mixture or which combination of chemicals and at what different concentrations these chemicals are able to produce such effect. This poses the major problem of causality facing the application of effect biomonitoring. To address this, Xia et al. (2020) in a recent study used high throughput transcriptomic approach to monitor the pathways activated by investigating the independent actions and concentration addition effects of single chemicals and chemical cocktails respectively in a concentration dependent manner. This strategy was used to create a model that can further predict, using independent actions of single chemicals, the concentration addition effects that is possible for combination of different chemicals that are present in the sample. While this strategy may still be at its infancy, it may be the way forward in consolidating the application of EBM techniques with the concentration effect of single compounds and chemical mixtures in attempt to assess water quality status.
Conclusions and recommendations on adoption of effect-based methods for pharmaceutical and organic chemical application

This review has highlighted that endpoint measurements with the aid of developed bioassays have proven useful in the determination of the biological effects of chemical contaminants in aquatic ecosystems. Although this can translate to possible water quality status, the different bioassays that are possible for one endpoint and the individual variations between any two bioassays will limit the ability to generalise or extrapolate results from different studies for informed decision or policy making. These individual variations include the type of reference chemicals, sensitivity of the bioassay and the type of data reporting format. To overcome this, different bioassays for individual endpoints can be standardised with the same samples and reference chemicals to calculate the sensitivity factor of each bioassay. Such calculated sensitivity factor can be taken into account for any bioassay to have a universally acceptable BEQ/EEQ value of any sample. In addition, bioassays alone are not suitable to attribute observed effect with causative chemicals. As such, there is need to accompany bioassays with quantitative techniques such as such as chromatographic and mass spectroscopic techniques will allow identification of specific chemicals in samples and possibility of linking observed effects from field samples with that produced from strategically testing pure and mixture of chemical samples in the lab.

Several studies reviewed here have successfully demonstrated that effect-based monitoring is not limited to assessment of water quality status but also in determining the efficiency of water treatment strategies as well as in the quantification of contaminants in aquatic environments. As noted by Omoruyi and Pohjanvirta,79 the application of bioassay in quantification of analytes may be problematic. Bioassays are only able to measure or quantify bioactive substances, and unable to detect compounds that require metabolism for activity. This however, must be taken into consideration, as is the case with carbamazepine, which has been reported to have five active metabolites that may be detected during wastewater treatment.144 Complementing bioassays with existing quantitative techniques will prevent any omissions.

From the studies reviewed here, it is evident that battery of bioassays evaluating endpoints such as estrogenicity, androgenicity, genotoxicity, aryl hydrocarbon receptor activation/dioxin-like activities and glucocorticogenicity to mention a few are valuable in water quality assessment based on biological activities that are elicited by these chemicals. While grab sampling was more practiced amongst the studies, evidence from studies that employed both sampling methods showed similarities in the effect detected in the samples. This will be provided if appropriate control measures are put in place such as frequency of sampling in grab sampling for instance. However, the use of sampling by POCIS or SPMD may produce different results dependent on the types of analytes picked up by the devices. Employment of either of SPMD and POCIS should thus be driven by carefully designed research question. This is well exemplified by the studies reviewed here. Most of the CEC (Table 1) that were analysed in samples collected by passive method utilised POCIS, which was likely based on the device compatibility with the polar groups present on such chemicals like carbamazepine, ciprofloxacin, diclofenac, erythromycin, sulfamethoxazole and venlafaxine. However, this consideration will not apply to grab sampling and this convenience might explain the reason for most studies employing this sampling method.

Evaluating the biological effect of a single individual chemical rather than mixture of analytes was a theme reviewed here. However, this is an unlikely scenario in real life as contaminated water bodies are often polluted with a mixture of chemicals rather than individual chemicals. While it is informative to know, for instance, the estrogenicity of diclofenac, the contribution of diclofenac amongst a mixture of hundreds of chemicals will be significantly influenced in the presence of these other chemicals such as carbamazepine, trimethoprim, ciprofloxacin, venlafaxine as common in studies reviewed here29,43,45,47,76,85 or any other possible combination of chemical cocktails. In addition, the causality effect of a specific chemical within a mixture of pollutant in a water sample in producing a particular effect is currently impossible with effect-based monitoring. As such, effect-based monitoring of pure samples of chemicals may have to be accompanied by determined, possible or suspected mixture of chemicals to paint the real life scenario.

Ultimately, consistent and robust bio-analytical methods for effect measurement underpin effective determination of bioactivities of pharmaceutical and organic compound contaminants in aquatic fauna. Assay development for biological endpoints is an unending endeavour for targeted and broad-scope effect-based measurement. This is applicable in an interdisciplinary approach to understand pharmaceutical and other organic pollutant activities within surface waters as a measure of water quality status. As toxicological research into biomonitoring of aquatic chemical contaminants continues to grow, standardisation of effect measures that will allow unification of results from different studies that will facilitate informed decision making both at local, continental and global scale remains pertinent.

Conflicts of interest

There are no conflicts to declare.

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