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Detection of Organic Compounds with Whole-Cell Bioluminescent Bioassays

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Abstract

Natural and manmade organic chemicals are widely deposited across a diverse range of ecosystems including air, surface water, groundwater, wastewater, soil, sediment, and marine environments. Some organic compounds, despite their industrial values, are toxic to living organisms and pose significant health risks to humans and wildlife. Detection and monitoring of these organic pollutants in environmental matrices therefore is of great interest and need for remediation and health risk assessment. Although these detections have traditionally been performed using analytical chemical approaches that offer highly sensitive and specific identification of target compounds, these methods require specialized equipment and trained operators, and fail to describe potential bioavailable effects on living organisms. Alternatively, the integration of bioluminescent systems into whole-cell bioreporters presents a new capacity for organic compound detection. These bioreporters are constructed by incorporating reporter genes into catabolic or signaling pathways that are present within living cells and emit a bioluminescent signal that can be detected upon exposure to target chemicals. Although relatively less specific compared to analytical methods, bioluminescent bioassays are more cost-effective, more rapid, can be scaled to higher throughput, and can be designed to report not only the presence but also the bioavailability of target substances. This chapter reviews available bacterial and eukaryotic

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whole-cell bioreporters for sensing organic pollutants and their applications in a variety of sample matrices.

Keywords

Bacterial luciferase; Bioavailability; Bioreporter; Bioluminescence; BTEX; Dioxin; Endocrine disruptors; Environmental monitoring; Firefly luciferase; Hydrocarbon; PAH; PCB

1 Introduction

Synthetic organic chemistry started in the nineteenth century when, instead of extracting urea from an animal kidney, the German chemist Friedrich Wöhler produced it from inorganic substances, and French chemist Marcellin Berthelot chemically synthesized fatty acids that are not found in nature [1]. Over the ensuing 200 years, chemists have subsequently learned to create an enormous number of organic compounds, both of natural and synthetic origin, that have become essential input materials for consumer products, agriculture, manufacturing, pharmaceutical, and life science industries, and myriad other applications. However, despite their industrial value, some of these compounds have unfortunately become associated with adverse health effects in humans and animals. For instance, exposure to benzene, a naturally occurring aromatic hydrocarbon found in crude oil, has now been linked to decreased circulating blood cell counts, leukemia, and immunotoxicity [2], as has exposure to the toxic synthetic compound dichlorodiphenyltrichloroethane (DDT), which was used extensively as an insecticide until it was banned from agricultural use worldwide because of its toxicity toward a wide range of organisms. These compounds, like many other organic pollutants, are persistent and prone to bioaccumulation, endowing them with a large potential to negatively affect the well-being of living organisms.

The increasingly large scale of industrial chemical production, and its corresponding increase in anthropogenic chemical consumption, has driven up a demand for effective tools and approaches that can both detect the environmental occurrence of these compounds as well as assess their potential biological effects following exposure. These environmental monitoring efforts have traditionally employed analytical methods such as high-performance liquid chromatography (HPLC) and gas chromatography coupled with mass spectrometry (GC/MS) to detect and quantify toxic chemicals, and the ability of these analytical methods specifically to measure chemical concentrations at high sensitivities has been instrumental for the evaluation of the level of contamination. However, these methods have proved to be time-consuming, expensive, and relatively difficult to perform and, more important, are not capable of measuring bioavailability and biological impact, two important aspects of the risk assessment paradigm.

To overcome these shortcomings, biological assays using living whole-cell bioreporters have been developed to provide more biologically relevant information. Bioluminescent bioreporters in particular, due to their ability to generate an easily measurable light signal, have been well validated in the laboratory and extensively applied in environmental monitoring. These bioreporters, being genetically transformed to express the luciferase

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genes of bacterial origin (*luxAB*), the full bacterial bioluminescent system (*luxCDABE*), or the firefly luciferase gene (*luc*) as a means of light production, provide a rapid, simple, and cost-effective complement to analytical chemical methods. As living entities, whole-cell bioreporters act as proxies for humans and other organisms to prewarn the occurrence of potentially toxic substances. Most environmental applications have traditionally employed bacterial-based bioreporters for this purpose, however, eukaryotic cell-based bioreporters are increasingly being utilized to provide more human- and animal-relevant data. This chapter reviews the development of both bacterial and eukaryotic cell-based bioluminescent bioreporters for sensing a broad range of organic compounds and provides an overview of the utility and limitations of these bioluminescent bioassays in practical applications.

2 Detection of Organic Compounds Using Bacterial Bioluminescent Assays

Being genetically easy to manipulate and displaying rapid and robust growth, bacteria have been extensively employed as hosts for bioreporter development. Despite the toxic nature of organic pollutants, evolution has provided some bacteria with unique genetic properties that allow them to adapt to the presence of toxic chemicals by utilizing them as carbon sources. As the generation of proteins involved in the degradation of exposed pollutants is an energy-consuming process and costs fitness under unexposed conditions, the catabolic process is carefully regulated at the transcriptional level in such a way that it is only initiated upon exposure to its corresponding chemical input. To exploit this unique feature, bacterial bioluminescent bioreporters are constructed by transcriptionally integrating reporter genes (*luxAB*, *luxCDABE*, or *luc*) within special catabolic pathways that specifically respond to the presence of target compounds. Fortunately, the diversity of catabolic capabilities and sophisticated characterization of responsible genetic components has provided a sizable toolbox for bioreporter construction. This section provides an overview of the genetic foundations for bacterial bioluminescent bioreporter development and their applications toward detection of a variety of common organic contaminants (Table 1).

2.1 Benzene, Toluene, Ethylbenzene, and Xylene

The monocyclic hydrocarbons benzene, toluene, ethylbenzene, and xylene (BTEX) are found across various environmental matrices such as water, soil, and other sediments due to contamination with petroleum products resulting from gasoline spills, underground storage container leaks, runoff from manufacturing plants, and so on. Traditionally, a sample suspected of BTEX contamination would be subjected to a lengthy extraction process to purify the available chemicals, followed by analytical-based testing to identify the compounds present. In an effort to ease the process of detecting and identifying chemical pollutants, bioluminescent reporter strains have been developed that modulate signal production in response to BTEX chemical exposure, indicating their presence in a sample. To accommodate the disparate needs of detection, a variety of reporter constructs has been developed over the years, ranging from the straightforward introduction of bioluminescent genes into naturally BTEX-degrading organisms to track their prevalence, to more complex introductions of both bioluminescent and BTEX-degrading genes into specialized reporter organisms that can be tailored to the needs of a particular investigation. Although this section focuses only on bioluminescent BTEX reporter organisms that modulate signal in

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response to compound detection, it should be noted that there is a variety of additional sources that have evaluated BTEX presence and toxicity using constitutively bioluminescent reporters as well [3–5].

Due to the prevalence of BTEX in the environment, a multitude of bioluminescent bioreporters have been developed across a variety of different host strains. The majority of these bioreporters function by leveraging the expression of the TOL plasmid degradation genes that were originally identified in *Pseudomonas putida* mt-2 [6]. These genes function across two distinct pathways consisting of an upper pathway and a *meta* pathway. In this system, BTEX compounds are first oxidized in the upper pathway, and then proceed to the *meta*-cleavage pathway where they are further broken down before ultimately being routed to the Krebs cycle [7–9]. Governing the expression of these pathways are two regulators, of which the primary XylR regulator is most often utilized for bioreporter design. This may seem counterintuitive because the *xylR* gene is constitutively expressed, but its resulting protein product remains inactive until physical interaction with a BTEX chemical imparts structural changes that permit it to bind to, and subsequently activate, the upper pathway promoter P_u [10]. Li et al. [11] were able to capitalize on this interaction and construct a pTOLLUX plasmid that utilized the *xylR* gene product to activate the transcription of an *Aliivibrio fischeri* (originally classified as *Vibrio fischeri* [12]) *luxCDABE* gene cassette that was fused to the native P_u promoter. When expressed in *Escherichia coli* DH5α, and assayed in 96-well microtiter plates, the resulting bioluminescence could be detected after a 2-h incubation with 7.5 μM toluene. However, because the XylR regulator can be activated by a variety of BTEX chemicals, a test with known concentration toluene spikes is required in parallel with all environmental samples. It was determined that, under these conditions, the reporter was capable of detecting a concentration of 168 μM of nonspecific BTEX compounds in soil, and 362 μM of nonspecific BTEX compounds in groundwater. However, additional testing is still required to determine which specific compound(s) are present.

In an alternative but somewhat similar approach, Willardson et al. [13] developed a BTEX reporter strain by employing the XylR regulator to govern expression of the firefly luciferase gene (*luc*). The resulting *xyl*R-P_u-*luc* fusion was housed on the pGLUTR plasmid and expressed in *E. coli* DH5α. The resulting reporter was then used to detect BTEX compounds in both soil and water samples [13]. Notably, the water samples used in these experiments were taken from near an underground storage tank known to have leaked BTEX compounds and were incubated directly with the reporter without preprocessing. Following this 1-h direct incubation, the samples were treated with luciferin substrate to induce signal generation and the total detectable BTEX concentration in the water sample was determined to be 215 μM of toluene equivalents. Unlike the water samples, this reporter still required soil samples to undergo an ethyl alcohol extraction to isolate any chemical pollutants prior to exposure. However, following this extraction and a subsequent dilution of the samples in medium, only a 1-h incubation was required before signal generation, and under these conditions 3.44 mM toluene equivalents could be detected. So although this method was still limited by its inability to report specific compounds, it was capable of detecting total BTEX compounds within 3 % of conventional detection methods, making it a powerful tool for general BTEX detection.

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In addition to reporters that utilize the TOL plasmid, a second class of reporter organism has been developed around the toluene benzene utilization pathway (*tbu*) from *Ralstonia pickettii* PKO1. Similar to the TOL-encoded pathway, the tbu pathway is regulated by the TbuT protein, which activates the *PtubA1* promoter in the presence of a BTEX inducer compound. Building upon this pathway, Tecon et al. [14] developed an *E. coli*-based bioreporter that harbors a pPROBE-LuxAB-TbuT plasmid whereby the TbuT regulator acts on the *PtubA1* promoter when a BTEX inducer compound is present to transcribe the *luxAB* genes from *A. fischeri*. Because only the *luxA*B genes were present, and not the full *luxCDABE* operon, all assays using this reporter required the addition of the n-decanal substrate prior to bioluminescent production. To determine its functionality under environmentally relevant conditions, this bioreporter was used to monitor an artificial oil spill. Using a 96-well microtiter plate assay, this bioreporter detected 4–12-μM toluene equivalent concentrations in contaminated seawater samples that were collected as early as 6 h after the spill. However, similar to the TOL-based systems, the inability to differentiate between BTEX compounds limits this reporter to only broad-spectrum compound detection.

The third class of reporters is typified by the bioluminescent *P. putida* TVA8 BTEX bioreporter developed by Applegate et al. [15]. This reporter, as do all members of its class, utilizes the *P. putida* T-2 toluene degradation pathway (*tod*) that was originally characterized by Zylstra et al. [16] and Wang et al. [17]. Similar to the previously described pathways, the *tod* pathway consists of a series of genes responsible for BTEX oxidation that are under the control of a BTEX regulatable promoter. Using this system, *P. putida* TVA8 was constructed by transposon insertion of the bioluminescent *luxCDABE* gene cassette downstream of the *todX* and *todR* genes for toluene recognition and transcriptional activation and validated in wastewater samples for its ability to detect BTEX compounds [18]. During the experiment, *P. putida* TVA8 was inoculated with a 1:10 ratio of wastewater and sampled every 30 min for 8 h to observe bioluminescence. Unlike the assays of Stiner and Halverson [19], no additional substrate was required because this construct contained the full *luxCDABE* cassette. Under these conditions, bioluminescence was detectable within the first 30 min [18] and significant bioluminescent responses were observed following treatment with 23 distinct compounds. Although this again highlights the inability of most BTEX bioreporter organisms to differentiate individual BTEX compounds from one another, it also shows their utility as detection systems for organic pollutants in general. Following this successful validation, the same reporter was later utilized to test groundwater located beneath an airfield site [20], where it successfully differentiated between contaminated and remediated sites as confirmed by analytical detection methods.

The final pathway that has been used for BTEX bioreporter construction is the isopropylbenzene (*ipb*) pathway. This pathway, which was originally identified in *P. putida* RE204 [21], consists of a regulatory protein (encoded by *ipbR*), an operator/promoter *ipbo/p*, and the isopropylbenzene dioxygenase gene (*ipbA*). To develop a BTEX bioreporter using this system, Selifonova and Eaton [22] transformed an *E. coli* HMS174 strain with a plasmid containing the *ipb* genes fused with the *lux* gene cassette of *A. fischeri*. This construct was then used to detect aromatic compounds in hydrocarbon mixtures (jet fuel, diesel fuel, and creosote) and for the direct detection of hydrocarbons extracted from

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sediments. It was demonstrated that the reporter could detect a concentration as low as 0.01 ppm in creosote hydrocarbon mixtures. Because the sediments contained a mixture of hydrocarbons in varying quantities, light detection was measured from dilutions of the total extracted hydrocarbons to determine if bioluminescent production increased corresponding to the increase of hydrocarbons. It was demonstrated that the reporter did produce bioluminescence corresponding to the sediment extraction dilutions and could detect hydrocarbons in a dilution as low as 1:500. However, because this reporter is not specific to BTEX compounds, the amount of light produced may not indicate the true concentration of BTEX compounds in mixed samples.

As a whole, it can be said that the varied classes of BTEX reporters have successfully met their goal of providing a faster and lower cost method for the detection of BTEX compounds. However, the major caveat for these reporters is their inability to differentiate individual BTEX compounds. Because each bioreporter was constructed to detect a number of compounds that are under the BTEX classification, pinpointing which contaminant(s) are present in the sample is not currently possible. Therefore, in order to determine the exact chemical contaminant further analysis of a sample still needs to be done. Despite this drawback, these reporters provide a simple and efficient method for the rapid screening of multiple categories of environmental samples, making them a valuable first-line analysis tool for large-scale monitoring projects.

2.2 Polycyclic Aromatic Hydrocarbons

Polycyclic aromatic hydrocarbons (PAHs) are compounds consisting of two or more fused benzene rings. Due to repeated spills and the seepage of petroleum products, they have become some of the most common soil and water contaminants and can be found around the globe. There is a pressing need for the identification and remediation of PAHs because they pose significant human health risks such as heart disease, cancers, and kidney and liver damage [23]. In order to meet this need, PAH-detecting bioreporters have been developed that utilize either the naphthalene, phenanthrene, or isopropylbenzene degradation pathways for control of their bioluminescent signals.

By far, the majority of the PAH bioreporters exploit the naphthalene degradation pathway in order to modulate their bioluminescent signal. This pathway consists of two separate systems, an upper pathway (*nahABCDEF*) that degrades naphthalene to salicylate, and a lower pathway that then degrades the salicylate to acetaldehyde and pyruvate [24]. A single regulatory gene, *nahR*, governs the expression of these genes in response to naphthalene, making it an ideal target for bioreporter design. Burlage et al. [25] were the first to make use of this pathway when they constructed *P. putida* RB1351. This strain contains the *luxCDABE* gene cassette under the control of the upper NAH promoter, P_{nah}, allowing bioluminescent expression to be modulated in response to naphthalene bioavailability. This reporter strain has been extensively investigated by Dorn et al., who refer to the strain as *P. putida* RB1353 [26–28]. From these studies, it has been concluded that the *lux* expression can be altered from a 1 °C temperature change and a change in pH of 0.2 [27], the reporter can successfully be integrated into a fiberoptic detection system for monitoring microbial

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activity in porous media in realtime [28], and that the fiber-optic detection system can be used for the realtime in situ monitoring of bioactive zone formation and dynamics [26].

The most widely known naphthalene pathway-based reporter, however, is likely *P. fluorescens* HK44, which has become one of the most evaluated microbial bioreporters ever to be developed. Similar to *P. putida* RB1351, HK44 expresses the upper naphthalene pathway (*nahABCDEF*) and the regulator gene *nahR*, however, in the lower pathway the *nahG* gene is fused to the *luxCDABE* cassette from *A. fischeri* [29]. Using this set-up King et al. [29] demonstrated a bioluminescent response to naphthalene at concentrations as low as 1.56 μM after 15 min of exposure in a chemostat culture. More important, however, it was also shown in this study that the bioluminescent signal generated by HK44 was capable of responding to naphthalene in a dose-responsive manner, allowing for realtime detection and monitoring. Throughout its widespread use, HK44 has since been tested against a variety of organic compounds and has been applied to monitor PAH occurrence in various environmental matrices (recently reviewed by Trögl et al. [30]). Although the majority of applications using strain HK44 have been performed on extracts of water, soil, and sediment samples, Valdman and Gutz [31] recently demonstrated the utility of agar gel-immobilized HK44 reporter cells for the detection of naphthalene and related compounds in the vapor phase. Various concentrations of naphthalene vapors were flowed into sampling tubes and bioluminescence was monitored in a luminometer. The limit of detection under this experimental design was determined to be 20 nM naphthalene, and a linear relationship between naphthalene concentration and bioluminescent response was obtained between the concentrations of 50 and 260 nM.

Because of the characteristics and popularity of the *P. fluorescens* HK44 bioreporter, it was selected as the model organism for a first of its kind multiyear controlled field release study. In 1996 *P. fluorescens* HK44 was released in a controlled environmental test site to monitor the long-term ability of a genetically modified organism to detect and degrade naphthalene. Over time, environmental naphthalene was detected in two ways, either through the detection of naphthalene vapors by HK44 biosensor modules interfaced with fiber-optic cables [32], or through direct interaction of HK44 bioreporter cells with naphthalene in the soil via observation of the resultant bioluminescent signal using a photomultiplier tube (PMT) [32]. The continued detection of HK44 throughout the 2-year study proved its ability to persist in the environment, and bioluminescent detection on-site demonstrated its utility as a continuous reporter for naphthalene bioavailability [32, 33].

It is important to remember, however, that despite the widespread use of *P. fluorescens* HK44 and its related naphthalene-based sensor organisms, other methods for PAH bioreporter construction have been employed. Among these has been the use of the phenanthrene degradative genes from the *Burkholderia* sp. strain RP007, which was first described by Laurie and Lloyd-Jones in 1999 [34]. Tecon et al. [126] exploited this operon for the construction of a bioreporter for the detection of naphthalene, phenanthrene, and related PAH compounds. The resultant reporter strain, *B. sartisoli* RP007, harnessed the regulatory genes *phnR* and *phnS* from the phenanthrene pathway to regulate expression of the *luxAB* genes. Under this design, when a PAH compound interacts with the PhnR protein it causes the downstream activation of the *phnS* promoter, which then allows transcription of

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the *A. fischeri luxAB* genes. Similar to other reporters that only contain the *luxAB* genes, this reporter is limited in that the substrate n-decanal must be added for the production of light concurrent with naphthalene exposure. However, when n-decanal is supplied, the reporter demonstrated a minimal detection limit of 0.17 μM after a 3-h incubation. When exposed to an artificial oil spill, bioluminescence was detected above the minimal detection limit after 3 h, and continued to produce a response after 5 days, demonstrating a longevity of signal that can be crucial for environmental monitoring applications.

2.3 Alkane Aliphatic Hydrocarbons

Alkanes are saturated hydrocarbon structures that can be deposited environmentally from a wide variety of sources. Most commonly, however, they originate from the natural seepage of crude oil deposits or from anthropogenic releases of fuel products and industrial lubricants. Due to their highly hydrophobic nature, they are not acutely bioavailable, and therefore have traditionally been difficult to detect. Historically, the predominant method for alkane detection by microbial bioreporters has been through expression of the regulatory region of the alkane-responsive *alk* operon from *Pseudomonas oleovorans*. In 1973 it was discovered that the genes responsible for octane degradation in *P. oleovorans* were located on a plasmid and could be transferred between organisms [35]. Although it was known at this time that the degradation function encoded by these genes was inducible in the presence of alkanes, it took another 15 years before the genetic structure of the operon was fully identified [36]. It was then a further 9 years before it was first exploited as a sensing component for bioreporter development [37]. The *alk* operon consists of two distinct sections, with the first encoding three genes for alkane catabolism (*alkBFG*) and second encoding a regulatory component (*alkS*) that can activate transcription in the presence of 6- to 10-carbon alkanes [38].

Sticher et al. [37] were able to remove the *alkS* regulatory component and coexpress it along with a fusion of the inducible *alkB* promoter and the *Vibrio harveyi luxAB* genes. This effectively governed the transcription of the *luxAB* genes in response to alkane presence, although it required an exogenous application of decanal to serve as the substrate for the *luxAB* luciferase in order to elicit a bioluminescent response. Following a survey of decanal concentrations, it was determined that the assay conditions had to be amended with 2 mM decanal to ensure that the results were indicative solely of the induction of the *luxAB* genes by alkanes, and not limited by a lack of substrate for the resultant bioluminescent reaction. Under laboratory conditions, this decanal-supplemented assay was capable of detecting 5- to 10-carbon chain length alkanes with a response time of 1–2 h. The minimum detection level for octane was determined to be 24.5 nM, however, induction at this level resulted in only a 1.4-fold increase in light production. It is also important to note that the assay could be inhibited by the presence of alicyclic hydrocarbons, aromatic hydrocarbons, alkylbenzenes, or biphenyls, which limited its use in environmental applications. Despite this handicap, however, the assay was used to monitor diesel-oil–contaminated groundwater samples, but was limited to reporting in octane equivalents inasmuch as it is not specific for individual hydrocarbon species. Similarly, because of the complex nature of diesel oil, a coassay was required to determine the level of inhibition caused by nonalkane chemicals, which then allowed a corrected octane equivalency to be determined. Although this assay format was

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not ideal, it represented the first time that a bioluminescent microbial assay was used to monitor for environmental alkane contamination, and provided a valuable first step toward the development of improved sensor moieties.

Building upon this system, Minak-Bernero et al. [39] were able to take further advantage of the remaining *P. oleovorans alk* operon genes and develop an alkane sensor that did not require the exogenous addition of decanal in order to produce a bioluminescent signal. To accomplish this, they constitutively co-expressed the *alkBFG* alkane catabolism genes, the *alkJ* alcohol dehydrogenase gene, and the *luxAB* genes. Under this system, the *alkBFG* gene products performed their native function of reducing the target alkanes to alcohols, the *alkJ* gene product then converted those alcohols into aldehydes, and the LuxAB luciferase proteins then used the resultant aldehydes as substrates for the generation of a bioluminescent signal. The ultimate result was a bioluminescent microbial bioreporter that could respond to the presence of alkanes within seconds after exposure to produce a detectable signal. This sensor was approximately as sensitive as the decanal-dependent sensor developed previously [37], giving a linear response to octane in the 10- to 200-μM range [39]. Additionally, because the *P. oleovorans alk* gene products were capable of modifying 5- to 12-carbon chain length primary alcohols and aldehydes [40], and the LuxAB luciferase protein could accept 6-carbon and longer chain length aldehydes [41], this sensor was theoretically capable of sensing any alkane between pentane and dodecane. However, because the system constitutively expressed all of the *alk* and *lux* genes, the sensor would report the detection of any bioavailable pathway intermediate products indiscriminately. This made it impossible to differentiate alkanes, alcohols, or aldehydes in a given sample. So although detection had become increasingly autonomous, the specificity of the system was reduced.

In 2010 there was a renewed interest in the detection of alkane hydrocarbons in seawater due to the highly publicized Deepwater Horizon oil spill in the Gulf of Mexico [42]. This spurred renewed testing with the available reporter strains and demonstrated that, although the detection characteristics were similar to those obtained in groundwater samples [37], the reporters could be inhibited by salt during in situ analysis with laboratory-contaminated seawater [14], limiting their use under environmental conditions. To overcome this deficiency, Zhang et al. [43] developed a bioluminescent reporter using the native *alk* operon system in *Acinetobacter baylyi* ADP1 rather than expressing a modified version of the *P. oleovorans alk* operon in *E. coli* as had been done previously. This reporter was constructed using homologous recombination to introduce the *luxCDABE* genes from *Photorhabdus luminescens* in place of the *alkM* alkane hydroxylase gene in the *A. baylyi* ADP1 chromosome, placing them under the control of the naturally alkane-inducible AlkR regulator protein. Although the detection limit was higher than that of *P. oleovorans*-based *E. coli* reporter strains [37], the *A. baylyi* ADP1 reporter strain was capable of detecting alkanes between 7- and 36-carbons in length, giving it a significantly enhanced detection profile [43]. *A. baylyi* ADP1 was also demonstrated to be significantly more tolerant to seawater than *E. coli*, which allowed the reporter to function without any interference from salt contamination. Another advantage of this reporter is that *A. baylyi* ADP1 was shown to adhere to the oil-water interface and emulsify the oil into small droplets by forming a single

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layer of cells around the droplet surface. This effectively presented an increased sample concentration for the reporter to detect, and aided in decreasing response time to 0.5 h. Testing with alternative contaminants such as salicylate and toluene did not show any significant induction of bioluminescent signal [44], suggesting that the *A. baylyi* ADP1 reporter may be more specific than previous *P. oleovorans*-based versions as well. One year later, similar inroads for the detection of longer chain alkanes were made by Kumari et al. [45], who expressed the *luxAB* genes under the control of the *alk* operon from *Alcanivorax borkumensis* SK2 in *E. coli*. However, they ultimately chose to focus on the development of an EGFP (enhanced green fluorescent protein)-based fluorescent reporter strain in place of the *luxAB*-expressing strain that required the addition of decanal to produce a bioluminescent signal.

2.4 Chlorinated Aliphatic Hydrocarbons

Chlorinated aliphatic hydrocarbons have been widely used throughout the chemical industry for decades. Through mishandling and environmental releases over this time frame, they have become widespread in soils and groundwater [46], and because of their widespread deposition and toxic nature, they now pose a significant risk to both human and environmental health [47, 48]. For these reasons, there is an increasing interest in the development of bioreporters that are capable of determining the location and bioavailability of these toxic compounds in order to direct remediation efforts for their disposal.

Classically, bioreporter-based chlorinated aliphatic hydrocarbon detection mechanisms have been established around the *tod* operon. This operon consists of three genes responsible for oxidation of toluene to cis-toluene dihydrodiol under the control of a regulatable promoter that is upregulated in response to increasing toluene concentrations [16]. Applegate et al. [15] were able to leverage this action by replacing the downstream *tod* genes with a complete *luxCDABE* operon to develop a *P. putida* strain (TVA8) capable of responding to challenges with either toluene or the chlorinated aliphatic hydrocarbon trichloroethylene (TCE) by production of a bioluminescent signal [49]. Using this reporter, it was possible to detect TCE at a lower detection limit between 1 and 5 μM and an upper limit of 230 μM, although the results of the analysis could be easily skewed in the presence of contaminating toluene. Despite this detriment, the *P. putida* TVA8 bioreporter was successfully deployed under environmental conditions, where it was able to detect TCE and 1,1,1 trichloroethane (TCA) in contaminated groundwater samples as confirmed by analytical analysis [20], further demonstrating its utility.

More recently, a second *P. putida* operon has been discovered that can be used as an alternative to the traditional *tod*-based approach. This operon, the *sep* operon, consists of three efflux pump-encoding genes that are regulated in response to a variety of common chemical solvents [50]. When the *luxCDABE* operon was cloned in place of the upstream *sepA* gene, the result was a strain that modulated bioluminescent activity in response to TCE availability. This strain was still susceptible to interference by the same contaminant chemicals as the TVA8 strain [15], however, it did present investigators with another tool for the realtime detection and monitoring of a wide range of halogenated solvents and chlorinated aliphatic hydrocarbons.

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Since the time the *tod* and *sep*-based reporter systems were first developed, a more specific reporter has emerged that can sense and respond to the presence of the chlorinated aliphatic hydrocarbon dichloromethane (DCM). The selectivity of this reporter is due to its utilization of the *dcm* operon from *Methylobacterium extorquens* DM4, which is able to grow on DCM as a sole carbon source. The *dcm* operon consists of the genes *dcmAR*, with the *dcmA* gene upregulated in the presence of DCM and the *dcmR* gene encoding a contrasting negative regulatory element. By cloning the *luxCDABE* genes under control of the *dcm* promoter, it was possible to elicit a bioluminescent response from aerosolized DCM at a range between 12 μM and 1.2 mM. Induction of the bioluminescent signal could be observed at 1 h posttreatment at the 1.2-mM level, but increased to 2.3 h at the 12-μM level. In the liquid phase, the reporter could detect DCM between a range of 1.2 μM–12 mM, and the induction time was relatively decreased compared to aerosolized samples, requiring only 0.5 h at the 12-mM concentration. Regardless of the medium used (aerosol or liquid) there was a correlation between bioluminescent output and DCM concentration at an R^2 value of 0.99 [51]. In contrast to the nonspecific reaction of *tod*- and *sep*-based systems, this level of specificity and dose–response kinetics highlights what can be achieved by modulating the selectivity of the upstream regulatory element that is used for bioreporter generation.

2.5 Biphenyl and Polychlorinated Biphenyls

PCBs represent some of the most widely distributed and persistent environmental contaminants due to their resistance to physical, chemical, and biological degradation. It is because of this exceptional stability that PCBs found extensive use as dielectric and coolant fluids in transformers, capacitors, and electric motors. Evidence of their consequent discharge can be found in nearly all environmental ecosystems, including water, sediments, soils, and air, and their tendency to bioaccumulate in living organisms magnifies their presence throughout the food chain as well. Microbiologically, there are a handful of known bacterial species that can utilize biphenyl as a sole source of carbon and energy, predominantly via oxidative degradation mediated by the biphenyl gene cluster (*bph*) [52]. Layton et al. [53] were the first to exploit the *bph* pathway for PCB bioluminescent biosensing by linking the *bph* R1 regulatory region to a plasmid-localized *luxCDABE* gene cassette that was inserted into *Ralstonia eutropha* to create the bioreporter ENV307(pUTK60). Validation was performed against biphenyl, 2-, 3-, and 4-chlorobiphenyl, and an Aroclor PCB mixture. However, due to the poor aqueous solubility of PCBs, a surfactant was added to the test samples to promote increased bioavailability. Minimum detection limits ranged from 0.80 μM for 4-chlorobiphenyl to 4.6 μM for Aroclor in a 96-well microtiter plate assay over a 6-h incubation period. The need to add surfactants to PCB samples then drove this group to create an improved toxicity-based bioassay because, in the standard Microtox test, the toxicity of the surfactants toward the *A. fischeri* reporters interferes with the toxicity profile of the PCB compounds [54]. To circumvent this limitation, Layton et al. [55] developed their toxicity assay using indigenous wastewater microorganisms displaying surfactant resistance (*Stenotrophomonas* sp. and *Alcaligenes eutrophus*) that were engineered to bioluminesce constitutively via plasmid insertion of a *luxCDABE* gene cassette. Results showed these two strains to be 400 times more resistant than *A. fischeri* to the commonly used surfactant polyoxyethylene 10 lauryl ether, signifying

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their potential practicality in PCB and other compound toxicity bioassays that require the addition of surfactants.

Pseudomonas sp. DJ-12 expresses a *meta*-cleavage dioxygenase via the *pcbABCD* operon that enables degradation of select biphenyl compounds. Park et al. [56] created plasmid-based gene fusions between the *pcbC* promoter and *luxCDABE* and *luc* to create two bioluminescent bioreporters in *E. coli* host cells. Bioassays performed in 96-well microtiter plates over 30-min exposure periods indicated responsiveness to biphenyl compounds between the 0.1 and 1-mM exposure concentrations analyzed. Testing at lower concentrations to establish true detection limits still needs to be performed, as well as compound specificity studies, but these bioreporters demonstrate a potential addition to the inventory of biphenyl-responsive bioreporters.

In bacteria and higher organisms, PCBs are biotransformed by cytochrome P-450 monooxygenases and metabolized to hydroxylated PCBs (OH-PCBs). Certain bacteria are able to use hydroxybiphenyls as sole carbon and energy sources via mediation of the *hbp* gene cluster under regulatory control of the *hbpR* gene [57]. Recognizing this, Turner et al. [58] linked the *hbpR* gene from *Pseudomonas azelaica* to the *luxAB* genes on a plasmid-based (pHYBP109) system that was inserted into *E. coli* to create a bioluminescent reporter for OH-PCBs. The bioreporter was tested against 27 OH-PCBs with dose-dependent responses successfully obtained with limits of detection in the range from 10^{-5} to 10^{-9} M in 4-h incubation assays followed by the addition of the n-decanal substrate. The bioreporter was also used by Tecon et al. [14] in a *luxAB*-based multibioreporter assay to monitor for oil spill constituents in aquatic ecosystems, subsequently allowing for the simultaneous detection of biphenyls, short-chain linear alkanes, and monoaromatic and polyaromatic compounds within a 3-h assay. Validation of the bioreporter was also applied diagnostically in human serum samples spiked with an individual OH-PCB (2-hydroxy-3′,4′-dichlorobiphenyl) as well as a mixture of 10 OH-PCBs, with demonstrated detection limits as low as 5×10^{-8} M within a 4-h bioassay time frame [58].

With a goal of expanding the bioreporter's chemical detection portfolio, Tropel et al. [59] then subjected this *hbpR* promoter/operator region to site-directed mutagenesis to modify its recognition specificity. Using this approach, they successfully created an *hbpR-luxAB P. azelaica* bioreporter that was capable of responding to both *m*-xylene and 2-hydroxybiphenyl. This ability to combine different regulatory pathways within a single bioreporter to enable biosensing across different chemical classes facilitates simplified multitargeted bioassays for expanded environmental monitoring.

2.6 Phenol and Derivatives

Phenols and their derivatives serve as some of the most common environmental pollutants in soil, water, and air. Deposition occurs through both natural events (i.e., decomposition of organic material, forest fires, and atmospheric degradation of benzene) and industrial activities where it is produced in massively high volumes (approximately 7 billion kg per year) as an important precursor component of plastics, epoxies, explosives, detergents, herbicides, and pharmaceutical drugs. Under such large-scale manufacturing demands, environmental impacts (especially in relation to wastewater discharges) are well recognized,

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with 12 phenolic compounds registered on the US Environmental Protection Agency's list of priority pollutants [phenol, 2-chlorophenol, 2,4-dichlorophenol, 2,4-dimethylphenol, 2,4-dinitrophenol, 4-nitrophenol, 2-nitrophenol, pentachlorophenol, 2,3,4,6-tetrachlorophenol, 2,4,6-trichlorophenol, 4-chloro-3-methylphenol (synonym 4-chlor-m-cresol), and 2-methyl-4,6-dinitrophenol (synonym 4,6-dinitro-o-cresol)]. Accordingly, microorganisms have evolved to utilize these phenols, which provides an inroad for the development of bioreporter assays based on their genetic pathways to detect and monitor these phenolic compounds [60]. Over two decades ago, Shingler et al. [61] isolated a *Pseudomonas* strain, CF600, capable of using specific phenols and derivatives as sole sources of carbon. Elucidation of its genetic pathway for doing so described the now well-understood *dmp* operon [62] that later became the platform for the *luxCDABE*-based bioluminescent bioreporter *P. fluorescens* OS8(pDNdmpRlux) [63]. Reporter OS8(pDNdmpRlux) demonstrated a response portfolio to a variety of phenols, including 2-, 3-, and 4-methylphenol (synonyms *o*-, *m*-, and *p*-cresol), 2,3-, 2,4-, 3,4-, and 2,6-dimethylphenol, resorcinol, and 5-methylresorcinol, with maximum detection limits achieved under 2-methylphenol (0.30 μM) and phenol (0.87 μM) exposures of a 4-h duration. As is customary with these types of bioreporters, specific phenols cannot be individually identified and the bioluminescent signal rather represents the total phenolic content in its bioavailable form. When applied to natural, mixed contaminant groundwater and semicoke dump leachates containing primarily phenol and methylated phenols, the bioreporter successfully bioindicated phenol bioavailability in nine of the ten samples analyzed, with this single negative sample hypothesized to contain phenolic constituents in a non bioavailable form [63]. Building upon these results, Wise and Kuske [64] devised a second generation bioreporter using mutated versions of the regulatory DmpR region of the *dmp* operon to increase the range of phenolics detected. This new reporter construct extended the range of detectable compounds to include 2-chlorophenol, 2, 4-dichlorophenol, 4-chloro-3-methylphenol, and 2- and 4-nitrophenol. Similarly, Gupta et al. [65] performed a more defined DmpR mutation approach and linked gene expression to firefly luciferase within an *E. coli* host cell to create a bioluminescent bioreporter (pRLuc42R) capable of detecting phenol at a lower limit of 0.50 μM within a 3-h assay time frame, however, its ability to detect phenolic compounds in realworld samples has yet to be tested.

Microbes belonging to the genus *Acinetobacter* also utilize phenol as a sole carbon source, and have thus also been transformed into bioluminescent bioreporters. Their genetic architecture consists of a *mop* operon wherein the MopR regulator activates phenol hydroxylase expression upon binding with phenolic compounds such as phenol, 3-chlorophenol, and 2- and 3-methylphenol [66]. Abd-El-Haleem et al. [67] developed the *Acinetobacter* bioreporter DF4-8 via linkage of this regulatory activity to the *luxCDABE* gene cassette to create a bioluminescent bioreporter capable of detecting phenol at a limit of detection of 0.03 mM within an approximate 4-h assay. When exposed to slurries of aged soils obtained from a phenol-contaminated industrial site, the bioreporter elicited a bioluminescent signal within approximately 6 h. This group then further developed a *luxCDABE*-based constitutively bioluminescent *Acinetobacter* bioreporter (DF4/PUTK2) for chemical toxicity assessment and showed its decreasing levels of bioluminescence in response to several phenolic compounds ranging from 50 to 500 ppm (EC_{50} = 333 ppm)

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[68]. They also immobilized the bioreporters in calcium alginate and demonstrated an 8-week storage capacity at 4 °C in a 96-well microtiter plate format, suggesting application toward a prepackaged, off-the-shelf sensor platform. A larger panel of bioreporters for monitoring phenol-related toxicity was developed by Wiles et al. [69] using four wastewater *Pseudomonas* isolates engineered to carry a chromosomally integrated *luxCDABE* cassette. When exposed to natural wastewater effluent samples in a 96-well microtiter plate format under a 5-min incubation period, this panel effectively bioindicated phenolic concentration shifts in concentrations ranging from approximately 10 to 800 ppm (EC_{50} = 454–757 ppm). In both this study and the previously described *Acinetobacter* reporter study, toxicity profiles were compared against the standard Microtox assay, where *A. fischeri* is used as the sensor microorganism [70–72], and in both cases Microtox performed less reliably. The advantage of using indigenous Pseudomonads or *Acinetobacter* strains in these studies is their natural robustness to the wastewater environment undergoing testing, whereas *A. fischeri*, being native to the marine environment, is less ecologically adapted and oftentimes responds less efficiently or requires additional sample preparation steps to adjust its performance efficiency [73].

3 Evaluation of Organic Toxicant-Induced Health Risks Using Eukaryotic Cell-Based Bioluminescent Assays

In addition to bacteria, eukaryotic cells, including the lower eukaryotic organism *Saccharomyces cerevisiae*, and cultured mammalian cell lines have been increasingly employed to serve as hosts for bioluminescent reporter assays against organic toxicants. However, unlike in bacterial bioassays where catabolic pathways for the biodegradation of target substances are exploited for reporter development, eukaryotic cells generally are not able to utilize such toxic organic compounds as carbon sources or lack exhaustively characterized catabolic pathways altogether. These deficiencies have been overcome as research on cell biology and toxicology have revealed that many essential receptor-mediated signaling transduction pathways for normal cellular functions can be disrupted by the binding of exogenous organic toxicants to endogenous receptors [74], which thus allows reporter development using similar techniques. Under these strategies, the characterization of key receptors and transcriptional response elements in these pathways forms the foundation for eukaryotic-cell–based bioluminescent bioreporters, which are commonly created by fusing bioluminescent reporter genes to the response element of a target pathway so that bioluminescent expression is transcriptionally modulated through chemical-receptor binding. This design requires coexpression of cognate receptors that are either endogenously present in some cell lines or, in the case of nonexpressing cell lines and *S. cerevisiae*, can be co-introduced with the reporter construct. This means that, because reporter gene expression is integrated into endogenous toxicity pathways, eukaryotic whole-cell bioreporters essentially measure chemical-triggered biological effects, making them ideal tools for providing pathway-specific risk assessment information. This is particularly important during environmental contaminant evaluation where multiple pollutants with a range of toxicological effects often coexist throughout a contaminated site. Because it is common for structurally different chemicals to exert similar toxic effects or for the same compound to induce multiple pathways (i.e., some PCBs have been shown to have both dioxin-like and

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estrogenic activity [75, 76]), eukaryotic cell-based bioassays can therefore serve as valuable tools for the estimation of potential harmful effects that may not be detectable using traditional analytical means.

Currently, the major use of these eukaryotic cell-based reporters has been for the detection of organic contaminants responsible for dioxin-like (Table 2) or endocrine-disrupting activities (Table 3). This section summarizes a variety of bioluminescent-based bioassays that have been developed for the detection of these two activities. Due to their extensive use in the literature, it is impractical and unnecessary to list all of the applications, however, a focus is drawn on highlighting the predominant application areas with recent examples to provide an overview of the usefulness and limitations of these unique reporter systems.

3.1 Bioassays for Dioxin and Dioxin-Like Compounds

In their most basic form, dioxins are any compound containing a heterocyclic 6-membered ring consisting of 2 oxygen atoms and 4 alternative atoms. Practically, dioxins are persistent pollutants that can bioaccumulate over time, leading to increased health risks for organisms of higher trophic levels, with the classic example being 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD). TCDD is the most toxic of the known dioxins, and is thus used as a representative model, with the relative toxicity of other chemicals expressed in toxic equivalency factors [77]. Collectively, chemicals that inflict toxic effects similar to TCDD are classified as dioxin-like, and can lead to hepatotoxicity, embryotoxicity, teratogenicity, immunotoxicity, dermal toxicity, carcinogenesis, or lethality [78, 79]. Dioxin-like activities have been found in various groups of organic compounds, including polychlorinated dibenzo-*p*-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs), some PCBs, and PAHs.

The mechanism of action for dioxin-regulated gene expression begins when the chemical binds to the aryl hydrocarbon receptor (AhR) in the cytosol. This chemical–AhR complex then translocates into the nucleus and forms a dimer with an AhR nuclear translocator (ARNT) protein. The dioxin:AhR:ARNT complex then binds to specific DNA sequences called dioxin-responsive elements (DREs), which results in the activation of the adjacent responsive gene(s) [80]. Because the resulting increase in gene expression is directly proportional to the toxicity of the binding chemical [81], this process provides an excellent platform for the development of regulatable bioluminescent bioreporter-based dioxin detection strategies.

The first dioxin-responsive bioluminescent reporter to be developed was used by Postlind et al. [82] to track expression of the *CYP1A1* and *CYP1A2* genes of the human cytochrome P450 gene family. This was accomplished by cloning the 5′ flanking region from either the *CYP1A1* or *CYP1A2* genes upstream of a *luc* gene in a human expression vector. These vectors were then introduced to a human hepatoma (HepG2) cell line and challenged with TCDD and other compounds. Under transient transfection conditions where cells were exposed to TCDD for 24 h the day after plasmid introduction, reporters expressing the *CYP1A2* 5′ flanking sequences displayed a detection minimum of 0.01 nM TCDD, whereas those expressing *CYP1A1* 5′ sequences were detectable down to a concentration of 0.001 nM. Each was capable of responding to TCDD treatment in a dose–response fashion, however, although the signal maximum for the *CYP1A2*-based reporter was 10-fold over

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control upon treatment with 10-nM TCDD, the *CYP1A1*-based reporter reached its signal maximum at 65-fold over control upon 100-nM treatment. This gave the *CYP1A1*-based reporter both a larger range of detection, as well as a greater signal intensity throughout that range. Commensurate with its lackluster performance compared to *CYP1A1*, the *CYP1A2*-based reporter construct was not able to function at all under stable expression conditions. Whereas the CYP1A1-based reporter was capable of stable expression and could detect 10-nM TCDD at 0.5 h postexposure, with increasing reporter activity positively correlating with exposure time up to 24 h. The EC_{50} for TCDD detection by this reporter was determined to be 0.35 nM, making it the first functionally useful dioxin-detecting human cell line.

Building upon this expression strategy, Garrison et al. [80] used a construct consisting of a mouse mammary tumor virus promoter mediated *luc* gene under the control of a 484 base pair 5′ upstream mouse *Cyp1a1* gene sequence that contained 4 DREs to generate a range of bioluminescent dioxin-responsive bioreporter cell lines. When treated with 1 nM TCDD for 24 h the day after plasmid introduction, all of these lines were capable of responding to TCDD challenge with a corresponding bioluminescent output. This demonstrated that the mouse DREs could be successfully recognized by the dioxin:AhR:ARNT complex across a wide range of species, and therefore that the assay could be performed in specific cell types to determine species-specific bioavailability of dioxins and dioxin-like compounds. Bolstered by these findings, a second set of stably transfected dioxin-responsive bioluminescent bioreporter cell lines were developed. Of these cell lines, the mouse H1L1.1c2 line (Hepa1-derived) was chosen as a model for characterization because it had the greatest level of induction and was found to respond reliably to TCDD treatment in a dose–response fashion (although this dose–response relationship was also reported for the human HepG2-derived HG2L1.1c3 cell line, no data were presented to support this claim at the time). The model H1L1.1c2 reporter cell line displayed a minimal detection limit between 0.1 and 1.0 pM of TCDD, with a maximal induction of 80-fold over control at 1.0 nM, providing an ED_{50} of 0.02 nM. This is less than the previous value of 0.35 nM established by Postlind et al. [82] with their human HepG2 cell line, and represented a lower maximal detection limit (1.0 vs. 100 nM) as well. Also unlike the Postlind study, Garrison et al. [80] were not able to detect a bioluminescent signal until 2 h posttreatment, and reached a maximum induction at 4 h posttreatment following application of 1.0-nM TCDD. However, despite being mostly in line with the Postlind study, the Garrison study remains notable for its demonstration of bio-reporter function across a wide variety of cell lines, and for demonstrating that the inducing chemicals did not act as competitive substrates for the luciferase enzyme, providing a significant advantage for the bioluminescent assay over older, more traditional chemical-based assays.

Following up these successful demonstrations against specific chemicals in laboratory settings, Murk et al. [83] transported the system to a rat hepatoma H4IIE cell line and rebranded its use as a chemical-activated luciferase expression (CALUX) assay. Using this new cell line they were able to demonstrate TCDD detection down to 0.5 fM, with a maximum detection limit between 100 pM and 1.0 nM, and an EC_{50} of 10 pM. Similar to the earlier experiments [80, 82], they were also able to demonstrate a dose-response

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relationship between TCDD and bioluminescent signal, and did not discover any significant substrate inhibition.

What solidified the CALUX assay as the predominant method for dioxin-like compound detection, however, was the successful demonstration of its use with environmental samples and its ability to mimic the results of existing, more complex and more costly in vivo assay results [83]. Murk and colleagues demonstrated that the *luc*-expressing H4IIE reporter cells could be exposed to extracted sediment and water samples to determine toxic equivalency factors rapidly and inexpensively. Although it was ultimately determined that unpurified sediment samples could become toxic to the H4IIE-*luc* cells, it is primarily highlighted that purification was not required for water samples, which significantly improved the speed at which they could be assayed. Perhaps more important, however, was the validation of the CALUX assay against the in vitro zebrafish early life stage assay. Although the presence of heavy metals led to a poor correlation between the two assays when performed directly in unprocessed environmental water samples, this correlation significantly improved following sample extraction. When compared side by side under laboratory controlled conditions, the EC_{50} as determined by the zebrafish early life stage assay was found to be 21 pM, whereas the EC_{50} of the CALUX assay was 10 pM. Moreover, the working range for the CALUX assay was improved compared to the zebrafish early life stage assay, and reduced both the time and cost involved in its performance.

Through its low cost, lack of substrate inhibition, ability to be adapted for high-throughput, and ability to function in disparate cellular backgrounds, the CALUX assay has become the predominant method for assaying dioxin and dioxin-like chemicals using mammalian cells. Since its early demonstrations as a tool for laboratory-based chemical toxicity testing and environmental pollutant screening, it has been used for a wide variety of applications including veterinary [84], food product testing [85–88], and human clinical sample analysis [89, 90]. And as the applications for the CALUX assay have expanded, so have the organisms that have been employed for its use. Although still predominantly performed in mammalian cell lines, recently the assay has been reconstituted in yeast by co-expressing the genes for AhR and ARNT with a DRE-mediated *luc* luciferase. This switch away from human cells and into the microbial eukaryote *S. cerevisiae* was done with the hope that it would provide a more robust and simplified expression system that could improve deployability and reduce costs. It was found that the yeast-based system could respond to TCDD treatment in a dose–response fashion, with a minimum detection time of 3.5 h and an EC_{50} value similar to that of the early human cellular reporters at 4.8 nM. Inasmuch as this assay was validated against the classical H4IIE-*luc* cell line-based CALUX assay and found to be in good agreement [91], it has since been used as a first stage in vivo screen for dioxin-like chemical load detection in composted sewage sludge [91], proving its worth as an alternative means of detection when human cell lines cannot be used.

As a final note, with the use of CALUX assays continuing to proliferate, it is important to recognize that the results of the assay can vary from lab to lab, and even from run to run [92]. In a validation study, Besselink et al. [93] found 14.6 and 26.1 % intralaboratory assay reliability levels for pure compounds and whole matrix, respectively, and 6.5 and 27.9 % interlaboratory assay reliability levels for pure compounds and whole matrix, respectively.

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Therefore, in light of these discrepancies, it is important to review the sample cleanup methods, the effects of the solvents used during extraction and testing, any known interaction with synergistic or antagonistic compounds used, the cell line utilized, and the analysis methods employed before comparing results between multiple sources in order to determine the validity of the comparison [92]. Despite these caveats, however, the CALUX assay remains the predominant method for bioluminescent screening of compounds eliciting dioxin-like activities.

3.2 Bioassays for Hormonally Active Chemicals

The vertebrate endocrine system consists of a group of signaling molecules collectively called hormones, which act through binding to their corresponding nuclear receptors in order to transcriptionally modulate expression of genes involved in different stages of an animal lifecycle including development, growth, and reproduction. Due to the critical roles of hormones, any interference with the endocrine system may lead to various adverse health effects in humans and wildlife [74], and a variety of organic compounds has been discovered that are considered as endocrine disruptors because of their ability to mimic or repress the function of these natural hormones in vivo [94, 95]. The presence of these chemicals in plastics, pesticides, herbicides, household products, cosmetics, and pharmaceuticals, and their wide use and discharge into the environment through anthropogenic activity and industrial waste has made the detection of endocrine disruptive activity an increasingly large concern.

The major mechanism of action for endocrine disrupting chemicals (EDCs) is to modulate the transcriptional activity of hormone receptors, which includes (but is not limited to) estrogen receptors (ER—with two isoforms ERα and ERβ), androgen receptors (AR), glucocorticoid receptors (GR), progesterone receptors (PR), and thyroid receptors (TR). Because of this mechanism of action, bioluminescent bioreporters for EDCs are constructed similar to the dioxin bioreporters by conditionally expressing a reporter gene (either *luc* or *luxCDABE*) under the control of the corresponding response element. Therefore, detection of organic compounds using these bioassays is activity—rather than structure-oriented. Cell-based reporter gene assays for EDCs including bioluminescent bioassays are routinely reviewed in the literature (for recent examples see [96, 97]) and are summarized here in Table 3.

Depending on the source of the target receptors, these bioreporters can be divided into two types, those that exploit endogenous receptors and those that require manual receptor cointroduction. For those that exploit endogenous receptors, the ER-positive human breast cancer cell lines MCF-7 and T-47D are the model platforms for bioassays of estrogenic and antiestrogenic activities. The first stable bioluminescent bioreporter for ER agonists and antagonists in mammalian cells was the MCF-7-derived MVLN reporter cell line developed by Pons et al. [98] and subsequently shown to be responsive to both estrogenic and antiestrogenic substances [99]. In these reporter cells, *luc* gene expression was placed under the control of the estrogen response element (ERE) derived from the 5′ flanking region of the *Xenopus* Vitellogenin A2 gene. Due to the limited techniques available at that time, the original tests with these cells were performed in a now rarely used 6-well plate format.

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Nevertheless, under these conditions treatment with the natural estrogen 17β-estradiol (E2) exhibited an EC_{50} of ∼20 pM after a 24-h exposure period. However, as technology has improved, this assay has been modernized, and is now commonly carried out in a more standard 96-well plate format and with a 48-h exposure [100, 101]. Concurrent with this modernization has been the introduction of additional bioreporter cell lines that function in a similar manner. For instance, building upon the work of Pons et al. [98], Balaguer et al. [102] later developed a similar MCF-7-derived MELN reporter cell line, in which *luc* expression was linked to an ERE and a β-Globin promoter. After 16 h of incubation, the MELN cell line was able to detect E2 concentrations as low as ∼1 pM with an EC_{50} value of ∼5 pM when performed in a 24-well plate. Other chemicals including a nonylphenol mixture, 4n-nonylphenol, 2,4′-dichlorodiphenyldichloroethylene (2,4′-DDE), and 4,4′-DDE (both of which are DDT breakdown products) also tested positive for estrogenic activity under the same assay conditions. The reproducibility and lab-to-lab variation of the MELN assay against a battery of compounds with known estrogenic or antiestrogenic activity was recently tested in a higher throughput 96-well plate format [103], and this study revealed that, although the individual laboratories maintained mean intralaboratory coefficients of variations of either 32.1 or 56.8 % for their EC_{50} values, both labs produced similar rankings of estrogenic or antiestrogenic potency of most of the chemicals, highlighting the utility of this assay for comparative applications.

Moving away from the MCF-7 cell line, Legler et al. [104] developed a T-47D cell-based ER-CALUX reporter cell line for detection of ER agonists and antagonists. Using a minimal TATA box promoter and three tandem repeats of ERE, this cell line (T-47D ER-CALUX) displayed very low background bioluminescence in solvent controls and a maximum induction of approximately 100- and 76-fold compared to unexposed background following a 24-h exposure to 30 pM E2 in 24- and 96-well plate formats, respectively. This bioassay was also capable of detecting E2 down to ∼0.5 pM and is the most sensitive among reported estrogen-specific assays. A similar T-47D-KBluc reporter developed by Wilson et al. [105] has a comparable EC_{50} value for E2 (10 pM in KBluc vs. 6 pM in ERE*tata*-luc) but possesses a larger dynamic detection range from 1 pM to 100 nM. However, despite these similarities, it is worth noting that controversial results were obtained in a follow-up study comparing the in vitro T-47D ER-CALUX assay with an in vivo transgenic zebrafish assay expressing the same reporter construct [106]. Despite the synthetic estrogen 17α-ethynylestradiol (EE2) testing 100 times more potent than E2 in the transgenic zebrafish assay, it showed equal estrogen agonistic activity compared to E2 in the cell-based in vitro assay. One possible explanation for these differences is that the binding affinities of test compounds may be different when interacting with ERs that have originated from different species, which would explain the poor performance of human cell-based assays to predict toxicokinetics in zebrafish models.

Under similar development strategies, bioluminescent bioreporters screening for AR agonists and antagonists have been generated as well. These reporters take advantage of AR-positive cell lines, such as T-47D and the human breast cancer cell line MDA-MB-453 in order to supply the receptors needed for successful activation of their chosen bioluminescent expression systems. Blankvoort et al. [107] were the first to develop a stable T-47D AR-

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LUX cell line for androgenic and antiandrogenic effects by using a rat probasin promoter-derived ARE-mediated *luc* reporter construct. This reporter cell line was shown to be capable of detecting methyltrienolone down to 46 pM after 24 h of exposure and detecting the environmentally relevant antiandrogenic compounds 4,4′-DDE and (RS)-3-(3,5-dichlorophenyl)-5-methyl-5-vinyloxazolidine-2,4-dione (vinclozoline) as well. However, because of the coexpression of other hormone receptors (such as ERα, ERβ, and PR) in the T-47D cellular background, there remains a possibility that nonspecific responses may have been detected. To overcome this issue, Wilson et al. [108] developed a bioassay for chemicals mimicking/blocking androgen and glucocorticoid activities utilizing the MDA-MB-453 cell line, which expressed high levels of AR and GR but showed undetectable or very low levels of alternative receptors [109, 110]. The resulting reporter cell line, named MDA-kb2, expressed the *luc* gene under the regulation of an AR- and GR-responsive mouse mammary tumor virus (MMTV) promoter and, because of the characteristic low-level expression of competing hormone receptors, provided a significant decrease in background activity that led to an increased signal-to-noise detection ratio.

This decrease in alternative receptor expression resulting from the use of the MDA-kb2 cell line helped to reduce cross-talk between different pathways [111], but was not the only means for accomplishing this goal. With the hope of providing a more receptor-specific bioassay, several cell lines with little to undetectable levels of nontarget receptors have been utilized through the introduction of specific receptors that are not natively expressed. Examples of this approach include the use of human cervical cancer cell line HeLa and osteosarcoma cell line U2-OS as parental cells for reporter development. In addition to the MELN reporter cell line, the HELNα and HELNβ bioreporters were developed by Balaguer et al. [102] by cointroducing ERα and ERβ, respectively. Although the E2-generated bioluminescent response of these cell lines is similar to that observed in MELN assays, it should be noted that TCDD can elicit an antiestrogenic response in HeLa cell-based assays compared to its demonstrated estrogenic activity in MELN assays. This differential behavior highlights an example of the effect of pathway cross-talk (in this case between the AhR- and ER-mediated pathways), which must always be accounted for during data interpretation. To reduce the prevalence of this cross-talk, a panel of CALUX bioassays has subsequently been developed for selective detection of chemicals interacting with ERα, ERβ, AR, GR, and PR using the U2-OS cell line, which demonstrates little or no natural activity of any of these receptors [112–114]. These reporter cell lines were generated by cointroducing a vector that conferred constitutive receptor expression, and a second vector that permitted target receptor-mediated expression of the *luc* gene. Using this approach, the ERα- and AR-CALUX bioassays were also shown to be well correlated with other animal-based assays (R^2 value of 0.46 and 0.87 for AR and ERα assays, respectively), making them useful tools for predicting potential in vivo activities with a reduced chance of cross-talk–based interference [115].

For similar reasons to those listed above, as the techniques for genetic expression continue to improve, there has been an increased interest in using the lower eukaryotic organism *S. cerevisiae* as a platform for hormonally active chemical screening. Due to their lack of human hormone receptor expression, fast and robust growth, and relatively simplified

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genetic manipulation techniques, yeast-based bioreporters can now be constructed using a stepwise transformation of a recombinant human hormone receptor (e.g., hERα, hERβ, and hAR) of interest and a receptor-responsive reporter gene. Several *luc*-based yeast bioreporters have been developed for the rapid profiling of estrogenic and androgenic potentials, and have demonstrated a lower detection limit for E2 and dihydrotestosterone of 30 and 50 pM, respectively [116, 117]. Despite this reduced sensitivity compared to mammalian cell-line–based assays, the yeast-based assays were capable of reporting relative potency of test chemicals more rapidly, with only a 2.5-h incubation time.

In particular, two yeast bioassays, BLYES [118] and BLYAS [119], which have been developed to detect estrogenic and androgenic activity, respectively, stand out from the other bioreporters mentioned above with respect to their choice of bioluminescent reporter genes. Instead of using the *luc* reporter gene, each of these utilizes a bioluminescent end point resulting from expression of the *luxCDABE* genes. The switch to the *lux* system eliminates the need for exogenous luciferin addition and/or cell lysis and permits autonomous bioluminescent signal generation and detection. This allows the assay to proceed more rapidly, and with near realtime signaling. Bioluminescent signal detection can occur as early as 1 h after exposure to 2.8 nM E2 in the BLYES assay [118], which allowed the BLYES and BLYAS assays to be used to evaluate the toxicity and potential endocrine-disrupting activities of a battery of 68 chemicals quickly and efficiently in a cost-effective manner [120].

Compared to bioreporters relying on endogenous receptor-mediated signaling, the test responses generated using recombinant yeast bioreporters and mammalian bioreporters with manually introduced receptors are less likely to be subject to nonspecific interactions, which may provide improved mechanistic insight. However, it still remains to be seen if an enhanced prediction of in vivo effects might be achieved using bioassays without exogenous manipulation of the signaling receptors. This creates a potential tradeoff that will need to be evaluated on a case-by-case basis.

3.3 Environmental Applications

The discharge of chemicals through anthropogenic activity and industrial waste in the environment urges a careful assessment of ecologically relevant compounds for their potential toxic effects. One particular phenolic compound of recent emerging importance is bisphenol-A (BPA). BPA is used extensively in the production of polycarbonate plastics and is widely and controversially implicated in causing negative health effects due to its biological action as an endocrine disruptor [121]. Its presence in drinking and wastewaters has become particularly relevant, along with other mid- to long-chain alkylphenols and alkylphenol ethoxylates that have been suggested to exhibit similar properties. For these reasons, a number of bioluminescent reporter systems using both mammalian cell lines and yeast have recently been employed to characterize its endocrine-disrupting activity. Michelini et al. [117] used a yeast-based hAR/ARE-*luc* bioassay to demonstrate the antiandrogenic potency of BPA with a half maximal inhibitory concentration (IC_{50}) of 5 μM against 10 nM testosterone. BPA's estrogenic potential was later supported by a number of different estrogen assays, yielding EC_{50} values of 2.8 and 0.8 μM using the BLYES [118]

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and T47D ER-CALUX [104] assays, respectively. In addition, several widely used industrial compounds, such as polyfluorinated iodine alkanes, nonylphenol isomers, and phthalates have also been evaluated by the MVLN and H4IIE-*luc* reporter assay for their estrogenic and dioxin-like potentials [101, 122–124]. These uses, although by no means exhaustive, highlight the utility of these eukaryotic cell-based bioluminescent bioassays to offer a high-throughput and relatively inexpensive route for profiling the potential toxicities of the ever-expanding number of chemicals that are routinely being used and released to the environment, providing valuable preliminary data for assessing the deleterious effects of their exposure.

The continuing release of organic compounds through urban and industrial wastewater has also raised concerns regarding the efficiency of its associated treatment processes, as any residual toxic chemical present in the treated effluent is directly discharged into surface water and can thus affect downstream aquatic ecosystems, and in some cases, drinking water supplies. Of particular interest has been the generation of disinfection by-products from oxidative chemical treatment and their potential as endocrine disruptors. To help elucidate this issue, several bioluminescent bioassays have been applied to determine the endocrine-disrupting potencies of industrial wastewater before and after ozonation treatment. For example, Schiliro et al. [125] utilized the MELN assay along with another non-bioluminescent cell-based proliferation assay to measure the E2 equivalents of pre-and postozone treated wastewater from a textile industrial wastewater treatment plant. The MELN bioluminescent bioassay estimated an average of 15.34 (±13.00) pM and 9.29 (±9.10) pM E2 equivalents in pre- and post-ozonation samples, respectively. Although they did note some discrepancy between the measured E2 equivalent values between the MELN assay and the proliferation assay (8.62 (±6.16) pM preozonation and 2.64 (±2.13) pM postozonation), they pointed out that both assays identified a comparable degree of reduction in estrogenic potentials as a result of the ozonation process. Furthermore, with respect to the possible generation of more toxic by-products during ozonation of naphthenic acid, a primary organic constituent of the wastewater produced during the hot water extraction of bitumen from oil sands in surface mining operations, He et al. [126] compared the endocrine-disrupting activity between untreated and ozone-treated oil sands process-affected water. This study demonstrated that antiandrogenic activity was reduced in ozone-treated water compared to untreated water through the use of a MDA-kb2 bioreporter assay. However, unlike in the study of Schiliro et al., and in disagreement with the general idea that ozone disinfection is an effective means to reduce estrogenicity [127], it was reported that the estrogenic potential of the oil sands wastewater was not affected by ozonation when evaluated with the T47D-KBluc bioreporter assay. It is therefore worth noting that wastewater treatment efficiency varies on a case-by-case basis, and that bioluminescent bioassays should therefore be used as a rapid preliminary screening method before applying comprehensive chemical analyses.

Last but not least, to help better understand the potential risks of chemical exposure, eukaryotic cell-based bioluminescent bioassays are increasingly being utilized in combination with chemical analysis to survey ecosystems affected by the discharge of toxic chemicals. Traditionally the pollutant composition would be fully characterized

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instrumentally, however, bioluminescent bioassays now provide a rapid and cost-effective means of simultaneously assessing potential biological effects as well. In addition, with an increased understanding that additive and possible synergistic effects of complex mixtures could contribute to the overall environmental impact [128], there is an understanding that chemical analysis alone may not generate sufficient information for risk assessment. Similarly, unidentified hormonal activity may be overlooked by instrumental analysis, which only looks for known targets. This was demonstrated by Fenet et al. [129] when they linked the concentrations of alkylphenols quantified by GC/MS with their contribution toward the total estrogenic activity in environmental samples using the MELN reporter assay. This study recognized varying degrees of correlation between chemically determined concentrations and total estrogenicity on a sample-by-sample basis, demonstrating that, although alkylphenols of GC/MS-determined concentrations could explain a large part of the estrogenic potency in the studied sediment samples, their abundance only provided little to a very low contribution toward the overall observed estrogenic effects in the water samples. These findings have since been repeated, suggesting the presence of other unintended or unknown estrogenic contaminants in studied sites [100]. However, despite their utility to assess biological effects rapidly, it is critical to acknowledge that these bioluminescent assays are not capable of identifying the causative agents, and therefore should not be used as a stand-alone technique for environmental evaluation. A comprehensive assessment of organic pollution requires thorough chemical and toxicological analyses and is often time-consuming. Therefore, the major role of cell-based bioluminescent assays should be to serve as a rapid and economical initial screening tool to prealert samples eliciting positive responses for further investigations and to reduce expense and labor on samples with negative responses.

4 Conclusions

Equipped with a bioluminescent reporter system, living whole-cell bioreporters are capable of sensing the presence of detrimental organic contaminants and internally transforming that input cue into an output signal in the form of light for easy detection. Compared to chemical analysis using costly instrumentations and complicated protocols, bioluminescent bioreporter-based assays are inexpensive, easy to perform, and capable of rapid and high-throughput detection. Bioreporters are often criticized for their compromised specificity, but it is important to note that they are not intended to replace analytical methods for the identification of the exact composition of a contaminated sample. Unlike analytical approaches, which can define structures and measure concentrations, whole-cell bioreporters are designed to survey biological potentials such as the biodegradation and toxicity potentials that are measured by catabolism-based bacterial reporters and toxicology-based eukaryotic reporters, respectively. With the major concerns of environmental monitoring being contamination evaluation and risk assessment, the most suitable application of bioluminescent bioassays is for the rapid prescreening of large numbers of samples to prioritize them for further in-depth examinations in combination with other analyses (including analytical methods) to provide biologically relevant data for comprehensive risk assessments. Realizing that eukaryotic-based bioreporters provide more human- centric biologically relevant information than the bacterial-based bioreporters, there is greater

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motivation toward their application in establishing toxicokinetic profiles for improved surveillance and modeling of human/animal health impacts. This includes both the lower eukaryotic yeast and mammalian cell lines that almost exclusively rely upon firefly luciferase as a signaling element, although newer versions of “humanized” bacterial luciferase capable of being expressed under eukaryotic genetic controls without the necessary addition of a light-activating substrate are becoming available for higher throughput, more data intensive, realtime chemical toxicity profiling [130]. As the inventory of bioluminescent bioreporters expands toward more chemical targets with greater specificity, sensitivity, and human relevance, it is clear that the bioreporter's role as an environmental sentinel is here to stay.

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Abbreviations

AhR	Aryl hydrocarbon receptor
AR	Androgen receptor
ARE	Androgen response element
ARNT	AhR nuclear translocator
BPA	Bisphenol-A
BTEX	Benzene, toluene, ethylbenzene, and xylene
CALUX	Chemical-activated luciferase expression
DDE	Dichlorodiphenyldichloroethylene
DDT	Dichlorodiphenyltrichloroethane
DRE	Dioxin-responsive element
E2	17β-estradiol
EDC	Endocrine disrupting chemical
EE2	17α-ethynylestradiol
ER	Estrogen receptor
ERE	Estrogen response element
GC	Gas chromatography
GR	Glucocorticoid receptor
HPLC	High-performance liquid chromatography
MS	Mass spectrometry
PAH	Polycyclic aromatic hydrocarbon
PCB	Polychlorinated biphenyls
PCDD	Polychlorinated dibenzo-*p*-dioxin

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PCDF	Polychlorinated dibenzofuran
PMT	Photomultiplier tube
PR	Progesterone receptor
T3	3,3′,5-triiodo-$_{L}$-thyronine
TCA	1,1,1 trichloroethane
TCDD	2,3,7,8-tetrachlorodibenzo-*p*-dioxin
TCE	Trichloroethylene
TR	Thyroid receptor

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Table 1

Bacterial bioluminescent bioreporter for organic contaminants and representative environmental applications

Reporter strain	Reporter construct	Target compound	Detection limit	References	Environmental application
E. coli DH5α (pPROBE-LuxAB-TubT)	*tubT-lux*AB	BTEX	0.24 μM toluene	[14]	Simulated aquatic oil spill [14]
E. coli DH5α pGLTUR	*xylR*-P_u-*luc*	Benzene, Toluene, Xylene	10–20 μM toluene 3 μM 3-xylene	[13]	Soil [13, 131]
P. putida TVA8	*tod-luxCDABE*	BTEX	0.03–50 ppm BTEX	[15]	Soil [3]
		TCE	1 μMTCE		Water [18]
E. coli HMS174 (pOS25)	*ipbR-luxCDABE*	Hydrophobic compounds	1 μM toluene 0.1 μM naphthalene	[22]	Soil [132]
E. coli DH5α pTOLLUX	*pR/xlyR*-P_u-*luxCDABE*	BTEX	N/A	[11]	Soil and groundwater [11]
B. sartisoli RP007 (pPROBE-phn-luxAB)	*phnS-luxAB*	PAH	0.17 μM naphthalene	[14]	Simulated aquatic oil spill [14]
P. putida pPG7-JAMA21	*nahR*-P_{sal}-*luxAB*	Naphthalene	0.50 μM naphthalene vapor	[133]	N/A[a]
P. fluorescens HK44 (pUTK21)	*nahR*-P_{nahG}-*luxCDABE*	PAH	12–120 μM	[29]	Soil [32, 131, 134]
E. coli DH5 (pGEc74, pJAMA7)	*alkS*-P_{alkB}-*luxAB*	6–10-carbon alkane	25 nM octane	[37]	Groundwater [20] Soil [131]
E. coli	*alkBFG, alkJ-luxAB*	5–12-carbon alkane	10 μM octane	[39]	N/A
Acinetobacter baylyi ADP1	*alkR*-P_{alkM}- *luxCDABE*	Alkane	3 nM octane	[43]	Seawater [43]
E. coli	*alkR*-P_{alkM}-*luxAB*	Alkane	5 nM octane	[45]	N/A
P. putida F1G4	*sep-luxCDABE*	TCE	1 mM TCE	[50]	N/A
Methylobacterium extorquens	*dcmR*-P_{dcmA}-*luxCDABE*	DCM	1 μM DCM	[51]	N/A
Ralstonia eutropha ENV307 (pUTK60)	*bphR1-luxCDABE*	PCBs	0.80 μM 4-chlorobiphenyl 4.6 μM Aroclor	[53]	N/A
E. coli XL1-Blue	P_{pcbC}-*luxCDABE*	PCBs	0.1 mM biphenyl (lowest concentration tested)	[56]	N/A
E. coli XL1-Blue	P_{pcbC}-*luc*	PCBs	0.1 mM biphenyl (lowest concentration tested)	[56]	N/A
E. coli (pHYBP109)	*hbpR*-P_{hbpC}-*luxAB*	OH-PCBs	1 nM 2-hydroxy-3′,4′,5-trichlorobiphenyl	[58]	Simulated aquatic oil spill [14] Human serum [58]
P. fluorescens OS8(pDNdmpRlux)	*dmpR*-P_o-*luxCDABE*	Phenolic compound	0.30 μM 2-methylphenol 0.87 μM phenol	[63]	Groundwater and semicoke-dump leachates [63]
E. coli (pRLuc42R)	*luc*	Phenolic compound	0.5 μM phenol	[65]	N/A
Acinetobacter DF4-8	*mopR-luxCDABE*	Phenolic compound	0.03 mM phenol	[67]	N/A

[a] N/A not available

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Table 2

Eukaryotic cell-based bioluminescent bioreporters for dioxin-like chemicals and recent environmental applications

Host cell (organism)	Reporter construct (reporter name)	Detection limit (TCDD)	EC_{50} (TCDD)	References	Recent environmental application
S. cerevisiae	hAhR and hARNT, DRE(×5)-*luc*	1 nM	4.8 nM	[91]	Sewage sludge [135] Sediment [91]
HepG2 (human)	DRE(×4)-*luc*	1 pM	0.35 nM	[82]	Solid municipal waste [136]
H4IIE (rat)	DRE(×4)-*luc* (H4IIE.Luc)	0.5 fM	10 pM	[83]	River water [137] Sediment [137]
Hepa1 (mouse)	DRE(×4)-*luc* (H1L1.1c2)	0.1–1 pM	30 pM	[80]	Storm water [138] Seawater [92]
RTH-149 (rainbow trout)	DRE(×4)-*luc* (RTL 2.0)	4 pM	64 pM	[139]	Seawater [140]
RHEK-1 (human)	DRE(×4)-*luc* (HKY1.7)	10 pM	200 pM	[141]	N/A[a]
Hepa1 (mouse)	DRE(×20)-*luc* (H1L7.5c3)	0.01 pM	10–16 pM	[142]	N/A

[a] N/A not available

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Table 3

Eukaryotic cell-based bioluminescent bioreporters for endocrine-disrupting chemicals and recent environmental applications

Host cell (organism)	Reporter construct (reporter name)	Detection limit	EC_{50}	References	Recent environmental application
Estrogenic and antiestrogenic					
S. cerevisiae	hERα, ERE(×2)-*luxCDABE* (BLYES)	45 pM E2	0.24 nM E2	[118]	Freshwater [143] Drinking water [143, 144]
S. cerevisiae	hERα, ERE(×2)-*lux* (BMAEREluc/ERα)	30 pM E2	0.5 nM E2	[116]	Wastewater [145]
S. cerevisiae	hERβ, ERE-*lux* (BMAEREluc/ERβ)	0.1 nME2	0.5 nM E2	[116]	N/A[a]
MCF-7 (human)	ERE-*lux* (MVLN)	1 pM	20 pM	[98, 99]	River water [137] Sediment [137] Wastewater [146]
MCF-7 (human)	ERE-*lux* (MELN)	1 pM E2	5 pM E2	[102]	Wastewater [125, 147] Freshwater [148, 149] Sediment [148, 149]
T-47D (human)	ERE(×3)-*lux* (T-47D ER-CALUX)	0.5 pM E2	6 pM E2	[104]	Wastewater [150, 151] Freshwater [151, 152] Sediment [151]
T-47D (human)	ERE(×3)-*lux* (T-47D-KBluc)	1 pME2	10 pM E2	[105]	Wastewater [126, 153, 154] Freshwater [153]
HeLa (human)	hERα, ERE-*lux* (HELNα)	1 pM E2	5 pM E2	[102]	Drinking water [155]
HeLa (human)	hERβ, ERE-*lux* (HELNβ)	1 pM E2	10 pM E2	[102]	N/A
U2-OS (human)	hERα, ERE(×3)-*lux* (ERα-CALUX)	0.8 pM E2	20 pM E2	[113]	Indoor dust [156] Drinking water, wastewater, and freshwater [157]
Androgenic and anti-androgenic					
S. cerevisiae	hAR, ARE(×4)-*luxCDABE* (BLYAS)	2.5 nM DHT	9.7 nM DHT	[119]	N/A
S. cerevisiae	hAR, ARE(×2)-*luc* (BMAARE luc/AR)	50 pM testosterone	10 nM testosterone	[116, 117]	Sediment [137]
		0.5 nM DHT	5.5 M DHT		
T-47D (human)	ARE(×2)-*luc* (AR-LUX)	46 pM methyltrieno-lone	86 pM methyltrieno-lone	[107]	Freshwater [158]
MDA-MB-453 (human)	MMTV-*luc* (final reporter known as MDA-kb20	0.1 nM DHT		[108, 126]	Wastewater [126, 159]
02-US (human)	hAR, HRE (hormone response element)-*luc*	3.6 pM DHT	0.13 nM DHT	[113]	Drinking water, wastewater, and freshwater [157]
Glucocorticoid receptor (GR) agonist and antagonist					
MDA-MB-453 (human)	MMTV-*luc* (final reporter known as MDA-kb2)	10 nM dexamethasone	N/A	[108]	Wastewater [159]
02-US	hGR, HRE(×3)-*luc* (final reporter known as GR-CALUX)	0.2 pM dexamethasone	0.37 nM dexamethasone	[113]	Wastewater [160]

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Host cell (organism)	Reporter construct (reporter name)	Detection limit	EC_{50}	References	Recent environmental application
Thyroid receptor (TR) agonist and antagonist					
PC 12 r(rat)	Avian TRα1, TRE(× 4)-*luc* (PC-DR-LUC)	30 pM 3,3′,5-triiodo-$_L$-thyronine (T3)	0.18 nM T3	[161]	Wastewater, drinking water, and surface water [162]
Progesterone receptor (PR) agonist and antagonist					
02-US	hPR, HRE(×3)-*luc* (PR-CALUX)	1.3 pM Org2058	0.09 nM Org2058	[113]	Indoor dust [156] Drinking water, wastewater, and freshwater [157]

[a] N/A not available

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