HHS Public Access

Author manuscript

Ecol Indic. Author manuscript; available in PMC 2015 November 18.

Published in final edited form as:

Ecol Indic. 2013 May 1; 28: 125–141. doi:10.1016/j.ecolind.2012.01.020.

Genetically modified whole-cell bioreporters for environmental assessment

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Abstract

Living whole-cell bioreporters serve as environmental biosentinels that survey their ecosystems for harmful pollutants and chemical toxicants, and in the process act as human and other higher animal proxies to pre-alert for unfavorable, damaging, or toxic conditions. Endowed with bioluminescent, fluorescent, or colorimetric signaling elements, bioreporters can provide a fast, easily measured link to chemical contaminant presence, bioavailability, and toxicity relative to a living system. Though well tested in the confines of the laboratory, real-world applications of bioreporters are limited. In this review, we will consider bioreporter technologies that have evolved from the laboratory towards true environmental applications, and discuss their merits as well as crucial advancements that still require adoption for more widespread utilization. Although the vast majority of environmental monitoring strategies rely upon bioreporters constructed from bacteria, we will also examine environmental biosensing through the use of less conventional eukaryotic-based bioreporters, whose chemical signaling capacity facilitates a more human-relevant link to toxicity and health-related consequences.

Keywords

Bioluminescence; Bioremediation; Bioreporter; Ecotoxicology; Fluorescence

1. Introduction

Bioreporters consist of prokaryotic (bacteria) or eukaryotic (fungi, algae, animal) cells that serve as living sensors for priority environmental pollutants and chemicals of toxicological concern. Evolution has provided these cells with unique genetic traits that permit their adaptation to (i.e., for metabolism) or defense against (i.e., a bactericidal toxin) the chemical agents to which they are exposed, thus facilitating their ability to functionally survive and propagate in nearly any ecosystem niche. With an understanding of the genetic mechanisms involved, genetic engineering, synthetic biology, or other nucleic acid-based manipulative techniques can be applied to convert cells into controllable on/off switches tuned to a

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particular chemical, chemical class, or toxicological effector, the result of which yields what are commonly referred to as ‘lights-on’ or ‘lights-off’ bioreporter constructs (Fig. 1). In a lights-on bioreporter, the cell emits light when exposed to a targeted chemical or toxin. This requires a fundamental understanding of the genetic mechanism(s) involved in that response and the genetic element, or promoter, that controls it. In the native cell, the promoter regulates the genes that respond to the chemical or toxin. In the bioreporter cell, the promoter’s link to these genes is disrupted and replaced with a reporter gene that now, when activated by the promoter, is transcribed and translated to a reporter protein that emits a bioluminescent, fluorescent, or colorimetric signal. Lights-on bioreporters are most often applied in environmental sensing and monitoring schemes, and can be designed to either specifically or nonspecifically report on their interactions with inducer chemicals. If specific, the promoter/reporter gene linkage directly associates with a particular chemical or chemical class, and the generation of light as well as the intensity of light identifies that chemical and indicates its overall concentration. If nonspecific, the promoter/reporter gene construct responds via an increase in light intensity to, for example, general stress or DNA damage related to exposure to a cytotoxic, mutagenic, or genotoxic chemical agent. Although the identity of the particular chemical causing the toxic interaction remains unknown, these bioreporters rapidly and efficiently pre-alert to environmental offenses that can then be more closely examined using chemical-specific bioreporters or conventional analytical techniques such as gas chromatography/mass spectrometry (GC/MS).

In a lights-off bioreporter, the cell is designed to continuously (constitutively) emit light. A measured decrease in light intensity after chemical exposure indicates that the chemical’s interaction with the bioreporter has caused cellular damage or disrupted general metabolism. Thus, although the chemical itself cannot be identified, it can be classified as displaying toxicity towards a living organism, and pre-warns of potentially analogous toxicity towards higher life forms (i.e., humans). For the most part, these bioreporters are designed around prokaryotic or lower eukaryotic cells that, to date, have served as suitable surrogates for more complex organismal systems, but this is gradually changing as we improve our abilities to manipulate higher eukaryotes towards defined bioreporter sensing strategies.

The emphasis of this review will center on bioreporter sensing technologies that have been applied within real-world environmental settings since this forms the foundation of their practical detection and monitoring capacities. The reader is reminded that there exists a much larger number of bioreporters than those discussed here that still remain laboratory bound, and with capacities to detect a wide array of chemicals and chemical interactions (see recent reviews by Robbens et al. (2010), Diplock et al. (2010), and Hynninen and Virta (2010) for more comprehensive examinations of bioreporter systems).

2. Common reporter elements

Whole-cell bioreporters most commonly incorporate reporter genes that code for signaling elements that emit bioluminescent, fluorescent, or colorimetric endpoints, with bioluminescence being derived from the bacterial (*lux*) or firefly (*luc*) genes, fluorescence from the green fluorescent protein (*gfp*) gene and its other colored variants, and colorimetric endpoints relying upon the β-galactosidase (*lacZ*) gene.

2.1. Bioluminescence

Bioluminescence–the chemical generation of light within a living organism–is a widely used reporter element in environmental biosensing. The chemical reaction yielding bioluminescence is catalyzed by an enzyme (luciferase) that reacts with a substrate (luciferin) to produce an excited state molecule that generates photons as it relaxes back to its ground state. Biotechnological exploitation of luciferase/luciferin reactions has generally focused on the bacterial bioluminescent system referred to as *lux* and the firefly bioluminescent system referred to as *luc*.

2.1.1. Bacterial luciferase (lux)—The luciferin substrate for the bacterial (*Vibrio*, *Photorhabdus*, and *Photobacterium* genera) bioluminescent reaction is a reduced riboflavin phosphate ($FMNH_2$) that is oxidized by the luciferase enzyme in association with a long-chain aldehyde and molecular oxygen. This reaction is controlled genetically by a five gene operon consisting of the *luxA*, *luxB*, *luxC*, *luxD*, and *luxE* genes (denoted as *luxCDABE* to designate the order of the genes in the operon) (Meighen, 1994). The *luxA* and *luxB* (*luxAB*) gene products form a heterodimeric luciferase while the *luxC*, *luxD*, and *luxE* (*luxCDE*) gene products supply and regenerate the long-chain aldehyde. The remaining required oxygen and $FMNH_2$ reactants are scavenged within the cell through ancillary metabolic processes. The end result is the emission of a blue/green 490 nm light signal. Two classes of *lux*-based bioreporters are used. The simplest integrates only the *luxAB* genes, resulting in bioreporters containing only the luciferase enzyme which then requires the exogenous addition of aldehyde, usually in the form of *n*-decanal, to the reaction. Although this creates a brighter and easier to detect signal due to substrate saturation, it makes signaling a time point dependent occurrence contingent upon the external addition of an activating compound. Nonetheless, numerous bioreporters harboring the *luxAB* genes have been designed around bacterial, yeast, and mammalian genetic systems and remain well tested within environmental, food, and water-based bioassays.

To permit continuous substrate-independent bioluminescent signaling, bioreporters can alternatively be designed to accommodate the complete *luxCDABE* gene cassette. These bioreporters contain the full complement of the luciferase/luciferin reaction and can generate bioluminescence spontaneously without any user mandated interventions. This advantageously endows the bioreporter with real-time to near real-time detection capabilities. The *luxCDABE* genetic operon has been synthetically optimized for efficient gene expression away from its native AT-rich state towards GC-rich microorganisms, thereby allowing for its integration into a wider variety of bacterial hosts (Craney et al., 2007). This is of value because it improves complementary matching of bioreporter hosts to test environments to better accommodate long-term bioreporter maintenance and survival under natural ecological conditions. The *luxCDABE* operon has also been synthetically restructured for efficient expression in mammalian cells, which is of significant advantage since it allows for the design of autonomously sensing eukaryotic bioreporters whose response kinetics become much more relevant towards human health than that suggested by analogous bacterial or lower eukaryotic bioreporter surrogates (Close et al., 2010).

2.2. Firefly luciferase (luc)

The *luc* gene, derived most commonly from the firefly *Photinus pyralis*, is a popular reporter gene due to its high light output and rapid response kinetics (Close et al., 2009). The Luc protein catalyzes the oxidation of a reduced luciferin substrate in the presence of ATP-Mg^{2+} and oxygen to generate a yellow/green 562 nm light signal, the quantum yield of which is the largest of any of the currently characterized bioluminescent systems. The Luc protein requires no post-translational modifications and is thus immediately available once translated. *luc* reporter systems require the exogenous addition of the luciferin substrate, so *luc*-based bioreporters are not able to react autonomously or monitor user defined targets in a continuous fashion. Nonetheless, its maximal light output translates into very sensitive assays that have been applied over a wide range of organic compounds, heavy metals, and environmentally significant estrogenic and endocrine disruptor agents.

2.3. Green fluorescent protein (GFP)

GFP is a photoprotein cloned from the jellyfish *Aequorea victoria* and is representative of a large family of natural and recombinant photoproteins capable of producing light in a palette of colors (Shaner et al., 2005). It requires no substrate to do so, but is dependent on an external light source to activate its fluorescent output and is thus always tied to instrumentation capable of performing this task. GFP and its variants are capable of functioning semi-continuously and perform well as near real-time sensors in environmental monitoring assessments. Due to their differently colored light emission outputs, they can additionally be implemented in dual-color formats where each color indicates a separate event (Hever and Belkin, 2006).

2.4. β-Galactosidase (lacZ)

The *lacZ* gene cloned from *E. coli* encodes a β-galactosidase (β-gal) enzyme that catalyzes the hydrolysis of substrate β-galactoside disaccharides into monosaccharides. *lacZ*-based bioreporters traditionally yielded a colorimetric signal when supplied with the substrate *o*-nitrophenyl-β-D-galactoside (ONPG). Commercially available kits such as the SOS Chromotest implement *lacZ*-based fusions to DNA responsive genes to monitor environmental samples for potential mutagenic or carcinogenic genotoxic compounds (Quillardet et al., 1982). Besides colorimetric endpoints, *lacZ* bioreporters can be used in conjunction with a variety of β-galactoside substrates that permit luminescent (Nazarenko et al., 2001), chemiluminescent (Jain and Magrath, 1991), or fluorescent (Rowland et al., 1999) endpoints. A disadvantage of *lacZ*-based biosensing strategies is that β-gal is endogenously present in natural environmental matrices and contributes to elevated background activity. Also, bioreporter cells must be permeabilized upon substrate addition to quantify β-galactosidase activity, which results in discontinuous and oftentimes delayed data accumulation, but electrochemical and amperometric interfaces can be implemented to bypass permeabilization steps (Ron and Rishpon, 2010).

3. Bacterial bioreporter applications in environmental assessment

Bacterial cells, being genetically easy to manipulate and robust enough to survive under various environmental conditions, have been employed as host cells in constructions of the

majority of whole-cell bioreporters. By exploiting intrinsic cellular processes such as the stress response, defense against toxins, and catabolism of various compounds, bacterial bioreporters can be designed to detect a variety of chemicals and physical conditions. A substantial number of bacterial bioreporters have been constructed and characterized (recently reviewed in Diplock et al., 2010; Eltzov and Marks, 2011; Ripp et al., 2011; van der Meer and Belkin, 2010), however, their applications in the field are relatively limited, primarily due to regulations limiting or excluding the environmental release of recombinant DNA. To explore what bacterial bioreporters can offer as environmental monitors, this section highlights recent applications of bacterial bioreporters in environmental assessment. Note that one of the primary advantages of bioreporters is their ability to report on bioavailability–that portion of the chemical that is freely available to cross the cellular membrane and therefore having a biological effect. The relationship between bioreporter sensing and bioavailability under environmental influences is a complex process that depends on the bioreporter's physiology, growth rate, membrane composition, transport mechanisms, and a host of other factors (Harms et al., 2006). Bioavailability is also specific in terms of the receptor, where, for example, a sample that is not bioavailable to bacteria may be bioavailable to plants or animals, or vice versa. Therefore, results obtained using bacterial bioreporters still need to be carefully interpreted as to what they actually measure, and a suite of different bioassays might be more appropriate to obtain comprehensive analyses to satisfy the goals of environmental assessments.

3.1. Bioreporters for the detection and monitoring of heavy metals

Heavy metals are important inorganic contaminants with regard to risk assessment due to their environmental prevalence and biological toxicity toward humans and wildlife. A long history of characterization of microbial metal resistance has accumulated significant knowledge of genetic elements and regulatory mechanisms that specifically respond to metals. Typically, bacteria counteract metal toxicity by means of efflux transporters that actively export the toxic metal ions to the outside of the cell and/or modification enzymes that transform the metals to less toxic forms (Nies, 1999). Because expression of the proteins involved in these defense machineries use energy, thus imposing extra metabolic burden to the host cells, they are only induced when metal ions are present at toxic concentrations. The induction of particular defense machinery often involves a specific transcriptional regulator, which upon metal-binding activates the transcription of downstream defense related genes. For example, defenses against mercury (Hg) and arsenic (As) are regulated by the transcriptional regulators MerR and ArsR, respectively. A generic metal bioreporter can be constructed by fusing the genes encoding the metal-specific transcriptional regulator as well as the promoter/operator of the defense related genes to a promoterless reporter gene (*luc*, *gfp*, etc.) or reporter genes (in the case of *luxAB* and *luxCDABE*). Upon exposure, the transcriptional regulator is activated by metal binding and subsequently turns on the expression of the reporter gene(s), finally leading to production of a measurable signal.

Because the toxicity of a particular metal is greatly dependent on the form in which it exists, analysis using bioreporters yields more biologically relevant information than conventional chemical methods. Many reports using artificially amended samples have demonstrated in

proof-of-concept that whole-cell bacterial bioreporters can be used for assessment of bioavailability and toxicity of metals in different sample matrices (Bondarenko et al., 2008; Brandt et al., 2006; Ivask et al., 2009, 2002) (a more detailed review is available in Hynninen and Virta, 2010). Rather than discussing the entire inventory of bacterial metal bioreporters, here we present a brief overview of their applications in field samples and refer the reader to Table 1 for a more comprehensive list.

3.1.1. Heavy metals in soil and sediment—Industry and mining contribute substantially to heavy metal soil contamination, but sources such as road runoff from automobiles and the spread of metal-containing livestock manure also function as environmental inputs (Bolan et al., 2004). Metal contamination in soils as well as sediments is traditionally evaluated by the total amount of metals determined using analytic chemical methods after acid extraction of solid samples. However, these types of methods cannot distinguish between bioavailable and non-bioavailable forms, and therefore do not indicate actual toxicity towards biological systems. Determination of the bioavailable fraction of metals in soil and sediment samples is of particular interest since a variety of sample attributes, such as soil type, organic matter content, pH, redox potential, and humidity can affect metal bioavailability (Hynninen and Virta, 2010). Therefore, chemically defined metal contents need more biologically relevant interpretations, which can be achieved using whole-cell bioreporters as living biosentinels tuned to heavy metal bioavailability.

Metals are generally present in soil and sediments in two forms, a soluble phase that can be readily extracted by water and a particle-absorbed solid phase that is not water extractable (Degryse et al., 2009). While it is widely accepted that the water extractable fraction is directly bioavailable (Giller et al., 2009), currently there is no agreement on the bioavailability of particle-bound metals (Magrisso et al., 2009). Soil–water suspensions obtained by mixing air-dried soil with water, and soil–water extract, which is the particle-free supernatant formed after centrifugation of the suspension, are the two commonly employed sample preparation methods (Hynninen and Virta, 2010). Bioavailable metal in the soluble fraction can be analyzed using water extracts, whereas bioreporter analysis of soil suspensions wherein bioreporter cells are in direct contact with soil particles allows for measurement of bioavailable metal including the absorbed fraction present in the sample, keeping in mind the imprecision of bioavailability (Harms et al., 2006).

Comparisons between the amount of bioavailable metal measured by bioreporter analysis using either water extracts or soil suspensions to the amount of total metal determined by chemical methods indicate that bioavailable metal only accounts for a small fraction of total metal and that the bioavailable fraction is not always correlated with total metal concentration. Ivask et al. (2007) analyzed 10 soil and sediment samples using an *E. coli merR-luxCDABE* strain and an *E. coli ars-luxCDABE* strain to examine Hg and As bioavailability in suspensions. The results showed that only 1.2–6.7% of total Hg and 0.9–4.9% of total As was available for detection by bioreporter cells. In a larger scale study, bioavailable Cd and lead (Pb) were found to be 0.5–56% of total Cd and 0.2–8.6% of total Pb across 50 different soil samples (Ivask et al., 2004). These and other similar findings suggest that chemically determined total metal concentration alone is not an adequate indicator for the purpose of ecological evaluation.

Bioreporter analyses of water extracts of field soil and sediment samples have also revealed that water extractable metal is not always bioavailable. Turpeinen et al. (2000) reported that only 4–6% and 13–43% of total water soluble Pb in humic surface soil and mineral soil sampled from the same site, respectively, were bioavailable as determined using a *luc*-based *Staphylococcus aureus* RN4220 (pTOO24) bioreporter. Similar observations were made by Liao and colleagues using a *cadCgfp*-based *E. coli* DH5α bioreporter in contaminated sediment and soil samples (Liao et al., 2006). The percentage varies by metal species and soil samples as well (Brandt et al., 2008; Maderova et al., 2011; Turpeinen et al., 2003). As a result, bioavailability (hence, effects on biological systems) cannot be well predicted by chemically measured water soluble metal concentration.

Bioreporter analysis of soil suspensions is of particular interest for another reason; it allows for the assessment of bioavailability of the particle-absorbed metal fraction. Whether absorbed metal is bioavailable is still uncertain, especially in contaminated field samples. A study in which fifty contaminated agricultural soils were tested using two *luc*-based bioreporters, *Bacillus subtilis* BR151 (pTOO24) and *S. aureus* RN4220 (pTOO24) specific for Cd and Pb, respectively, showed that 115-fold more Cd and 40-fold more Pb were bioavailable in soil suspensions than in soil/water extracts (Ivask et al., 2004). Contradictorily, another study by Magrisso et al. (2009) reported that absorbed Pb was unavailable to a *luxCDABE*-based bioreporter *Cupriavidus metallidurans* AE1433, as indicated by no bioluminescence induction with soil suspensions despite a chemical analysis acid extraction procedure that suggested significant amounts of total Pb in the soil sample. The uncertainty in bioavailability of bound metals is then translated to the lack of confidence in the evaluation of contamination scales, remediation strategies and efficacies if only chemical methods are used. Therefore, bacterial bioreporters can be included as a complementary analytic tool to conventional chemical methods for more comprehensive assessments of metal contamination in soils and sediments.

3.2. Heavy metals in water

Heavy metal contamination in water systems impacts the growth of waterborne organisms, and with respect to human health, drinking water quality. Therefore, there is an increasing need for rapid, sensitive and cost-effective screening protocols to monitor natural and drinking water systems. Bacterial bioreporters are well suited for this task for several reasons. Since bacteria are very sensitive to toxic heavy metals and have evolved to rapidly eliminate the toxic effects, very low detection limits can be accomplished using bacteria as host cells for bioreporter construction (Hynninen and Virta, 2010). In fact, most heavy metal bioreporters can detect concentrations below the drinking water safety limit (Ripp et al., 2011). Bacterial bioreporter assays are less time-consuming and less expensive than chemical analysis, making them a suitable method for Tier I screening in large scale surveys.

Arsenic serves as one of the most serious water contaminants (Nordstrom, 2002). Several bacterial bioreporters have been developed for arsenic by taking advantage of the arsenic resistance mechanism found in most bacteria (reviewed in Diesel et al., 2009). The arsenic bioreporter *E. coli* DH5α (pJAMA-arsR) containing the *luxAB* genes as reporter elements

has been applied in a large scale field testing wherein 194 different groundwater samples from Vietnam were screened for arsenic (Stocker et al., 2003; Trang et al., 2005). After a 1.5 h incubation, this strain exhibited a lower detection limit of 7 μg As/L groundwater with a linear response between 10 and 100 μg As/L. When validated against chemical analysis, the bioreporter assay was more reliable than chemical field test kits, yielding only 2.4% false positive and 8.0% false negative results if using the World Health Organization guideline of 10 μg/L as the threshold value for safety. This advocates the potential monitoring capabilities of arsenic bioreporters, especially in disadvantaged areas where access to expensive chemical analysis equipment is limited.

Another commonly examined metal in aqueous environments is iron (Fe), whose bioavailability is typically monitored in relation to primary production in aqueous ecosystems because it is an essential nutrient for phytoplankton. Fe-responsive bioreporters are normally constructed using ecologically relevant cyanobacteria species belonging to the *Synechococcus* and *Synechocystis* genus (Bullerjahn et al., 2010). These bioreporters usually carry a transcriptional fusion of the *Vibrio harveyi luxAB* genes and the *isiAB* promoter responsive to iron deficiency. *Synechococcus* sp. strains PCC 7942 and PCC 7002 have been engineered to carry such reporter constructs, and the resulting bioreporters, designated as KAS101 (Durham et al., 2002) and CCMP2669 (Boyanapalli et al., 2007; Bullerjahn et al., 2010), respectively, have been used to assess Fe bioavailability in the Great Lakes (Hassler et al., 2009; McKay et al., 2005; Porta et al., 2005) and marine environments (Boyanapalli et al., 2007). These bioreporters function such that the bioluminescent response increases with reducing concentration of intracellular Fe^{3+}, making them suitable for low concentration detection. In addition, the sensitivity of the *isiAB* promoter to Fe^{3+} repletion permits very low detection limits (<1 nM).

With the aim of understanding the fate of gaseous elemental Hg during atmospheric mercury depletion events (AMDEs), Larose et al. (2011) employed an *E. coli mer-luxCDABE* bioreporter strain to characterize bioavailable Hg in arctic snowpacks. Hg(0) in gas phase is transported from lower latitudes to polar regions, causing local mercury contamination even without significant anthropogenic sources. By evaluating the bioavailable fraction of Hg in surface and basal snow, it was possible to determine the contribution of AMDEs and other deposition pathways to the bioavailable Hg pool, as well as to obtain a better understanding of Hg cycling within the snowpacks. This could not be achieved using traditional analytical methods because the total amount of Hg, both in bioavailable or non-bioavailable forms, is measured in these methods.

3.3. Bioreporters for the detection and monitoring of organic pollutants

Anthropogenic activities have released various types of organic compounds into the environment. These compounds, despite their industrial value, represent another major group of environmental pollutants as they can evoke adverse effects on human health. Microorganisms have evolved transcriptionally regulated catabolic pathways for the degradation of organic compounds (Tropel and van der Meer, 2004). Similar to the case of metal resistance, the regulation of catabolic pathways at the level of transcription is accomplished by an effector-activated regulatory protein stimulating expression of the genes

encoding degradation enzymes. Bacterial bioreporters for organic chemicals are therefore designed around these regulatory proteins and corresponding promoters. For example, a plasmid containing a *luc* gene fused to the *xylR* gene encoding the toluene-binding regulatory protein XylR and the XylR-responsive promoter P_u was constructed and introduced into *E. coli* DH5α, yielding a bioluminescent bioreporter that responded to toluene and related compounds (Willardson et al., 1998). Bioreporters can also be constructed using bacterial strains that mineralize target compounds, allowing for coupled reporting and degradation. Such bioreporters are of particular interest in the aspect that in addition to detecting the presence of target compounds, they can provide insight towards the catabolic potential of bioremediation. Bioreporters using degrading and non-degrading bacteria have been developed for middle-chain alkanes (Sticher et al., 1997), simple aromatic hydrocarbons (such as BTEX (benzene, toluene, ethylbenzene and xylene)) (Applegate et al., 1997, 1998; Selifonova and Eaton, 1996; Stiner and Halverson, 2002; Willardson et al., 1998), two to three ring polycyclic aromatic hydrocarbons (PAHs) (such as naphthalene and phenanthrene) (King et al., 1990; Tecon et al., 2009, 2006), phenolic compounds (Abd-El-Haleem et al., 2002; Hay et al., 2000; Leedjarv et al., 2006; Shingler and Moore, 1994), and polychlorinated biphenyls (PCBs) and their metabolites (Feliciano et al., 2006; Layton et al., 1998). A detailed discussion of these bioreporters is addressed by Tecon and van der Meer (2008) and their applications in field experiments are summarized in Table 2.

3.3.1. Organic pollutants in soil—The fate of organic compounds released into soils is a complicated process that encompasses biodegradation, leaching, volatilization, sequestration, and/or bioaccumulation (Semple et al., 2003). In terms of risk assessment, the fraction undergoing biodegradation is of the most interest, and serves as a suitable monitoring endpoint for bacterial bioreporters. King et al. (1990) constructed the first organic chemical bioreporter *Pseudomonas fluorescens* HK44 for the detection of naphthalene. Containing a plasmid-borne *nahG-luxCDABE* fusion, HK44 emits light upon exposure to naphthalene, salicylate, and substituted analogs. A controlled field release of strain HK44 into soils in contained lysimeters was initiated in 1996 to demonstrate bioreporter monitoring of a bioremediation event (Ripp et al., 2000). HK44 cells were sprayed within a PAH contaminated soil matrix and naphthalene-induced bioluminescence was detected over a 2 year monitoring period, with signal strength diminishing in parallel with the bioremediative loss of naphthalene.

Bioreporter assays of soil samples are more conventionally performed on organic solvent extractions of contaminated soils rather than directly within the soil itself. As an example, Dawson et al. (2008) applied the *Pseudomonas putida* TVA8 *luxCDABE* BTEX bioreporter to methanol extracts of BTEX-impacted soils to estimate degradation and toxicity. Bioluminescent response was achieved within a 2 h incubation of bioreporter cells and diluted methanol soil extracts. It was also demonstrated that changes in BTEX concentration and toxicity over time could be correlated with the bioluminescent response to methanol soil extracts during the process of degradation. However, additional concerns associated with solvent extraction are raised. First, the extractable amount of organic contaminants from soil samples may vary by different solvents (Kelsey et al., 1997). Second, the alcohol solvent

(such as methanol and butanol) used for extraction can be toxic to the bioreporter cells, resulting in loss in viability and a corresponding loss in signal. Third, solvent toxicity can damage the cell membrane, thus increasing the supply of fatty acids to the cell that then serve as a substrate for bacterial luciferase to erroneously increase the bioluminescent response. Although dilution is commonly performed to minimize solvent effects, dilution steps can reduce the test concentration below the bioreporter detection limit, leading to an underestimation of the amount of contaminant present in the original sample. Another problem of solvent extraction is, once again, associated with bioavailability. Many physico-chemical properties of soils can impact the bioavailability of organic compounds (Semple et al., 2003), whereas solvent extraction cannot distinguish between biologically available and unavailable fractions. The bioavailability issue is critical when determining biodegradation potential (i.e., the extent of contaminants that can be degraded by microbes). As demonstrated by Paton et al. (2009), bacterial bioreporters can be used to address these concerns. In their experimental design, soils historically contaminated with naphthalene were subjected to bioremediation by adding a natural naphthalene-degrading microbial species, followed by chemical analysis to determine the amount of naphthalene that had been degraded. A range of non-exhaustive extraction techniques was used to extract naphthalene from the soils prior to biodegradation. By comparing the bioluminescent response of the naphthalene bioluminescent bioreporter HK44 to each extract with the amount of biodegradable naphthalene that was determined chemically, the authors demonstrated a correlation between extractable and bioavailable naphthalene. In addition, the extract was immediately useable in the bioreporter assay without a dilution step, suggesting a more straightforward assay format.

3.3.2. Organic pollutants in water—Compared to soil samples, organic compound detection in water using bacterial bioreporters is more straightforward since the aqueous sample can be directly used in bioreporter assays without prior solvent extraction. Willardson et al. (1998) applied the previously mentioned *xylR*-P_u-*luc* bioreporter *E. coli* DH5α (pGLTUR) for detection of toluene and related compounds in a deep water aquifer. The bioreporter accurately measured contaminant concentrations within 3% of those measured by conventional chemical methods after 1 h of incubation with the water sample.

Detection of hydrocarbons in groundwater and wastewater has also been demonstrated using the BTEX bioluminescent bioreporter *P. putida* TVA8. Because strain TVA8 also responds to some chlorinated aliphatic hydrocarbons (CAHs) (Shingleton et al., 1998), Bhattacharyya and colleagues employed a set of different constitutive and inducible bioluminescent bioreporters including TVA8 to assess several groundwater samples that were contaminated with CAHs including a known TVA8 inducer trichloroethylene (TCE) (Bhattacharyya et al., 2005). Strain TVA8 linearly responded to TCE within the range from 0 to approximately 2000 μmol/L, covering the concentrations of TCE in the groundwater samples. It was demonstrated that the samples inducing the highest bioluminescence were also the ones that contained the highest chemically-determined TCE concentrations (Bhattacharyya et al., 2005). Kuncova et al. (2011) compared the bioluminescent response of TVA8 to contaminated wastewater influent and effluent after treatment. The removal of the majority of BTEX contamination was indicated by an approximate 100-fold decrease in the

maximum bioluminescence induced by the effluent than that induced by the influent. The bioreporter results also agreed with side-by-side chemical analysis, which measured approximately 1000-times less BTEX compounds in the effluent sample. However, the bioreporter assay failed to respond to a groundwater sample with known high concentrations of BTEX, probably due to the presence of uncharacterized toxicants within the sample. Dilution schemes can alleviate such toxic interactions, and the inclusion of constitutive 'always on' bioreporters as controls can indicate the presence of interfering toxicants that reduce cell viability.

A phenol bioreporter *P. fluorescens* OS8 (pDNdmpRlux) containing a plasmid-borne *dmpR*-P_o-*luxCDABE* fusion was constructed and used to detect phenolic compounds in groundwater and dump leachates (Leedjarv et al., 2006). With a laboratory-determined detection limit of 0.08 mg phenol/L, this bioreporter was able to elicit a detectable bioluminescent response to nine out of ten samples tested. Substantial variation of the bioavailable fractions across samples was also observed, ranging from 6% to 95% of the total amount of phenol determined by chemical methods. One sample, although chemically determined to contain phenol at a total concentration approximately 140-times the bioreporter detection limit, was unsuccessful in inducing bioluminescence in the bioreporter, suggesting that phenol present in the sample was probably not bioavailable. Such results thus support the importance of taking bioavailability into account in environmental risk assessment.

In a contaminated site, for instance an oil spill site, it is not uncommon that different types of pollutants coexist. Tecon et al. (2010) recently demonstrated the application of a suite of multiple bacterial bioreporters for monitoring hydrocarbon mixtures in marine environments. Five bioreporters for the detection of short chain linear alkanes, monoaromatic and polyaromatic compounds, biphenyl, 2-hydroxybiphenyl, and DNA damage were used to evaluate artificial crude oil spills in seawater over a period of 7–10 days. Three strains including the short chain alkane bioreporter *E. coli* DH5α (pGEc74, pJAMA7), the BTEX bioreporter *E. coli* DH5α (pPROBE-*luxAB*-TbuT), and the naphthalene and phenanthrene bioreporter *Burkholderia sartisoli* RP007 (pPROBE-*phn-luxAB*) were able to detect significant amounts of inducing compounds in oil-contaminated seawater samples. The short chain alkane bioreporter detected a maximum equivalent octane concentration between 200 and 600 nM 6 h after the spill in individual replicates, whereas chemical analysis also measured maximum concentrations of C_{11} and C_{12} alkanes at approximately 200 nM 2 to 6 h after the spill. Similarly, the equivalent naphthalene concentration detected by the bioreporter assay peaked at approximately 1 μM (equivalent to 0.13 μg/mL) 2–3 days after the spill, which was comparable to total naphthalene and phenanthrene concentrations of 0.18–0.80 μg/mL as measured by chemical methods in three other replicates. Although chemical analysis of monoaromatic compounds was not performed due to the discrepancy in the types of compounds that can be measured between chemical methods and bioreporter assays, the concentration of toluene equivalents detected by the BTEX bioreporter evolved in a manner similar to that of octane, with a peak 6 h after the spill followed by a gradual decrease. The authors therefore stated that the trend of concentrations of each type of organic compound determined by bioreporter assays was

generally in agreement with chemical analysis as well as data from other studies. Light and volatile species such as alkane and BTEX peaked a few hours after the spill, followed by a gradual decrease. On the other hand, heavier PAH constituents (i.e., naphthalene) were bioavailable to the bioreporters in aqueous phase for several days. They also concluded that this multi-strain bioreporter platform could be used as a simple and rapid tool for monitoring hydrocarbon mixture contamination and potentially as an indicator to predict the time scale of oil spills.

3.4. Bioreporters for the detection and monitoring of environmental toxicity

In addition to specifically sensing single compounds or classes of compounds, whole-cell bioreporters can be designed to detect effects without necessarily identifying the chemical nature of the analytes. These effects can be general, such as cytotoxicity, or somewhat specific, such as genotoxicity, protein damage, or oxidative stress. Bioreporters for cytotoxicity detection are usually constructed by expressing the reporter gene under the control of a strong constitutive promoter and function in a lights-off mode. Exposure to toxicants is reflected by a decrease in reporter signal due to inhibited cellular metabolism. Alternatively, reporter genes can be coupled with a promoter responsive to certain types of stress, allowing for semi-specific detection in which exposure to samples capable of causing the given stress results in an elevated level of signal. With regard to the choice of stress-responsive promoters, the promoter of the *recA* gene involved in DNA repair is commonly used for the construction of genotoxicity responsive bioreporters. Promoters of genes involved in the heat shock response such as *grpE* and *dnaK* are usually employed to detect protein damage. For monitoring environmental samples where the coexistence of various toxic chemicals is routine, using toxicity sensitive whole-cell bioreporters is advantageous in that the collective toxicity from all contaminants present in the sample is reported. Applications of genetically modified whole-cell bioreporters for toxicity assessment in environmental samples are summarized in Table 3.

Because of its autonomous nature and easily measurable light signal, the *luxCDABE* gene cluster is the most commonly used reporter element for the construction of toxicity responsive bioreporters. In fact, naturally bioluminescent marine bacteria such as *Vibrio fischeri* were originally exploited for toxicity monitoring. The widely used and commercially available Microtox test was developed on the basic principle that exposure to toxicants can be reflected by the reduction of bioluminescence in *V. fischeri* (Chang et al., 1981). One limitation, however, is that because *V. fischeri* is a marine species, the assay needs to be performed in saline, limiting its application in more complex environmental samples. Using recombinant DNA techniques, other environmentally relevant yet naturally non-luminescent bacterial species can be engineered to express the bioluminescence genes and function as toxicity bioreporters. An example is *P. fluorescence* Shk1, which was constructed by introducing the *luxCDABE* genes into a *P. fluorescence* strain isolated from the activated sludge of a wastewater treatment plant (Kelly et al., 1999). Strain Shk1 was shown to respond to a range of toxicants including cadmium, dinitrophenol, and hydroquinone. It also has been successfully used to monitor toxicant loads in wastewater influents (Kelly et al., 2004; Lajoie et al., 2003; Ren and Frymier, 2003). Additionally, plasmid pUCD607 containing the *luxCDABE* genes isolated from *V. fischeri* (Shaw and

Kado, 1986) has been introduced to non-luminescent bacteria for bioreporter development. Two strains constructed using pUCD607, *E. coli* HB101 (pUCD607) (Rattray et al., 1990) and *P. fluorescens* 10586 (pUCD607) (Aminhanjani et al., 1993), have been utilized to assess toxicity in a variety of soil and water samples (Bhattacharyya et al., 2005; Bundy et al., 2001; Dawson et al., 2007; Flynn et al., 2002, 2003; Nissen et al., 2009; Paton et al., 2006a,b; Tiensing et al., 2002). A bioluminescently tagged *Acinetobacter* strain DF4/pUTK2 was also developed and successfully applied to monitor for heavy metal toxicity in industrial and municipal wastewater samples (Abd-El-Haleem et al., 2006). This group has also designed a lights-on bioreporter that monitors for nitrate and nitrite toxicity in wastewater. Just like heavy metals, these inorganic compounds can have significantly deleterious effects on aquatic life when released in effluent waters (Abd-El-Haleem et al., 2007). By fusing the nitrate/nitrite responsive *nasR*-like promoter from *Klebsiella* to the *luxCDABE* gene cassette, they demonstrated real-time biosensing of these compounds in the influent and effluent waters of Egyptian sewage and industrial wastewater treatment plants, with laboratory demonstrated detection limits of <10 ppm.

In addition to simply detecting the presence of toxicants, lights-off bioreporters can be used to determine bioremediation constraints. Sousa et al. (1998) used the environmentally relevant, constitutively bioluminescent strain *P. fluorescens* 10586s (pUCD607) as a proxy to evaluate the metabolic burden on bioremediation under various BTEX contaminated sediment and water samples. By assessing the bioluminescent response under different sample manipulations, it was possible to determine if the environmental condition was favorable for microbial remediation to occur, as well as to identify and alleviate potential constraints. When assessing complex samples for a specific target, the compound-specific lights-on bioreporter might be interfered with by the sample matrix, causing inhibition or signal quenching (Brandt et al., 2006). A constitutive toxicity bioreporter therefore can be used in parallel to correct for this potential interference.

4. Emerging whole-cell eukaryotic bioreporters

While bacterial-based bioreporters are well established for the detection and monitoring of a wide variety of environmental conditions, there is an increasing trend towards the use of eukaryotic systems in this role. This shift owes to the increasing desire for determination of the effects and bioavailability of environmental contaminants as they relate to humans and other animals. Bacterial bioreporters are handicapped in this regard for several reasons. One chief concern is that the different ploidities of bacterial organisms can affect the mutagenic or carcinogenic actions of environmental contaminants that trigger the reporter (Cavenee et al., 1983), or can lead to situations where genetic alteration cannot be detected. In addition, these bacterial reporters may simply lack the required components for interacting with the target analyte, as is the case with estrogenic monitoring (Gu et al., 2002). Whole-cell eukaryotic bioreporters overcome these problems because their detection of specific compounds provides bioavailability data that is directly relatable to humans and other animals (Struss et al., 2010).

4.1. Available classes of eukaryotic bioreporter proteins

Eukaryotic whole-cell bioreporters are designed using the same fluorescent and bioluminescent systems that have been incorporated into bacterial bioreporters–fluorescent GFP, bioluminescent *lux* and *luc*, and colorimetric *lacZ*–with each presenting more or less the same advantages and disadvantages as that established in their bacterial counterparts. Also, analogous to bacterial bioreporters, their incorporation into eukaryotic hosts can be implemented constitutively wherein the loss of signal indicates a toxic effect or inducibly wherein an increase in signal indicates the presence of a targeted agent. GFP and its variants are commonly used in eukaryotes to detect environmental contaminants, and can allow for parallel detection of multiple contaminants when several fluorescent proteins with non-overlapping emission wavelengths are used (Shibasaki et al., 2001). However, unlike when expressed in bacteria, their application in eukaryotic cells is hindered by the presence of additional naturally fluorescent compounds within the host. This can lead to high levels of background fluorescence under standard imaging conditions, and therefore reduces the detectability of the reporter signal, especially at lower cell population sizes or under conditions of weak induction. Bioluminescent reporter systems, however, are not subject to these high background levels in eukaryotic hosts and are therefore often preferred over their fluorescent counterparts for imaging of smaller cell population sizes or detection of weakly induced signals. The *luc* gene is the most commonly used of the bioluminescent reporters, however, because it requires the addition of a chemical substrate prior to bioluminescent emission, it is not well suited for remote or online monitoring, and can itself be toxic to the eukaryotic host (Hollis et al., 2001). The bacterial *lux* genes were recently optimized for expression under eukaryotic regulatory controls (Close et al., 2010; Gupta et al., 2003). With no substrate addition required, these are the only reporter systems capable of repetitive, real-time signaling, although their resulting bioluminescence emission is not as bright as *luc*.

4.2. Environmental monitoring using lower eukaryotic hosts

Lower eukaryotes, such as yeast, have proven invaluable as biomonitoring tools due to their ease of use, plentiful genetic manipulation techniques, and single celled nature (Walmsley and Keenan, 2000). Combined with their eukaryotic genetic architecture, this makes them logical replacements for bacterial bioreporters when accessing bioavailability or investigating eukaryotic specific metabolic pathways. The most common use of yeast-based environmental bioreporters has been for the detection and bioavailable assessment of estrogenic or androgenic compounds (Table 4). The similarities between yeast and bacterial growth and maintenance, combined with the ability of yeast cells to express the human estrogen receptor (Metzger et al., 1988), has made them a preferred model organism for the detection and measurement of estrogenic/androgenic compounds from environmental samples.

With the optimization and re-engineering of the *lux* cassette to function in *S. cerevisiae* (Gupta et al., 2003), it became possible to use bioluminescent production as a measure of both the presence and bioavailability of estrogenic/androgenic chemicals in a host with relevance to humans. Upon exposure to estrogen, the bioluminescent yeast strains are capable of initiating expression of the *lux* genes, ultimately leading to the production of bioluminescence. When compared with the traditional *lacZ* colorimetric-based assays, the

lux-based system is able to demonstrate similar dynamic ranges (4.5×10^{-11} to 2.8×10^{-9} M for each) and EC_{50} values ($2.4 (\pm 1.0) \times 10^{-10}$ for the *lux* system and $4.4 (\pm 1.1) \times 10^{-10}$ for the colorimetric system), however, the *lux*-based system can do so much faster, producing results in as little as 1 h compared to the minimum of 3 d for the colorimetric system (Eldridge et al., 2007; Sanseverino et al., 2005). In a similarly human-relevant application, Bakhrat et al. (2011) proposed that a modified version of the yeast-based *lux* reporter system be used to evaluate the effectiveness of sunscreens. By replacing the promoter governing expression of the *lux* genes with the DNA damage-responsive UFO1 promoter, they were able to detect changes in the dose of UV radiation received by yeast expressing the full *lux* cassette under different sunscreen protection regimens.

More traditional *luc*-based bioluminescent bioreporters have been incorporated into yeast for the evaluation of heavy metal and other environmental contaminants (Table 4). Lankinen et al. (2011) demonstrated the value of yeast-based biosensing in their comparative analysis of nickel (Ni) responsive fungal, bacterial, and yeast bioreporters. Using fungal bioreporters, it was determined that Ni became cytotoxic at a concentration of 20 mg/L. This was in contrast to the results obtained when using bacterial bioreporters (85 mg Ni/L) and especially that of *luc*-based yeast bioreporters, which did not display a cytotoxic response until Ni concentrations reached 294 mg/L. Had these tests been performed using only one of the bioreporter organisms, it would not have been possible to draw relevant conclusions concerning the bioavailability of Ni across the full array of community members. The inclusion of the yeast bioreporter is specifically relevant, as it provides the best indication of Ni toxicity to humans and other higher animals.

Similar results were demonstrated in a second study that evaluated the detection of polyhalogenated organic pollutants in river sediments (Leskinen et al., 2008). In this study, detection of these chemicals was compared between traditional chemical analysis and *luc*-based biodetection using either yeast or rat cells as bioreporter hosts. The various detection strategies all had varying levels of sensitivity, as well as disparate strengths and weaknesses. The mammalian cell-based bioreporters were determined to have EC_{50} values 15–500 times lower than their yeast-based counterparts, but were not as robust, nor as inexpensive to maintain. As such, the authors suggested that the yeast-based detection system could function as a valuable pre-screening tool for the early detection of polyhalogenated pollutants in environmental samples. Those samples that tested positive for the presence of contaminants with yeast could then be subjected to the more sensitive mammalian or analytical-based detection strategies.

Novel *luc*-based bioreporters for heavy metal sensing were recently constructed in the ciliate *Tetrahymena thermophila* (Amaro et al., 2011). The *luc* reporter gene was fused to heavy metal responsive metallothionein promoters, and these bioreporters emitted increased bioluminescence in proportion to increased heavy metal concentrations. Since ciliates do not have a cell wall in their vegetative state, they more readily take up the chemicals to which they are exposed, and as a consequence display greater sensitivity than their yeast bioreporter counterparts (down to low nanomolar concentrations) and within rapid timeframes (~2 h).

The incorporation of GFP into yeast reporters was uniquely demonstrated by Radhika et al. (2007) in a construct capable of “smelling” the presence of 2,4-dinitrotoluene (DNT), a mimic for the explosive compound trinitrotoluene (TNT). This was accomplished by introducing both the mammalian olfactory receptor Olfr226 and a *gfp* gene under the control of the G-coupled protein receptor responsive CREBP promoter. Upon detection of DNT by the olfactory receptor, a traditional G-coupled protein receptor cascade was initiated that culminated with the activation of the CREBP promoter and expression of GFP. By exciting the reporter cells at set intervals, it was then possible to continually monitor for the presence of DNT in the environment. By altering the specificity of the olfactory receptor, it should also be possible to screen for alternative chemicals, opening up the possibility of a remote sensor array with direct relevance to human cytotoxicity.

4.3. Environmental monitoring using human whole-cell bioreporters

Driven by the same concerns that lead to the evolution from bacterial to yeast and other lower eukaryotic bioreporters, many research groups are choosing to forego the use of yeast as proxies for human relevance and instead opting to survey for environmental contamination directly in a human cellular host. There are several advantages to this strategy, namely, that the bioavailability of the compound being tested should correlate directly to humans as a whole, and that, through the use of multiple human cell types, the effects of the compound in question can be evaluated in light of individual organ systems. This level of specificity and relevance is impractical in bacterial reporters and has allowed human cell-based reporter systems to gain widespread popularity in the biomedical and biotechnology communities.

Just as with yeast-based reporters, the most popular sensing targets for human cell-based bioreporters have been estrogenic and androgenic compounds. Liu and Lu (2011), for example, modulated the expression of GFP through activation of the human estrogen response element in human lung carcinoma cells. While the main target of this investigation was the estrogenic surfactant nonylphenol, this could be considered a proof-in-principle demonstration of their system since the estrogen response elements used should display broad reactivity to many other estrogenic compounds.

The toxic effects of PAHs were evaluated in a high-throughput assay using *luc*-incorporated mammalian cell lines stably transfected with the *luc* gene under the control of a dioxin responsive promoter (Machala et al., 2001). Bioluminescence emission from reporter cells exposed to increasing concentrations of PAHs signaled concentrations at which the toxicants became available to the cell. This methodology allows for rapid detection and screening of multiple conditions simultaneously, and the bright nature and low background of the *luc* system allows for detection of even weakly induced cells.

The incorporation of *lux* within mammalian cells was demonstrated by Close et al. (2012) using human kidney cells constitutively expressing bioluminescence. These investigators were able to demonstrate the bioavailability of a toxic aldehyde compound while simultaneously demonstrating during which time periods of the exposure the target cells were processing the aldehyde. This ability is unique to the autonomous bioluminescent expression offered by the *lux* cassette and, if proven to be a successful technique over time,

should be able to offer researchers increased levels of data collection with minimal changes to their existing experimental protocols.

5. A needs assessment of whole-cell bioreporters for environmental applications

Whole-cell bioreporters have some noteworthy advantages. As self-propagating entities, a substantial number of bioreporters can be easily obtained at low cost, bioreporter assays are fast and simple to perform, they maintain sensitivity that typically meets or exceeds necessary standards, they report on chemical bioavailability rather than mere total concentrations, evolution and nature provide a substantial selection of bioreporters for biosensing needs, and fairly straightforward genetic manipulation techniques allow for desired modifications. Despite these advantages, bioreporters rarely if ever reach commercialization stages or conventional field application status due to several persistent obstacles, foremost of which, from a marketing standpoint, is the inability to patent most bioreporters since the reporter gene technology they incorporate is well substantiated and lacks novelty. Without patent protection, there is little profit to be made and consequent little interest in the pursuit.

The classification of bioreporters as recombinant organisms significantly affects their application capacity in real-world environments. The potential for recombinant DNA to relocate from its original host to other members of the microbial community is a complex and poorly understood event whose consequences may impact environmental and public health safety. Government-mandated restrictions and guidelines strictly limit the introduction of recombinant organisms into the environment, and make the process of moving a bioreporter from the lab to the field a challenging, lengthy, and costly undertaking that typically exceeds the resources of an academic lab and proves unprofitable for a commercial enterprise. As a consequence, real-world bioreporter applications are more focused on biosensors where the bioreporter remains entrapped within a monitoring device and is thus not freely released to the environment (Fleming, 2010). However, the challenge then becomes finding an immobilization or encapsulation matrix that sustains long-term bioreporter cell viability and maintains the bioreporters in an immediately responsive state. Many different types of encapsulation matrices are available, with none yet providing the model long-term, instantly responsive characteristics that are needed (Date et al., 2010). There are clever alternatives, such as using sporulating bacteria as the bioreporter scaffold, which allows the bioreporter to be stored as a spore for exceedingly long durations with germination being triggered upon interaction with key environmental intermediates (Knecht et al., 2011). There are pressing needs for other such alternatives.

The definitive metric of a bioreporter is its sensitivity, and with bioreporters as of yet not approaching the sensitivity (nor specificity) of analytical chemical methods, many end-user applications remain off limits. To improve sensitivity, researchers have focused on finding promoter elements that have evolved greater sensitivity or genetically modifying promoter elements to be more sensitive (Behzadian et al., 2011; Norman et al., 2005; Peng et al., 2010). Innovative synthetic biology techniques will certainly assist in these latter efforts and ultimately propel molecular biological activities towards much needed optimizations and

restructurings of bioreporter elements (see Ghim et al., 2010; Close et al., 2010; Yagur-Kroll and Belkin, 2011 for examples). Many bioreporters also respond to more than a single target, making it difficult to determine the occurrence and quantity of a specifically desired chemical within a complex mixture. Modifications of the transcriptional regulator involved in the sensing pathway can provide a potential method to improve specificity. Ambiguousness in chemical identification caused by low specificity may even be resolvable without actually improving the bioreporter itself. As demonstrated by several groups, testing with an array of different bioreporters in combination with pattern learning algorithms or decision tree models can potentially identify a chemical by the unique pattern or 'fingerprint' of bioreporter signals (Elad et al., 2008; Jouanneau et al., 2011). It is still important to point out that bioreporters are not intended to fully replace chemical analytical methods. On the contrary, bioreporters serve as an ideal complementary chemical analysis approach. Environmental monitoring ordinarily occurs over large geographic areas, and bioreporter assays very effectively provide rapid 'snapshots' of contaminant presence/absence, thus delineating where further, more precise analytical sampling should and, more importantly, should not occur. It is this movement away from blind sampling that reduces the exorbitant costs associated with analytical methods that often yield large numbers of unproductive samples simply labeled as 'below detection limits'.

6. Conclusion

Whole-cell bioreporters, whether existing within the walls of the laboratory or applied as bona fide environmental sensors, have proven to be popular and practical tools for the detection and monitoring of contaminants of ecological concern. Considering the advantages of small size, massive population numbers, robustness, adaptability, and information processing power that even on a per-cell basis exceed current silicon-based technologies, it is clear that living cells are particularly well suited for biosensing applications. Additionally, with researchers endlessly fine-tuning the genetics and reporter gene scaffolding within bioreporter organisms, the biosensing attributes of speed, sensitivity, and specificity will continue to improve. More of a concern at this juncture is how one deals with the massive data downloads that will emanate from collections of bioreporter organisms each continuously and unremittingly monitoring a specific chemical or chemical interaction over hours, days, months, or perhaps even years. The scale of these resulting databases will be unmanageable using the current toolbox of data management software, and we will begin entering the deluge of 'data-intensive' science that has been pre-warned by Bell et al. (2009) and Hey et al. (2009). The challenge may therefore not lie within the bioreporter's ability to successfully gather and communicate data, but in our ability to process and meaningfully interpret what the bioreporter is trying to tell us.

Acknowledgments

Portions of this review reflecting work by the authors were supported by the National Science Foundation Division of Chemical, Bioengineering, Environmental, and Transport Systems under award number CBET-0853780, the Division of Biological Infrastructure under award number DBI-0963854, the National Institutes of Health, National Cancer Institute, Cancer Imaging Program under award number CA127745-01, the USDA National Institute of Food and Agriculture Biotechnology Risk Assessment Program under grant number 2009-39210-20230, and the Army Defense University Research Instrumentation Program.

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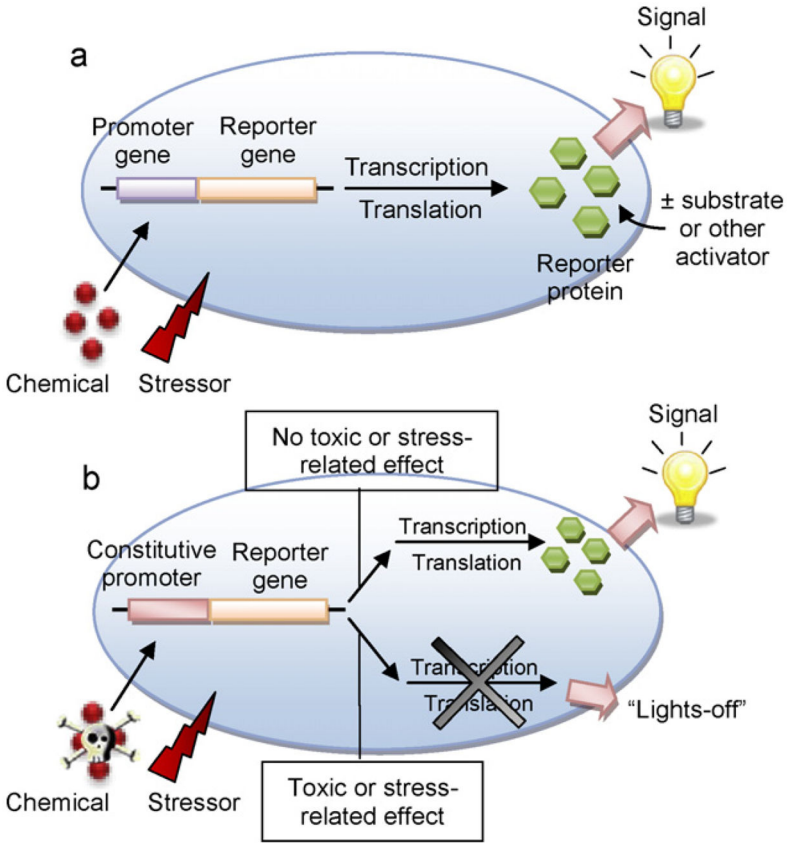

Fig. 1.

The fundamentals of whole-cell bioreporter sensing. (a) A 'lights-on' bioreporter emits light when its promoter gene is activated upon exposure to a target of interest, and can function either specifically to identify a chemical or chemical class or nonspecifically to identify a toxic or stress-related cellular interaction. (b) A 'lights-off' bioreporter emits light continuously and signals the presence of a toxic chemical or stress-related interaction with its constitutive promoter via a reduction in its light emission intensity.

Table 1

Field applications of bacterial bioreporters for heavy metal bioavailability assessment.

Bioreporter					**Field application**		
Host strain	**Reporter construct**	**Analyte**	**Response time**	**Detection limit**	**Matrix**	**Response time**	**Reference**
Bacillus subtilis BR151 (pTOO24) (Tauriainen et al., 1998)	*cadC-luc*	Cd	3 h	3.3 nM (Cd) (Tauriainen et al., 1998) 3.7 μg/L (Cd) (Hakkila et al., 2004)	Sediment (water suspension and water extract) Soil (water suspension and water extract)	2 h 2 h	Hakkila et al. (2004) Ivask et al. (2011) Ivask et al. (2004)
E. coli CM1166 (pC200) (Corbisier et al., 1993)	*ars-luxAB*	As, Sb	1 h	<0.1 mg/L (Flynn et al., 2002)	Soil and sediment (water extract)	1 h	Flynn et al. (2002, 2003)
E. coli DH5α (pVLCD1) (Liao et al., 2006)	*cadC-gfp*	Cd, Pb, Sb	2 h	0.1 nM (Cd(II)), 10 nM (Pb(II)), 0.1 nM (Sb(III)) (Liao et al., 2006)	Sediment and soil (water extract)	2 h	Liao et al. (2006)
E. coli DH5α (pJAMA-arsR) (Stocker et al., 2003)	*arsR-luxAB*	As	0.5 h	4–7 μg/L (Stocker et al., 2003; Trang et al., 2005)	Groundwater Tap water	1.5 h 1 h	Trang et al. (2005) Stocker et al. (2003)
E. coli JM109 (pNM2) (Larose et al., 2011)	*merRT-luxCDABE*	Hg	2 h	0.5 ng/L (Larose et al., 2011)	Snowpack	2 h	Larose et al. (2011)
E. coli MC1061 (parsR*luxCDABE*) (Hakkila et al., 2002)	*arsR-luxCDABE*	As	3–4 h	141 μg/L (As(V)), 18 μg/L (As(III)) (fiber-optic immobilized); 80 μg/L (As(V)), 8 μg/L (As(III)) (free bacteria) (Ivask et al., 2007)	Sediment (water suspension)	3–4 h	Ivask et al. (2007)
E. coli MC1061 (pmerBR$_{BS}$ luc) (Ivask et al., 2001)	*merRB-luc*	Organic Hg	2 h	0.2 nM (methyl Hg), 1 nM (phenyl Hg), 10 μM (dimethyl Hg) (Ivask et al., 2001)	Sediment (water suspension)	2 h	Hakkila et al. (2004)
E. coli MC1061 (pmerR*luxCDABE*) (Hakkila et al., 2002)	*merR-luxCDABE*	Hg	2–3 h	2.6–6.3 μg/L (immobilized) (Hakkila et al., 2004; Ivask et al., 2007); 0.03 μg/L (free) (Ivask et al., 2007)	Sediment (water suspension)	2–3 h	Hakkila et al. (2004), Ivask et al. (2007)
E. coli MC1061 (pSL*cueR*/ pDNP*copAluc*) (Hakkila et al., 2004)	*cueR*, copA-*luc*	Cu	2 h	NR	Sediment (water suspension)	2 h	Hakkila et al. (2004)
E. coli MC1061 (pTOO11) (Virta et al., 1995)	*merR-luc*	Hg	1–2 h	0.1 fM (Virta et al., 1995); 6.7 mg/L (Hakkila et al., 2004)	Sediment (water suspension)	2 h	Hakkila et al. (2004),

Bioreporter					Field application		
Host strain	**Reporter construct**	**Analyte**	**Response time**	**Detection limit**	**Matrix**	**Response time**	**Reference**
							Lappalainen et al. (2000), Lappalainen et al. (2000)
E. coli MC1061 (pTOO31) (Tauriainen et al., 1999)	*arsR-luc*	As	1 h	33 nM ($NaAsO_2$), 33 μM (Na_2 $HAsO_4$) (Tauriainen et al., 1999); 6.1 μg/L (Hakkila et al., 2004)	Sediment (water suspension) Soil (water suspension and water extract)	2 h 2 h	Hakkila et al. (2004) Turpeinen et al. (2003)
P. fluorescens DF57-Cu15 (Tom-Petersen et al., 2001)	Cu-inducible promoter-*luxAB*	Cu	1.5 h	0.005 mg/L (Cu^{2+}) (Maderova et al., 2011)	Soil (water suspension and water extract)	1.5 h	Brandt et al. (2006, 2008), Maderova et al. (2011)
Ralstonia eutropha AE 1239 (Corbisier et al., 1996)	*cop-luxCDABE*	Cu	NR	NR	Incinerator fly-ashes (acetate extract and medium suspension)	4–5 h	Corbisier et al. (1996)
R. metallidurans AE1433 (Corbisier et al., 1996)	*cupS-luxCDABE*	Pb, Zn, Cd, Co	5 h	0.4 ppm (Pb) (Magrisso et al., 2009)	Soil (water suspension)	3 h	Magrisso et al. (2009)
R. metallidurans AE2515 (Tibazarwa et al., 2001)	*cnrYXH-luxCDABE*	Ni, Co	4–6 h	0.1 μM (Ni), 9 μM (Co) (Tibazarwa et al., 2001)	Soil (medium suspension)	Continuous monitoring	Everhart et al. (2006)
Staphylococcus aureus RN4220 (pTOO24) (Tauriainen et al., 1998)	*cadC-luc*	Cd, Pb	2 h	10 nM (Cd), 33 nM (Pb) (Tauriainen et al., 1998) 1 nM (Sn)	Sediment (water suspension) Soil (water suspension and water extract) Soil (water extract)	2 h 2 h 2 h	Hakkila et al. (2004) Ivask et al. (2004) Turpeinen et al. (2000)
Synechococcus sp. CCMP 2669 (Boyanapalli et al., 2007)	P_{isiAB}-*luxAB*	Fe^{3+}	12 h	1 nM (Boyanapalli et al., 2007)	Seawater	12 h	Boyanapalli et al. (2007), Breitbarth et al. (2009)
Synechococcus sp. KAS101 (Durham et al., 2002)	P_{isiAB}-*luxAB*	Fe^{3+}	12 h	0.45 nM (Hassler et al., 2006)	Lake water	12 h	Breitbarth et al. (2009), Hassler et al. (2009), McKay et al. (2005), Porta et al. (2005)
Synechocystis sp. PCC 6803 *coaLux* (Peca et al., 2008)	*coaR*-P_{coaT}-*luxAB*	Co	3 h	0.3 μM (Co^{2+}) (Peca et al., 2008)	Soil (acetate extract)	3 h	Peca et al. (2008)
Synechocystis sp. PCC 6803 *nrsLux* (Peca et al., 2008)	*nrsR*-$P_{nrsBACD}$-*luxAB*	Ni	3 h	0.2 μM (Ni^{2+}) (Peca et al., 2008)	Soil (acetate extract)	3 h	Peca et al. (2008)

NR: not reported.

Table 2

Applications of bacterial bioreporters for monitoring organic pollutants in environmental samples.

Bioreporter					Field application		
Host strain	**Reporter construct**	**Analyte**	**Response time**	**Detection limit**	**Matrix**	**Response time**	**Reference**
Burkholderia sartisoli RP007 (pPROBE-*phn-luxAB*) (Tecon et al., 2010)	*phn-luxAB*	Naphthalene, phenanthrene	3 h	0.17 μM (naphthalene) (Tecon et al., 2010)	Seawater	3 h	Tecon et al. (2010)
E. coli DH5α (pGEc74, pJAMA7) (Sticher et al., 1997)	*alkS*, P_{alkB}-*luxAB*	C_6-C_{10} alkane	15–75 min	24.5 nM (octane) (Sticher et al., 1997)	Soil (DMSO or water extract) Groundwater Seawater	1 h 2 h 2 h	Bundy et al. (2001) Bhattacharyya et al. (2005) Tecon et al. (2010)
E. coli DH5α (pHYBP103M3) (Beggah et al., 2008)	*hp*	2-hydroxylbiphenyl and biphenyl	2 h	0.30 μM (2-hydroxylbiphenyl) (Tecon et al., 2010)	Seawater	2 h	Tecon et al. (2010)
E. coli DH5α (pHYBP109) (Jaspers et al., 2000)	*hbpR*-P_{hbpC}-*luxAB*	2-hydroxylbiphenyl	2 h	0.30 μM (2-hydroxylbiphenyl) (Tecon et al., 2010)	Seawater	2 h	Tecon et al. (2010)
E. coli DH5α (pLZCapR) (Shin et al., 2005)	*capR-lacZ*	Phenolic compounds	5–7 h	0.1 μM (phenol) (Shin et al., 2005)	Hospital water Soil (water extract)	5–7 h 5–7 h	Shin et al. (2005) Shin et al. (2005)
E. coli DH5α (pPROBE-LuxAB-TbuT) (Tecon et al., 2010)	*tbuT-luxAB*	BTEX	2 h	0.24 μM (toluene) (Tecon et al., 2010)	Seawater	2 h	Tecon et al. (2010)
E. coli HMS174 (pOS25) (Selifonova and Eaton, 1996)	*ipbR-luxCDABE*	Hydrophobic compounds	100–250 min	Varies among compounds (Selifonova and Eaton, 1996)	Sediment (ethanol extract) Soil (HPCD extract)	100–250 min 2 h	Selifonova and Eaton (1996) Diplock et al. (2009)
E. coli pGLTUR (Willardson et al., 1998)	*xylR*-P_u-*luc*	Toluene	1 h	10–20 μM (Willardson et al., 1998)	Soil (ethanol extract) and deep aquifer water Soil (DMSO or water extract)	1 h 1 h	Willardson et al. (1998) Bundy et al. (2001)
P. fluorescens HK44 (pUTK21) (King et al., 1990)	*nahR*-P_{nahG}-*luxCDABE*	Naphthalene	8–24 min	12–120 μM (Heitzer et al., 1992)	Soil (DMSO and water extract) Soil Soil (methanol and HPCD extract)	1 h in situ monitoring 1 h	Bundy et al. (2001) Ripp et al. (2000) Paton et al. (2009)
P. fluorescens OS8 (pDN*dmpRlux*) (Leedjarv et al., 2006)	*dmpR*-P_o-*luxCDABE*	Phenolic compounds	4 h	0.08 mg/L (phenol), 0.03 mg/L (2-methylphenol) (Leedjarv et al., 2006)	Groundwater and dump leachate	4 h	Leedjarv et al. (2006)

Bioreporter					Field application		
Host strain	**Reporter construct**	**Analyte**	**Response time**	**Detection limit**	**Matrix**	**Response time**	**Reference**
P. putida TVA8 (Applegate et al., 1998)	*tod-luxCDABE* (Ch)	BTEX	1–4 h	0.03–50 mg/L (Applegate et al., 1998)	Soil (HPCD extract) Soil (water extract) Groundwater Wastewater and groundwater	30 min 2 h 2 h Continuous monitoring	Diplock et al. (2009) Dawson et al. (2008) Bhattacharyya et al. (2005) Kuncova et al. (2011)
Ralstonia eutropha JMP 134-32 (Hay et al., 2000)	*tfdRP*$_{DII}$*-luxCDABE*	2,4-Dichlorophen-oxyacetic acid	20– 60 min	2.0 μM	Soil	1 h	Toba and Hay (2005)

Ch: integrated into the chromosome.

Table 3

Environmental toxicity monitoring using genetically modified bacterial bioreporters.

Bioreporter			**Field application**		
Host strain	**Reporter construct**	**Effector**	**Matrix**	**Pollutants**	**Reference**
Acinetobacter baylyi ADP1 recA-lux (Song et al., 2009)	*recA-luxCDABE*	DNA damage	Groundwater	Phenolic compounds	Song et al. (2009)
Acinetobacter DF4/pUTK2 (Abd-El-Haleem et al., 2006)	*luxCDABE*	General	Wastewater	Metals	Abd-El-Haleem et al. (2006)
B. subtilis BR151 (pCSS962/pBL1) (Lampinen et al., 1992)	*luxCDABE*	General	Soil (water suspension and water extract)	Cd	Ivask et al. (2011, 2004)
E. coli DPD2511 (Belkin et al., 1996)	*katG-luxCDABE*	Oxidative stress	Water source from treatment plant and stream water	Not specified	Gu et al. (2001)
E. coli DPD2540 (Bechor et al., 2002)	*fabA-luxCDABE*	Membrane damage	Water source from treatment plant and stream water	Not specified	Gu et al. (2001)
E. coli DPD2794 (Vollmer et al., 1997)	*recA-luxCDABE*	DNA damage	Tap water and river water Water source from treatment plant and stream water	Not specified Not specified	Eltzov et al. (2009) Gu et al. (2001)
E. coli HB101 (pUCD607) (Rattray et al., 1990)	*luxCDABE*	General	Groundwater Water source from treatment plant and stream water Sediment and soil Soil (water or solvent extract) Soil (water extract) Soil (water extract)	CAH Not specified As and Sb Hydrocarbon Cu and Ni Phenyltin compounds	Bhattacharyya et al. (2005) Gu et al. (2001) Flynn et al. (2002) Flynn et al. (2003) Bundy et al. (2001), Diplock et al. (2009) Paton et al. (2006a) Paton et al. (2006b)
E. coli MC1061 (pTOO02) (Hakkila et al., 2004)	*luc*	General	Sediment (water suspension)	Metals	Hakkila et al. (2004)
E. coli MG1655 (pJAMA8-*cda*) (Tecon et al., 2010)	*cda-luxAB*	DNA damage	Seawater	Hydrocarbon	Tecon et al. (2010)
E. coli TV1061 (Van Dyk et al., 1994)	*grpE-luxCDABE*	Protein damage	Tap water and river water Water source from treatment plant and stream water	Not specified Not specified	Eltzov et al. (2009) Gu et al. (2001)
P. fluorescens 10586 (pUCD607) (Aminhanjani et al., 1993)	*luxCDABE*	General	Groundwater Sediment and soil Soil (water extract) Soil (water extract) Sediment and groundwater	CAH As and Sb Cu and Ni Phenyltin compounds BTEX	Bhattacharyya et al. (2005) Flynn et al. (2002) Flynn et al. (2003) Paton et al. (2006a) Paton et al. (2006b) Sousa et al. (1998)
P. putida F1 (pUCD607) (Weitz et al., 2001)	*luxCDABE*	General	Soil (water or solvent extract)	Hydrocarbon	Bundy et al. (2001), Dawson et al. (2008), Diplock et al. (2009)

Bioreporter			Field application		
Host strain	**Reporter construct**	**Effector**	**Matrix**	**Pollutants**	**Reference**
P. fluorescens DF57-40E7 (Tom-Petersen et al., 2001)	*luxAB*	General	Soil (water suspension and extract)	Cu	Brandt et al. (2006, 2008), Maderova et al. (2011)
P. fluorescens OS8 (pDNLux) (Leedjarv et al., 2006)	*luxCDABE*	General	Groundwater and dump leachate	Phenolic compounds	Leedjarv et al. (2006)
P. fluorescens Shk1 (Kelly et al., 1999)	*luxCDABE*	General	Wastewater	Metals	Kelly et al. (2004), Lajoie et al. (2003)
S. aureus RN4220 (pTOO02) (Ivask et al., 2004)	*luc*	General	Soil	Pb and Cd	Ivask et al. (2004)
Salmonella typhimurium TA1535 (pSWITCH) (Baumstark-Khan et al., 2005)	P_{colD}-*luxCDABE*/P_{lac}-*gfpuv* (bicistronic)	General and DNA damage	Groundwater	Not specified	Baumstark-Khan et al. (2005)

Table 4

Selected environmental applications of eukaryotic bioreporters.

Bioreporter					Field application		
Host strain or cell line	**Reporter construct**	**Analyte**	**Response time**	**EC_{50}/Detection limit**	**Matrix**	**Response time**	**Reference**
S. cerevisiae/AhR (Leskinen et al., 2008)	DRE-*luc*	AhR ligands	3.5 h	5.5 nM DHT/0.5 nM DHT	Sediment	3.5 h	Leskinen et al. (2008)
Rat hepatoma cell line H4IIE DR-CALUX (Murk et al., 1996)	DRE-*luc*	AhR ligands	24 h	0.01 nM TCDD/0.5 fM TCDD	Sediment Soil Indoor dust	24 h 24 h 24 h	Dindal et al. (2007), Hilscherova et al. (2010), Houtman et al. (2006), Keiter et al. (2008), Murk et al. (1996), Wolz et al. (2008) Andersson et al. (2009), Sidlova et al. (2009) Suzuki et al. (2010)
Human breast carcinoma cell line MDA-kb2 (Wilson et al., 2002)	ARE-*luc*	Androgenic activity	Overnight	NR/0.1 nM DHT	Water	24 h	He et al. (2011)
S. cerevisiae hERα (Gaido et al., 1997)	ERE-*lacZ*	Estrogenic activity	20 h	3.5 nM DHT/2.19 nM DHT	Wastewater	20 h	Nakada et al. (2004), Nelson et al. (2007)
S. cerevisiae BLYES (Sanseverino et al., 2005)	ERE-*lux*	Estrogenic activity	4–6 h	0.24 nM E2/0.045 nM E2	Surface water and drinking water	4–6 h	Bergamasco et al. (2011)
S. cerevisiae hERα (Leskinen et al., 2005)	ERE-*luc*	Estrogenic activity	2.5 h	0.5 nM E2/0.03 nM E2	Wastewater	2.5 h	Salste et al. (2007)
S. cerevisiae hER YES (yeast estrogen screen) (Routledge and Sumpter, 1996)	ERE-*lacZ*	Estrogenic activity	72 h	0.44 nM E2/0.045 nM E2 (Sanseverino et al., 2005)	Wastewater Sediment Surface and ground water Sewage	72 h 24 h 72–96 h 72 h	Fernandez et al. (2007), Nelson et al. (2007), Thorpe et al. (2006) Grund et al. (2011) Aneck-Hahn et al. (2008), Beek et al. (2006), Leusch et al. (2010) Leusch et al. (2010)
S. cerevisiae hERα (Balsiger et al., 2010)	ERE-*lacZ*	Estrogenic activity	2 h	0.145 nM E2/100–200 pM	Wastewater Sediment	2 h 2 h	Balsiger et al. (2010) Jeffries et al. (2011)
Human breast carcinoma cell line MCF-7 MELN (Balaguer et al., 1999)	ERE-*luc*	Estrogenic activity	16 h	5 pM E2/1 pM E2	Sediment Sewage Wastewater Surface and ground water	16 h 16 h 16 h 16 h	Louiz et al. (2008) Hernandez-Raquet et al. (2007), Leusch et al. (2010) Cargouet et al. (2004), Dagnino et al. (2010) Cargouet et al. (2007) Cargouet et al. (2004), Leusch et al. (2010)
Human breast carcinoma cell line T47D-KBluc (Wilson et al., 2004)	ERE-*luc*	Estrogenic activity	24 h	0.01 nM E2/1 pM E2	Wastewater Water Sewage	24 h 24 h 24 h	Wehmas et al. (2011) He et al. (2011), Leusch et al. (2010) Leusch et al. (2010)

Bioreporter					Field application		
Host strain or cell line	**Reporter construct**	**Analyte**	**Response time**	**EC_{50}/Detection limit**	**Matrix**	**Response time**	**Reference**
S. cerevisiae BMA64/luc (Leskinen et al., 2005)	*luc*	Toxicity	0.5 h	NR	Soil	0.5 h	Lankinen et al. (2011)

hER: human estrogen receptor; hAR: human androgen receptor; AhR: acyl hydrocarbon receptor; ERE: estrogen response element; ARE: androgen response element; DRE: dioxin response element; E2: 17β-estradiol; T: 17β-testosterone; DHT: dihydrotestosterone; TCDD: 2,3,7,8-tetrachlorodibenzo-*p*-dioxin; NR: not reported.