Application of Bioanalytical Tools for Water Quality Assessment

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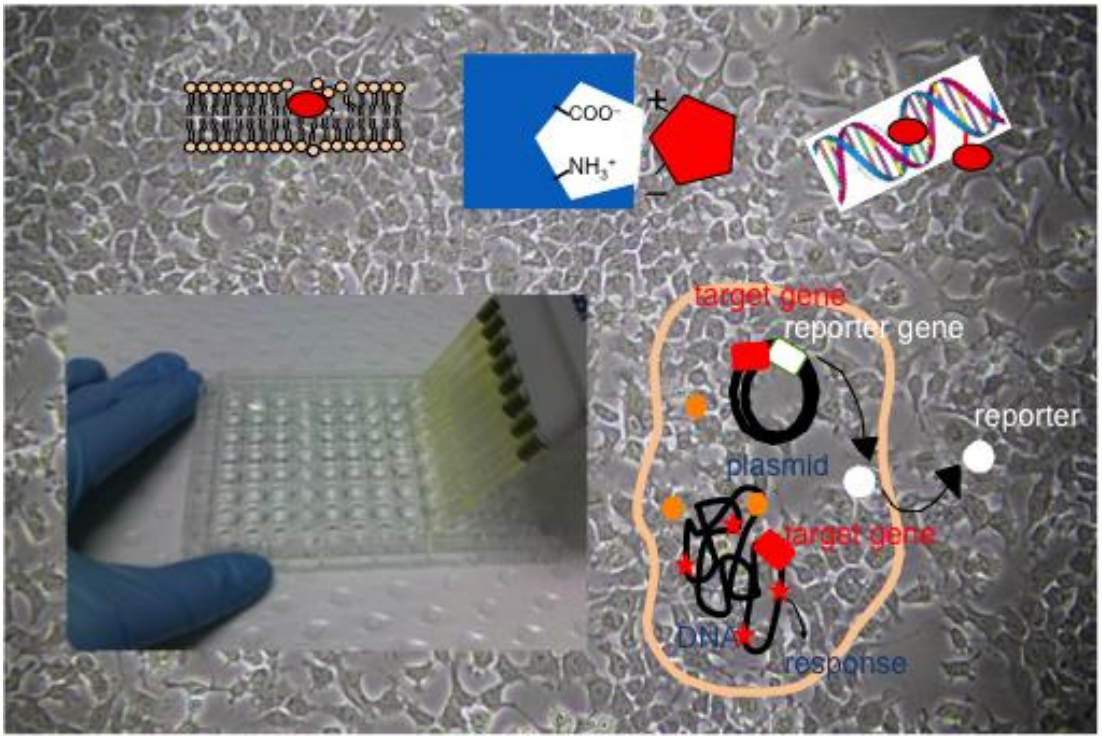

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The Urban Water Security Research Alliance (UWSRA) is a $50 million partnership over five years between the Queensland Government, CSIRO's Water for a Healthy Country Flagship, Griffith University and The University of Queensland. The Alliance has been formed to address South East Queensland's emerging urban water issues with a focus on water security and recycling. The program will bring new research capacity to South East Queensland tailored to tackling existing and anticipated future issues to inform the implementation of the Water Strategy.

For more information about the:

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Description: Collage: Cells, plating a 96-well plate and principle of a reporter gene assay.

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FOREWORD

Water is fundamental to our quality of life, to economic growth and to the environment. With its booming economy and growing population, Australia's South East Queensland (SEQ) region faces increasing pressure on its water resources. These pressures are compounded by the impact of climate variability and accelerating climate change.

The Urban Water Security Research Alliance, through targeted, multidisciplinary research initiatives, has been formed to address the region's emerging urban water issues.

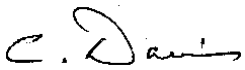
As the largest regionally focused urban water research program in Australia, the Alliance is focused on water security and recycling, but will align research where appropriate with other water research programs such as those of other SEQ water agencies, CSIRO's Water for a Healthy Country National Research Flagship, Water Quality Research Australia, eWater CRC and the Water Services Association of Australia (WSAA).

The Alliance is a partnership between the Queensland Government, CSIRO's Water for a Healthy Country National Research Flagship, The University of Queensland and Griffith University. It brings new research capacity to SEQ, tailored to tackling existing and anticipated future risks, assumptions and uncertainties facing water supply strategy. It is a $50 million partnership over five years.

Alliance research is examining fundamental issues necessary to deliver the region's water needs, including:

- ensuring the reliability and safety of recycled water systems.
- advising on infrastructure and technology for the recycling of wastewater and stormwater.
- building scientific knowledge into the management of health and safety risks in the water supply system.
- increasing community confidence in the future of water supply.

This report is part of a series summarising the output from the Urban Water Security Research Alliance. All reports and additional information about the Alliance can be found at http://www.urbanwateralliance.org.au/about.html.



Chris Davis
Chair, Urban Water Security Research Alliance

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EXECUTIVE SUMMARY

As part of the South East Queensland Urban Water Security Research Alliance, this report reviews historical and current application of bioanalytical tools for monitoring of water quality. The aim of the review is to provide an up to date evaluation of the state of research within this field in Australia and New Zealand. Basic theory and concepts are detailed before worldwide applications are outlined to set the local resources into global context.

For the purpose of this review, bioanalytical tools are defined as *in vitro* cell-based and low complexity *in vivo* bioassays indicative of modes of toxic action that are relevant for human and/or environmental health. These include whole cell and reporter gene assays, tests with unicellular organisms as well as enzyme assays. Existing assays are classified into three major groups of modes of toxic action; non-specific, specific and reactive toxicity. Non-specific toxicity assays are crucial in providing an estimate of the overall toxic burden of all chemicals within a mixture and include the very popular Microtox® assay and all cell viability/proliferation assays. Specific toxicity assays target particular toxicant groups through detection of specific endpoints. Typical bioassays applied for monitoring of specific toxicity include reporter gene assays capable of detecting the induction of nuclear receptors, such as the estrogen, androgen, thyroid, aryl-hydrocarbon and retinoic acid receptor. Bioanalytical capacities are advancing worldwide and in Australia by covering more receptor-mediated toxicity endpoints.

Reactive toxicity includes any mode of action that involves the chemical reaction between chemicals and biological molecules, including DNA damage (genotoxicity, mutagenicity), reactivity towards proteins, peptides and lipids as well as oxidative stress. Until recently, the main focus has been on detection of genotoxicity using the classic Ames test and assays indicative of the SOS response, e.g. the *umuC* and SOS Chromo tests. Lately, some genotoxicity tests based on mammalian cell lines with detection of DNA damage using the COMET assay and/or the micronucleus assay have also been introduced to water quality testing. Test battery type approaches combine a number of assays within and/or across the above categories, enabling a more comprehensive characterisation of various aspects of toxicity.

Cell-based assays have been applied for monitoring of water quality worldwide since the 1970s. Most research has focused on surface waters and domestic and industrial wastewaters. Scattered studies on pulp and paper mill effluents as well as oilfield produced effluents are also found in the literature and, in recent years, screening of advanced water treatment processes, disinfected drinking water and recreational waters have emerged. Improved sample preparation and sample enrichment methods, as well as the introduction of more sensitive bioassay endpoints, have allowed progression from highly contaminated water samples to high quality water such as recycled water and drinking water.

Overall, Australia and New Zealand are well-positioned, active players in this increasingly important field of research. Bioanalytical tools are routinely applied throughout the region for water quality monitoring and for surveillance of the efficacy of treatment processes.

1. INTRODUCTION

1.1. Background

Organic micropollutants make up a group of man-made chemicals including pesticides, industrial chemicals, consumer products and pharmaceuticals, but also include natural compounds such as hormones (Schwarzenbach *et al.*, 2006). The widespread distribution of organic micropollutants in our waterways poses a threat to aquatic life and to humans through the consumption of food and drinking water. Micropollutants enter the aquatic environment via direct sources such as agriculture, industry and through sewage effluent discharge. Due to the complex nature of the chemical mixtures present in the source water for water recycling schemes, conventional wastewater treatment is not always sufficient to remove the entire contaminant load. Disinfection steps such as ozonation and chlorination are used to enhance the control of human pathogens. While biological and advanced treatment processes are very effective in eliminating some unwanted pathogens, they may introduce other potentially harmful substances such as disinfection by-products and transformation products.

Chemical monitoring provides a quantitative assessment of single contaminant concentrations in a water sample but cannot account for unknown compounds including most transformation products. Effect-based monitoring complements chemical analysis. Classical ecotoxicological tests used in water quality assessment include *in vivo* fish and aquatic invertebrate assays that measure e.g. mortality, growth and feeding responses. The sensitivity of *in vivo* tests has greatly improved with the development of e.g. the zebrafish embryo toxicity test, which is also considered to be of higher ethical preference than adult fish tests. Fish and invertebrate species are, however, not appropriate models for mammalian toxicology, which is more relevant for human exposure scenarios (e.g. drinking water). *In vitro* molecular and cell-based assays are sensitive, cost- and time-effective alternatives to whole animal testing. Implementation of human and other mammalian cell lines has facilitated evaluation of toxicological endpoints relevant for human health risk assessment.

For the purpose of this review, we define bioanalytical tools as cell-based and low-complexity *in vivo* bioassays indicative of specified endpoints that are relevant for human and/or environmental health. These include whole cell and reporter gene based cell assays and tests with unicellular organisms and enzymes, but excludes immunoassays unless in combination with a cell bioassay. Studies that rely on passive sampling are generally not included, although a few examples are used to illustrate the development of bioanalytical tool applications by the project partners.

A major advantage of bioanalytical tools is their capability of detecting the combined toxicity of mixtures of known and unknown compounds with the same toxic mode of action, whereas chemical analysis can only quantify known, targeted chemicals. By measuring the sum toxicity of a water sample, the bioassay approach is also more risk-oriented as it explicitly accounts for the differential in toxicity across different chemicals. A suite of bioassays further enables toxicity assessment across multiple compound groups of varying toxic modes of action. In this way, a comprehensive bioanalytical test battery provides an integrated measure of the toxicity of all or many of the biologically active substances in a water sample.

1.2. Objectives

This review details current applications and recent developments of bioanalytical tools for water quality assessment, with a focus on *in vitro* cell-based assays. The objectives of the review are to:

- describe current application of bioanalytical tools for water quality assessment in Australia within a global context;
- outline local (Australia and New Zealand) resources for water quality assessment; and
- identify key strengths and limitations in the field.

Following a brief introduction to the principles and use of cell-based assays in water quality monitoring, a review of worldwide research in the field to date sets local activities into a global context. Australian and New Zealand applications and developments are then reviewed, starting with research conducted by the project partners, Griffith University and The University of Queensland, before expanding to research carried out by institutions throughout the region.

2. BIOANALYTICAL TOOLS

For the purpose of this review, we define bioanalytical tools as low-complexity *in vivo* and *in vitro* bioassays used to measure the toxicity of single chemicals or mixtures of known and unknown organic micropollutants and their transformation products. Previous reviews had wider or narrower definition. For example, Behnisch *et al.*(2001) included biomarkers and enzyme immunoassays (EIA, ELISA), while Eggen and Segner (2003) only included assays that describe a defined chemical-biological interaction excluding general cytotoxicity assays.

***In vivo* assays** are whole organism exposure tests used to determine the toxicity of a chemical(s) or effluent of interest to a target organism and/or tissues/organs/cells of the organism. ***In vitro* assays** are often cell-based but also include isolated tissue (e.g. metabolically active liver homogenate) and enzyme extracts. As cell lines (e.g. mammalian, fish, yeast and bacteria) can be obtained and grown without sacrificing test animals, molecular and cell-based assays have the advantage of being of low ethical cost compared to *in vivo* assays (Blaauboer, 2002; Hartung, 2010). Due to the high volume requirements of many *in vivo* assays, these are most suitable for exposure to whole effluent or spiked water solutions. Cell-based assays generally require less space (lower volumes) and are often more practical for assessment of less contaminated environmental samples for which sample concentration and clean-up are prerequisites (further detailed in Section 3). Additional advantages of cell-based assays are their high sensitivity and time- and cost-effectiveness. Some *in vivo* assays share some of the advantages of *in vitro* assays. The fish embryo test, for instance, is a recommended alternative to traditional ecotoxicological protocols, although its replacement of the standard fish acute toxicity test is still to be validated (Embry *et al.,* 2010). *In vivo* biomarkers such as vitellogenin, a marker for estrogenicity, have also proven sensitive and informative indicators of endocrine disruption (Purdom *et al.,* 1994). Yet, while *in vivo* assays are valuable for ecotoxicological assessment, applications for monitoring of water quality are generally limited to whole effluent testing and low-complexity assays including those based on biomarker responses. Use of *in vitro* bioassays in monitoring programs to date have been limited due to difficulty in determining their relevance to well-established *in vivo* toxicity tests and predicting effects in whole organisms. Recent advances in molecular toxicology and system biology, which were taken up by the Tox21 program of the National Institute of Health jointly with the United States Environmental Protection Agency (US EPA) (Gibb, 2008), have, however, led to a paradigm shift (Hartung, 2010). *In vitro* bioassays now gain acceptance provided an (ideally mechanistic) *in vitro* to *in vivo* extrapolation model exists.

Cell-based bioassays target particular endpoints or mechanisms of toxicity and can be divided into two groups:

- Bioassays with primary cells and cell lines; and
- Bioassays with recombinant cell lines.

Native cells typically respond to all chemicals in a given sample and are suitable for assessment of non-specific toxicity. Non-specific toxicity is typically measured in cytotoxicity tests that quantify cell growth/viability. Cytotoxicity assays can be more specific if cells (be it primary cells or cell lines) are derived from particular tissues, e.g. pulmonary epithelial cells or liver cells. The differential toxicity between different cell types can further give an indication of the mode of action of the chemicals in the sample. Some cells react specifically to groups of chemicals with common modes of toxic action by expressing a specific physiological response, e.g. direct inhibition of photosynthesis in algae or proliferation of breast cancer cells in the presence of estrogens.

Recombinant cell bioassays have emerged in the last few years to detect and amplify specific responses. Examples include hormone-mimetic activity or induction of the aryl-hydrocarbon receptor. The general design of recombinant cell bioassays is the integration of a reporter plasmid into a cell (e.g. human or mammalian immortal cell line), which carries a responsive element for a certain receptor followed by a reporter gene that encodes a measurable feature such as an enzyme (e.g. β-galactosidase) or the green fluorescent protein. The amount of response quantified via the enzyme activity or the fluorescence intensity of the green fluorescent protein is proportional to the amount of chemical bound to the receptor.

Most cell-based assays target a particular mode of toxic action and/or a particular recipient (e.g. human vs. fish cell line). Comprehensive risk assessment thus requires a battery of bioassays in order to cover all or many modes of toxic action and/or recipients relevant for the water sample of interest. Two distinct approaches can be applied to design a test battery; one is driven by consideration of the protection goal, while the other is driven by detection of chemical groups of concern.

I. Protection-goal oriented test battery design

A protection goal can be to minimise human cancer occurrences or to ensure healthy fish reproduction. Protection goals set the context for all chemical risk assessment legislation and are often translated into specific assessment endpoints. Depending on the protection goal, the test battery must be designed to include the relevant assessment endpoints. In selecting a suite of bioassays, it is important to consider what we seek to protect (e.g. human health vs. aquatic ecosystem health, marine species vs. freshwater species) as well as the suite of tools necessary to conduct the assessment. The most appropriate exposure route (and recipient tissue) must be carefully selected. If exposure to humans is via drinking water, for instance, the oral route is the most significant exposure pathway. If, on the other hand, exposure is to recreational water via swimming, dermal contact is more likely to govern, with limited exposure via ingestion. When the relevant organism(s) and the potential risks posed to that organism(s) are determined, the associated bioassays can be selected. Assays to be included will be specific to protection goals identified as part of hazard identification within a risk assessment framework.

II. Chemical oriented test battery design

Chemicals with a common mode of toxic action act according to the mixture toxicity concept of concentration addition. Thus mode-of-action specific bioassays can identify relevant toxicant groups present in a sample. Application of a **mode-of-action-based test battery** can help generate a more complete picture of the toxic potency of a water sample than chemical analysis alone, because all known and unknown chemicals with a common mode of action will contribute to mixture toxicity in an associated bioassay. It is sensible, for instance, to include a test indicative of endocrine disruption if the sample is suspected to contain hormones (e.g. wastewater) or a phytotoxicity assay if herbicides are likely to be present (e.g. agricultural runoff). Effect-based batteries can be further advanced by incorporating several chemical specific assays for a given toxic mode of action. Estrogenic activity, for example, can be induced by direct binding of the estrogen receptor (ER) by estrogenic compounds but also by indirect mechanisms such as activation of the aryl hydrocarbon receptor by polyaromatic hydrocarbons (PAHs).

Application of broad test batteries comprising a range of specific endpoints as well as non-specific cytotoxicity endpoints allow the assessor to account for unexpected toxicant groups that may otherwise go undetected. In the chemical oriented design, priority is given to quantification of the risks posed by relevant groups of chemicals. Bioassays of high sensitivity towards the toxicant group of interest may hence be selected irrespective of their (lack of) direct relevance to the protection goal. For example, in order to protect our drinking water from herbicides, even though the water tested is destined for human consumption and the protection goal is to achieve good human health, it may be appropriate to include an algal assay, simply because photosynthetic organisms are particularly sensitive to herbicide exposure.

Both test battery approaches may lead to very similar and often overlapping sets of bioanalytical tools as chemicals cannot be viewed independently of their mode of action. When researchers design test batteries, they will often include considerations related to both approaches. It must also be noted that not all bioassays are fully selective and 100 % indicative of a given mode of toxic action. In all cases, a cell-based bioassay will be influenced by a combination of non-specific and specific toxicity. In a water sample, there will be thousands of chemicals, only a fraction of which will respond specifically to the endpoint featured in the applied assay. Within a range of concentrations, a window will typically exist where the specific effect sets in but is not yet compromised by overlaying cytotoxicity. The wider this window is, the more useful a given bioassay is for application in complex water matrices.

3. MODES OF TOXIC ACTION

A **mode of action** (MOA) or mode of toxic action is a common set of physiological and behavioural signs that characterise a particular type of adverse biological response (Rand, 1995). These responses can be caused by a range of **mechanisms of action** (or mechanisms of toxic action), which represent the crucial biochemical processes and/or xenobiotic-biological interactions underlying a given mode of action. It must be noted that the MOA is not a universal property of a chemical but is related to the target organism or target organ/tissue. As a given chemical can exhibit multiple mechanisms of toxicity, the MOAs displayed may vary with exposure duration (acute vs. chronic) and organism.

Toxicity pathways are defined as "the cellular response pathways after chemical exposure expected to ultimately result in adverse health effects" (Collins *et al.*, 2008). The initiating event is the macromolecular interaction between the toxicant and receptors or other biomolecules. This interaction triggers a cellular response (e.g. activation of certain genes, production or depletion of proteins or altered signalling) and ultimately leads to observable endpoints or disease. For improved environmental risk assessment, cellular response pathways can be taken a step further by linking these individual health effects to population level effects using conceptual adverse outcome pathways (AOPs) (Ankley *et al.*, 2010). Mechanisms of toxicity can be classified according to the type and degree of interaction of a chemical pollutant with the target molecule or target site (Escher and Hermens, 2002). The main targets for environmental pollutants are (membrane) lipids, proteins and peptides and DNA (Table 1).

Depending on the type of interaction with the target, one can differentiate between **non-specific, specific and reactive toxicity**. Non-specific toxicity involves partitioning to the target site only, whereas specific effects are the results of three-dimensional interactions including specific H-donor/acceptor interactions and ionic interactions between the chemical and a target molecule. MOAs are classified as reactive when covalent bonds are formed between the chemical and its target or when chemical reactions are involved (e.g. oxidative stress) (Escher and Hermens, 2002). This generic classification scheme can be further refined by differentiation between more specialised target sites such as specific enzymes and receptors. Especially prominent is the nuclear receptor super family, a class of proteins that sense hormones (hormone responsive elements) and regulate gene expression.

Throughout the report all tables are colour coded to assign the tests to one of the three major classes of modes of toxic action.

Non-specific toxicity
Reactive toxicity
Specific and receptor mediated toxicity

Table 1: Mode of action classification scheme (adapted from Escher and Hermens, 2002).

MOA class	Target site	MOA	Molecular mechanism(s)
Non-specific	All membranes	Baseline toxicity	Non-specific disturbance of membrane structure and functioning
	Energy transducing membranes	Uncoupling	Ionophoric shuttle mechanisms
Specific	Energy transducing membranes	Uncoupling	Ionophoric shuttle mechanisms
	Energy transducing membranes	Inhibition of the electron transport chain	Blocking of quinone and other binding sites etc.
	Energy transducing membranes	Inhibition of ATP synthesis/ depletion of ATP	Blocking of proton channels and other transport channels
	Photosynthetic membranes	Photosynthesis inhibition	Blocking of photosynthetic electron transport
	Specific enzymes	Inhibition e.g. acetylcholinesterase	Binding to enzymes
	Specific and nuclear receptors	Inhibition or induction of (nuclear) receptors e.g. AhR, ER etc.	Binding to (nuclear) receptors
	Specific enzymes and receptors	Indirect mutagenicity (DNA repair, recombination, regulation)	Non-covalent or covalent binding to enzymes of the nucleic acid metabolism, effect on replication or repair
Reactive	All membranes	Degradation of membrane lipids and membrane proteins	Formation of reactive intermediates (e.g., reactive oxygen species) causing peroxidation of membrane lipids and membrane proteins
	All proteins, peptides	Damage and depletion of biomolecules	Electrophilic reactivity, alkylation and oxidation of proteins and glutathione (GSH)
	Specific enzymes and receptors	Indirect mutagenicity (DNA repair, recombination, regulation)	Non-covalent or covalent binding to enzymes of the nucleic acid metabolism, effect on replication or repair
	DNA, RNA	Direct mutagenicity (frameshift, cross-links, strand breaks, deletion, etc.)	Base modification and damage: electrophilic (alkylation) and oxidative damage, bulky adducts

AhR = arylhydrocarbon receptor, ATP = adenosine triphosphate, ER = estrogen receptor, MOA = mode of (toxic) action

4. SAMPLE PREPARATION

The topic of sample preparation is beyond the scope of this review but a brief primer is given here as sample collection, storage and preparation are as important as the choice of the appropriate bioassay.

In "direct toxicity assessment" or "whole effluent testing", the investigated organism (or cell line) is exposed to effluent, environmental or spiked water samples without prior clean up and/or concentration steps. Raw samples are, however, rarely practical for conservative assessment as the concentration of micropollutants is often too low to achieve any measurable effect, especially for highly treated waters (e.g. drinking water). More polluted samples, such as untreated sewage, industrial effluent or pesticide runoff, in contrast, often contain high concentrations of matrix components, such as salts and metals, which may conceal the effect of the organic micropollutants. In addition, the pH can influence bioassay results. When organic micropollutants are targeted using bioanalytical tools, they must therefore be separated from the matrix and enriched prior to testing. The importance of sample preparation cannot be overstated. The sample extraction process must be standardised and fully validated as its thoroughness will directly influence the quality of the results, irrespective of the quality of the analysis method.

Most common is the use of solid phase extraction (SPE) techniques, where samples are passed through absorbing material (sorbent) packed in a cartridge. The sorbent retains specific compounds depending on its chemistry (for example C18 and HLB cartridges will retain various organic compounds). The compounds bound to the sorbent are eluted with solvent into a small aliquot, which can be tested. This step concentrates the toxicants in the sample, thus improving the method detection limit.

In previous comparison studies of a large series of different solid phases, C18, ENV (and mixtures thereof) and HLB were validated as suitable matrices for SPE (Escher *et al.*, 2005b; Leusch *et al.*, 2006a). Current development focuses on recovery of nitrosamines and other disinfection by-products, which may be better recovered by coconut charcoal cartridges (Supelco), as well as improving recovery for the more polar (water-soluble) compounds, which may not be efficiently retained on HLB cartridges.

5. WORLDWIDE APPLICATIONS OF BIOANALYTICAL TOOLS IN WATER QUALITY ASSESSMENT

5.1. Overview and Historic Development

Bioanalytical tools that respond to relevant initiating triggers in a toxicity pathway or are linked to a defined mode of toxic action have the potential to be applicable for water quality assessment. Assay robustness and specificity in the presence of matrix components and other chemicals must be characterised and endorsed prior to their implementation as monitoring tools. This overview focuses therefore only on cell-based assays that have been applied for water quality monitoring to date, excluding those that are limited to chemical risk assessment of individual chemicals and defined mixtures and those that rely on passive sampling.

Cell-based assays have been applied for water quality assessment for decades. Most early work focused on assays indicative of carcinogenicity (reactive toxicity) and general (non-specific) toxicity. The Ames, or *Salmonella* mutagenicity test (Ames *et al.*, 1975), for instance, has been utilised in water monitoring since the 1970s (Simmon and Tardiff, 1976; Pelon *et al.*, 1977) and is still widely used. The Microtox® assay (Beckman Instruments Inc., 1980) was first used to measure cytotoxicity of water samples in the early 1980s (Chang *et al.*, 1981; Timourian *et al.*, 1982). Specific toxicity assays emerged in the field in the 1990s, particularly when endocrine disrupting chemicals (EDCs) became a topic of much concern due to growing public awareness that they can cause adverse effects to wildlife and, by inference, to humans at environmentally relevant exposure concentrations.

Numerous studies have applied *in vitro* assays for monitoring of water quality and cannot all be listed within the scope of this report. Below are some examples of applied cell-based assays within the three main MOA classes. A few case studies are used for illustration of the diverse applicability of such bioassays and some recent advances in the field are highlighted. A range of additional valuable assays exists. For an overview, albeit not comprehensive, of the many assays and their worldwide applications in water quality assessment, please refer to Appendix I.

5.1.1. Non-Specific Toxicity

The Microtox® assay remains the most widely used assay for measurement of cytotoxicity in water samples. The assay utilises light emission in naturally bioluminescent marine bacteria (*Vibrio Fischeri* or *Photobacterium phosphoreum*) as a measure of general 'energy status'. For waters containing complex chemical mixtures, a lowered energy status (light output) will largely reflect the underlying baseline toxicity of all chemicals in the mixture. The Microtox® assay is thus a suitable tool for detection of non-specific toxicity. In addition to the low cost and simplicity of the assay, its application for various water types is well established in the literature providing a large volume of comparative information. Microtox® applications include effluents of coal gasification (Timourian *et al.*, 1982), oil refineries (Chang *et al.*, 1981), pulp mills (Rosa *et al.*, 2010) and sewage treatment plants (Farré *et al.*, 2002) as well as environmental waters (Dizer *et al.*, 2002) and drinking water (Guzzella *et al.*, 2004). Several staining assays are also available for quantification of cell growth/viability in water samples (Appendix 1).

5.1.2. Specific Toxicity

Ongoing reports of observations of sexual disruption in aquatic wildlife (e.g. Smith, 1981; Purdom *et al.*, 1994; Jobling *et al.*, 1998) have led to the development of a multitude of *in vitro* assays specific for detection of hormone and hormone mimicking activity. In particular, estrogenicity has received much attention, but more recently many other members of the nuclear receptor family have been targeted in bioassays.

The E-SCREEN (Soto *et al.*, 1995) and the yeast estrogen screen (YES; Routledge and Sumpter, 1996) assays are among the most common screening tests for estrogenic activity in environmental waters. The YES assay is capable of detecting activation of the estrogen receptor (ER) in recombinant yeast (*Saccharomyces cerevisiae*), while the E-SCREEN assay measures cell proliferation in human breast cancer cells that are dependent on estrogen and/or estrogen-like chemicals for growth. Both assays were first applied in water quality assessment with the screening of sewage waters in the late 1990s (Desbrow *et al.*, 1998; Körner *et al.*, 1999). It is beyond the scope of this review to detail all existing estrogen assays; the Global Water Research Coalition (GWRC), however, recently reviewed all available estrogen assays (GWRC, 2006).

French researchers developed the recombinant reporter cell line, MELN, for detection of estrogenicity by stable transfection of the human breast cancer cell line, MCF-7 (Balaguer *et al.*, 1999). The MELN assay has been applied successfully for screening of surface water and wastewaters in France (Balaguer *et al.*, 1999; Cargouet *et al.*, 2004; Muller *et al.*, 2008a; Jugan *et al.*, 2009; Miege *et al.*, 2009; Creusot *et al.*, 2010; Dagnino *et al.*, 2010) and overseas (e.g. Mahjoub *et al.*, 2009; Leusch *et al.*, 2010). Pillon *et al.*(2005) optimised the MELN assay by inclusion of the inhibition test of MELN activation, which enables the analyst to differentiate between high and low affinity estrogens. High affinity estrogens, such as the free estrogens 17β-estradiol, estriol and ethinyl estradiol, bind directly to the ER. Low affinity estrogens such as PAHs and dioxins activate the ER indirectly via binding to the AhR, which then forms a complex with the ER (Ohtake *et al.*, 2003). The concept is carried out in practice by initial measurement of total estrogenicity of a water sample in the original MELN assay. Then the assay is repeated with prior addition of recombinant ER-α, which inhibits subsequent ER activation in MELN cells by free estrogens as these can also bind to the recombinant ER-α. A reduction in ER activity in the inhibition test thus indicates the presence of high affinity estrogens in the sample. By means of the inhibition assay, Pillon *et al.* (2005) showed that river sediments contained low affinity estrogens in high concentrations in contrast to the overlying water, which contained high affinity estrogens in low concentration. The group further developed immobilised ER-α columns for separation of ER and AhR active compounds. All EDCs act primarily via receptor-mediated toxicity.

The nuclear receptor super family comprises a large number of different receptors with 48 (including homologues, e.g., ERα, ERβ) known for the human genome (Zhang *et al.*, 2004). With such diversity in toxic pathways, a range of reporter gene assays using recombinant cell lines have been developed in addition to the ER-responsive assays (e.g. the YES assay). Nuclear receptors targeted for monitoring of water quality include the aryl-hydrocarbon (AhR), androgen (AR), glucorticoid (GR), pregnane X (PXR), progesterone (PR) and thyroid (TR) receptors. The AhR, for example, is responsive to carcinogens such as dioxins, dioxin-like chemicals and polyaromatic hydrocarbons (PAHs). Several AhR specific assays are available and include the AhR-CALUX (chemical-activated luciferase gene expression; Murk *et al.*, 1996) and the AhR-CAFLUX (chemically activated fluorescence expression; Nagy *et al.*, 2002) assays.

The retinoic acid and retinoid X receptors, RAR and RXR, respectively, are important for regulation of early life stage development. These receptors are still relatively novel targets in water quality assessment. Schoff and Ankley (2002) applied a mouse embryonic cell line (F9S:1) with a stable transfection of the RAR/RXR to evaluate the RAR/RXR disruptive potential of a paper mill effluent. The effluent samples did not activate the retinoic acid responsive element (RARE) inserted in the cell line, indicating an absence of retinoic acids in the samples. When retinoic acid heterodimers (all-*trans* RA (atRA) and 9-*cis* RA (9cRA)) were added to activate the RARE however, the effluents were found to inhibit atRA induced activity, whereas 9cRA stimulated activity remained unchanged. In this way, Schoff and Ankley (2002) indirectly demonstrated the presence of RAR/RXR disrupting chemicals in the effluent. The original yeast two-hybrid assay was developed by Nishikawa *et al.* (1999). Shiraishi *et al.* (2003) applied this yeast in an estrogenicity assay, which was subsequently adapted by Kamata *et al.* (2008) for screening of xenobiotically induced RAR activity. In recent years, the yeast two-hybrid assay has been applied for testing of surface and wastewaters in China (Cao *et al.*, 2009b; Zhen *et al.*, 2009) and Japan (Inoue *et al.*, 2009a; Inoue *et al.*, 2009b; Inoue *et al.*, 2010). In the study by Cao *et al.* (2009b), the yeast two-hybrid assay was one of a battery of four assays used to evaluate the toxicant removal efficiency across several steps of advanced sewage treatment.

Enzyme activity (induction/inhibition) is another marker of specific toxicity. The EROD (ethoxyresorufin-*O*-deethylase) assay targets induction of cytochrome P450 via measurement of enzymatic (EROD) activity and responds to dioxin-like chemicals. The EROD assay was first developed in 1974 (Burke and Mayer, 1974). Huuskonen *et al.* (1998) were among the first to apply a microplate version of the EROD assay for water quality monitoring when they assessed the toxicity of lake water receiving paper mill effluents. The AChE (acetylcholinesterase) inhibition assay is useful for detection of neurotoxic insecticides such as organophosphates and carbamate insecticides and was first developed by Ellman *et al.* (1961). Hamers and co-workers (2000) optimised the assay for use with environmental samples and validated it using rainwater.

5.1.3. Reactive Toxicity

The first applications of the Ames test in water quality monitoring were conducted as early as in the 1970s, when the assay was applied for mutagenicity screening of various water types including surface water (Pelon *et al.*, 1977; Vankreijl *et al.*, 1980), ozonated recycled water (Gruener, 1978), coal gasification process water (Epler *et al.*, 1978), drinking water (Simmon and Tardiff, 1976; Nestmann *et al.*, 1979; Cheh *et al.*, 1980), marine water (Kurelec *et al.*, 1979), pulp and paper mill effluents (Bjorseth *et al.*, 1979; Carlberg *et al.*, 1980) and different wastewaters (Rappaport *et al.*, 1979; Saxena and Schwartz, 1979). The SOS Chromo (Quillardet *et al.*, 1982) and SOS *umu/umuC* (Oda *et al.*, 1985) assays were developed in the following decade and are popular screening tools for genotoxicity in environmental waters. Both SOS techniques respond to genotoxic chemicals through colorimetric detection of the SOS response, which is activated by DNA damage. The SOS *umu* and Chromo tests were optimised for high throughput screening of surface waters in the beginning of the 1990s (Reifferscheid *et al.*, 1991; Langevin *et al.*, 1992). Another commonly used method for detection of reactive toxicity in polluted waters is the Comet assay (or Single Cell Gel Electrophoresis (SCGE) assay), which relies on the different migration behaviour of intact and damaged DNA in an electric field (Rydberg and Johanson, 1978; Ostling and Johanson, 1984). Initially, this assay could only detect double strand breaks but it was later optimised for detection of single strand breaks by Singh *et al.* (1988). The Comet assay was first used for water quality analysis in 2001, when it was applied to assess the genotoxicity of river water in Germany and China (Schnurstein and Braunbeck, 2001; Zhong *et al.*, 2001). Biomarker responses such as those indicative of oxidative stress (OS) and reactive oxygen species (ROS) are also used as warning signs of reactive toxicity in water samples (Marabini *et al.*, 2006).

While most research in the field has focused on surface water (those of rivers and lakes) and wastewater, chemical disinfection of drinking water (using e.g. chlorine, chlorine dioxide and ozone) can form potentially harmful disinfection by-products (DBPs) from natural and synthetic organic matter. As many as 600 DBPs have already been identified, but more than 50% of the organic halides formed during chlorination processes and an equal fraction of the assimilable organic carbon produced during ozonation of drinking water remain unidentified (Richardson *et al.*, 2007). Researchers at the University of Illinois have worked on methods for biological characterisation of relevant groups of DBPs including well established DBPs such as the halonitromethanes (Plewa *et al.*, 2004a) and the haloacetic acids (Plewa *et al.*, 2004b), as well as emerging DBPs such as the haloacetamides (Plewa *et al.*, 2008). The Illinois group applies bacterial and mammalian cell assays for detection of DBP cytotoxicity and genotoxicity. *Salmonella typhimurium* is utilised in a microplate cytotoxicity assay and a modified Ames mutagenicity assay (Kargalioglu *et al.*, 2002; Plewa *et al.*, 2004b). For evaluation of DBP toxicity in mammalian cells, the microplate cytotoxicity assay and the SCGE Comet assay were adapted for use with Chinese hamster ovarian cells (CHO) (Plewa *et al.*, 2002; Plewa *et al.*, 2004a). Recreational pool waters contain comparatively high levels of DBP precursors such as urine, sweat and sunscreen. Recently, the CHO SCGE assay was applied to screen a variety of pool waters (Liviac *et al.*, 2010). Genotoxic responses were higher in all tested pool waters compared to the source tap water and varied with conditions of illumination and disinfection type. In a study that screened pool waters in the Ames assay, pool waters were found to be of similar mutagenicity to drinking water (Richardson *et al.*, 2010).

As noted, the reviewed techniques are only a few examples of the many methods available for detection of non-specific, specific and reactive toxicity in waters. Further examples of assays and their applications in water quality assessment are provided in Appendix I.

5.2. Application of Test Batteries

Researchers have applied single and multiple assays for water quality assessment for decades but battery applications have dramatically increased over the last decade and testing was expanded from contaminated sites and effluents to surface waters and highly treated waters, including drinking water.

Sanchez *et al.* (1988) were among the first to use a battery of five acute toxicity assays (three bacterial, one *in vivo* and one molecular) and three mutagenicity assays (the Ames test, and the *E. coli* and *S. cerevisiae* reverse mutagenicity assays) to evaluate the toxicity of industrial effluents. Since this publication, the number of studies applying batteries of three or more tests (including a minimum of one cell-based assay) has grown steadily, demonstrating increasing endorsement of the test battery approach (Figure 1). The motives behind the choice of assays vary however, causing some limitations in the comparability of the resulting data. As discussed, MOAs can be divided into three major groups – non-specific, specific and reactive toxicity. The three groups target differing types of toxicity and, hence, differing types of toxins. To date, individual studies often target a certain MOA or a certain contaminant group. While such a tactic has some advantages, it also has certain shortcomings. A project covering estrogenicity in three different assays, for example, has the advantage of ensuring that all estrogenicity is detected and accounted for but cannot elucidate which compound group(s) caused the observed responses (except in few cases where more selective assays are applied, such as the inhibition test of MELN activation). Similarly, a study exclusively screening for reactive toxicity cannot be used to assess whether the water source in question also contains EDCs.

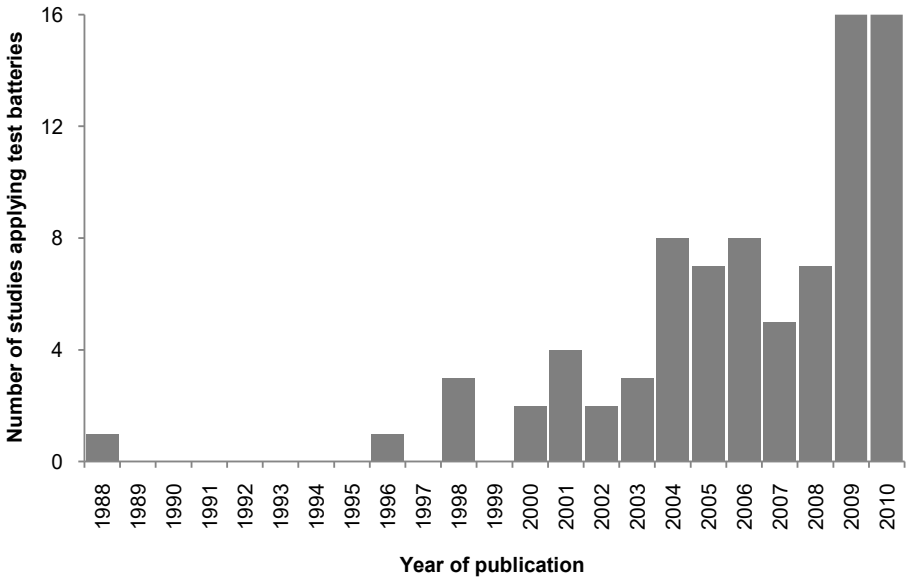

Figure 1: Historical trend in number of worldwide studies applying test batteries. Includes batteries applying three or more assays plus a minimum of one cell-based assay.

Application of a well designed **MOA based test battery** targeting all of the three main MOA categories, as well as a range of specific MOAs, can enable optimal coverage of all or many relevant MOAs for a given water type. However, of 83 studies found to apply test batteries since 1988, only 11 represented all three MOA groups and, more specifically for the purpose of this review, only seven of these were entirely cell-based or included a cell-based assay in all three categories (Table 2). To illustrate this further, the test battery applications published in 2010 were graphed and colour coded according to the MOA categories used in each study (Figure 2). Only two of a total of sixteen studies cover all three categories. Ideally, a MOA based test battery should cover not only all three main MOA groups, but also a wide range of specific MOAs within each group. As reviewed in the following section, these are goals The University of Queensland and Griffith University, as well as other researchers, have been working towards in the past decade.

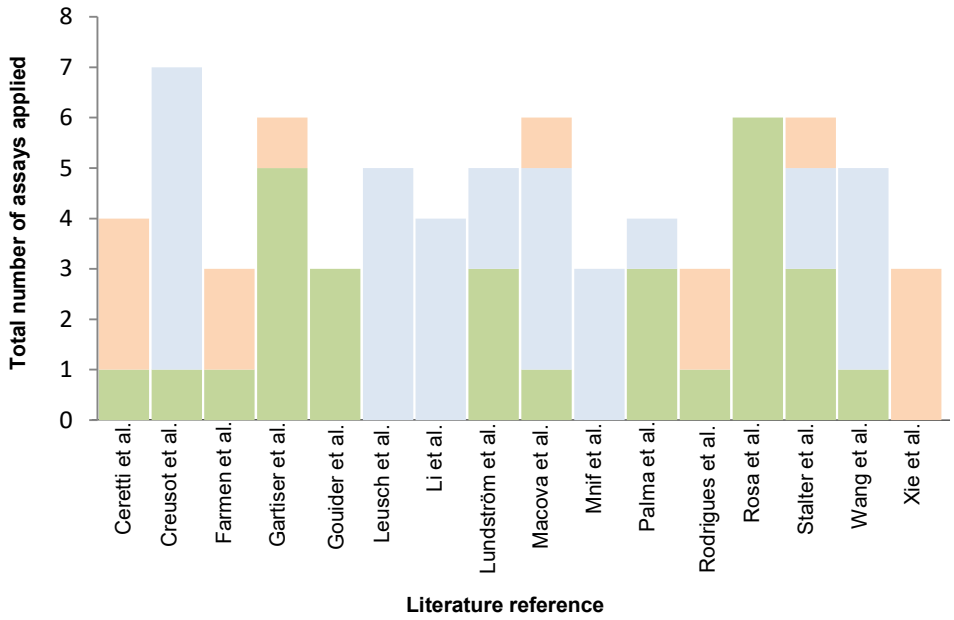

Figure 2: Worldwide application of cell-based test batteries (≥ 3 assays) in 2010. The coloured sections in the bars represent the three main categories of MOAs. The batteries comprise cell-based and low-complexity in vivo assays including a minimum of one cell assay.

Table 2: Worldwide application of test batteries applying three or more assays including at least one cell-based assay.

Sample type	Non-specific toxicity	Specific toxicity								Reactive toxicity			References listed historically
	Growth/cytotoxicity	Dev	Endocrine ER	Endocrine other	Imm	Neuro	PAH/Dioxin	PSII	Rep	Genotox	OS	ROS	
Mixed	xxxxx									xxx			(Sanchez et al. 1988)
LF	xxxxxxxx												(Clement et al. 1996)
WW	x		xx										(Gagné and Blaise 1998)
Mixed	xxxxxxxx												(Rojícková-Padrtová et al. 1998)
WW	xxx												(Tarkpea et al. 1998)
R, WW	xxxxx												(Blinova 2000)
GW	xx								x				(Gustavson et al. 2000)
WW	xx									x			(Castillo et al. 2001)
WW			xxx										(Garcia-Reyero et al. 2001)
WW	xxxx												(Guerra 2001)
OR	xxxx						X						(Schirmer et al. 2001)
R, WW	x					x				xx			(Dizer et al. 2002)
R, WW			xxx										(Murk et al. 2002)
LF	xxxx												(Isidori et al. 2003)
WW (mix)	xxxxxx									x			(Manusadzianas et al. 2003)
R, WW			xxxxx										(Pawlowski et al. 2003)
WW	xx	x	x							x			(Aguayo et al. 2004)
ADWT	xxx									xx			(Buschini et al. 2004)
ADWT	xx									xxxx			(Guzzella et al. 2004)
ADWT	x									xx			(Klee et al. 2004)
R, WW	xxxxxx												(Latif and Licek 2004)
Mixed	xxxxx		x				X			x			(Pessala et al. 2004)
WW	x		xx										(Rutishauser et al. 2004)
WW	xx		x										(Schiliró et al. 2004)
R	xxxx												(Di Marzio et al. 2005)
WW	xxx												(Emmanuel et al. 2005)
DWT										xxxx			(Lah et al. 2005)
AWWT	x		x				X						(Ma et al. 2005))
R			xxx										(Matsuoka et al. 2005)
R			xx				X						(Pillon et al. 2005)
ADWT	xx									xxxx			(Zani et al. 2005)

Sample type	Non-specific toxicity	Specific toxicity								Reactive toxicity			References listed historically
R, PME, WW			x	xxx									(Bandelj et al. 2006)
WW										xxx		xxxxxx	(Fatima and Ahmad 2006)
ADWT	xx									xxx			(Guzzella et al. 2006)
R, PME, WW	xx	x	x							xx			(Keiter et al. 2006)
WW			xxx	x									(Leusch et al. 2006b)
ADWT	x										x	x	(Marabini et al. 2006)
Lake	xx									xxx			(Pellacani et al. 2006)
AWWT	xxxxx												(Petala et al. 2006b)
WW	x		x	x									(Allinson et al. 2007)
WW		x					X			x			(Gustavsson et al. 2007)
WW	x		x							xx			(Isidori et al. 2007)
DWT	xxx									xx			(Marabini et al. 2007)
WW	xxxxxx												(Wadhia et al. 2007)
R, WW	xx							x					(Escher et al. 2008a)
R, WW	xx		x			x		x		x			(Escher et al. 2008b)
WW										xxxx			(Krishnamurthi et al. 2008)
AWWT	xxxx				x								(Kontana et al. 2008)
OFPW	x						X			x			(Li et al. 2008a)
R	xxxxxxx	x							x				(Mankiewicz-Boczek et al. 2008)
DW, R, WW			x	xxx									(Van der Linden et al. 2008)
AWWT	xxxx												(Antonelli et al. 2009)
PME						xxxxxxxx							(Basu et al. 2009)
AWWT	x	xx								x			(Cao et al. 2009b)
AWWT	xx		x			x		x		x			(Escher et al. 2009)
WW (mix)	xxxx									xx			(Gartiser et al. 2009)
R		xx	x	xx									(Inoue et al. 2009b)
AWWT	xxxxxx				xx								(Kontana et al. 2009)
DWT	xxx									xxx			(Maffei et al. 2009)
WW			x	x		x							(Mahjoub et al. 2009)
WW	xxxxxx		x										(Mendonca et al. 2009)
WW	xxx												(Ostra et al. 2009)
R	(x)				(x)xxx								(Pool and Magcwebeba 2009)
WW			x	xx									(Shi et al. 2009a)
ADWT	x									xx	xxx	x	(Shi et al. 2009b)
AWWT	x		xx										(Wu et al. 2009)

Sample type	Non-specific toxicity	Specific toxicity								Reactive toxicity			References listed historically
Mixed	x									xx			(Zegura et al. 2009)
DW	x									xxx			(Ceretti et al. 2010)
WW	x		x	xxx			Xx						(Creusot et al. 2010)
OFPW	x										x	x	(Farmen et al. 2010)
PME	xxxxx									x			(Gartiser et al. 2010)
WW	xxx												(Gouider et al. 2010)
Mixed			xxxxx										(Leusch et al. 2010)
WW			x	xxx									(Li et al. 2010a)
AWWT	xxx	xx											(Lundstrom et al. 2010)
AWWT	x		x			x	X	x		x			(Macova et al. 2010)
AWWT			x	x			X						(Mnif et al. 2010)
R	xxx								x				(Palma et al. 2010)
OR	x									xx			(Rodrigues et al. 2010)
PME	xxxxxx												(Rosa et al. 2010)
AWWT	xxx		x						x	x			(Stalter et al. 2010)
PC	x								xxxx				(Wang et al. 2010)
DWT										x	xx		(Xie et al. 2010)

x = in vitro incl. algae, **x = in vivo**, **x = plant**. **Sample type codes:** ADWT = advanced drinking water treatment, AWWT = advanced wastewater treatment, DW (T) = drinking water (treatment), LF = landfill leachate, OFPW = oil field produced water, OR = oil refinery, PC = petrochemical, PME = pulp mill effluent, R = river, WW = wastewater. **Toxic endpoints:** Dev = developmental toxicity, ER = estrogen receptor, Imm = immunological toxicity, Neuro = neurotoxicity (mainly acetylcholine esterase (AChE) inhibition), OS = oxidative stress, PAH/dioxin = PAH/dioxin-like activity (aryl hydrocarbon receptor (AhR)), PSII = photo system II inhibition (photosynthetic algae), Rep = reproductive toxicity, ROS = reactive oxygen species.

6. AUSTRALIAN AND NEW ZEALAND APPLICATIONS OF BIOANALYTICAL TOOLS IN WATER QUALITY ASSESSMENT

6.1. Griffith University and The University of Queensland

The most common application of cell based assays for water quality assessment in Australia is for evaluation of wastewater effluents with the main focus on estrogenic and androgenic compounds. Griffith University has been active in this area since the early 2000s (Leusch *et al.*, 2005) applying various estrogen specific cellular assays (estrogen receptor binding assay (ERBA), ER competitive binding assay, ER-CALUX, E-SCREEN, YES, MELN and T47D-KBluc (a human breast cancer cell-based reporter gene assay) as well as an AR competitive binding assay. The research has included assessment of estrogenicity and androgenicity in Queensland and New Zealand wastewater effluents (Leusch *et al.*, 2005; Bandelj *et al.*, 2006; Leusch *et al.*, 2006a; Leusch *et al.*, 2006b; Tan *et al.*, 2007; Leusch *et al.*, 2010) and in effluents from New Zealand and Canadian pulp mills (Bandelj *et al.*, 2006). The Landsborough wastewater treatment plant (WWTP) in South East Queensland, Australia, was assessed for its efficacy to remove estrogen active compounds during advanced wastewater treatment using the ERBA and E-SCREEN assays (Leusch *et al.*, 2005). The study found secondary treatment removed 95 per cent of the total water estrogenicity, whilst tertiary treatment (including ozonation, activated carbon filtration and UV treatment) brought estrogenic activity to below detection (< 75 ng/L EEQ). Recently, five estrogen specific *in vitro* assays (ER-CALUX, E-SCREEN, MELN, T47D-KBluc and YES) were compared for their capability to detect estrogenicity in Brisbane groundwater and river water plus raw and treated sewage water (Leusch *et al.*, 2010). The bioassay results corresponded well with chemical analysis and the five assays were of comparable sensitivity with the YES assay being the least sensitive.

As outlined in previous sections, no single bioassay can provide a complete picture of the toxicity of environmental and sewage waters as these consist of complex mixtures of pollutants. Presently, the Smart Water Research Centre (SWRC) at Griffith University is introducing a comprehensive health outcome approach for water quality assessment in a collaborative effort with the Australian Water Quality Centre (AWQC) in South Australia (Table 3).

Table 3: Bioanalytical test battery jointly used by SWRC and AWQC.

Mode of toxicity	Mechanism	Endpoint	Bioassay
Non-specific	Cytotoxicity	Basal cytotoxicity to gastrointestinal cells	Caco2-NRU
	Cytotoxicity (liver cells)	Basal cytotoxicity to liver cells	Hepcytotoxicity assay
Specific	ED: estrogenic	ER-mediated reporter gene activation	ER-CALUX, T47D-kBluc
	ED: androgenic	AR-mediated reporter gene	AR-CALUX, MDA-kb2
	ED: glucocorticoid	GR-mediated reporter gene	GR-CALUX
	ED: progesteronic	PR-mediated reporter gene	PR-CALUX
	ED: thyroidal	TRβ-mediated reporter gene	TRβ-CALUX
	Immunotoxicity (limited measure)	Immunomodulation of cytokine production by monocytes	THP1 cytokine production assay
	Neurotoxicity (limited measure)	Acetylcholinesterase (AChE) inhibition	AChE assay
	Hepatotoxicity	CYP450 induction in liver cells	Hep-CYPi
Reactive	Mutagenicity	Formation of histidinerevertants	Ames test
	Genotoxicity	Micronucleus formation	FCMN assay

AR = androgen receptor,Caco2 = human cancer colon cell line, CALUX = chemical activated luciferase gene expression,CYP450 = cytochrome P450, ED = endocrine disruption, ER = estrogen receptor, FCMN = flow cytometry micronucleus,

GR = glucocorticoid receptor, MDA-kb2 = stably transfected breast cancer cell line, NRU = neutral red uptake,PR = progesteronic receptor, T47D-kBluc =stably transfected human breast cancer cell line, THP1 = human acute monocytic leukemia cell line, TRβ = thyroid receptor β.

Over the last decade, researchers at The University of Queensland's National Research Centre for Environmental Toxicology (Entox) (previously also based at the Swiss Federal Institute for Aquatic Science and Technology, Eawag, Switzerland) have worked towards development and application of various bioanalytical tools for water quality assessment. The ToxY-PAM phytotoxicity assay, which measures (inhibition of) photosynthetic yield in algae with photosystem II (PSII) (Schreiber *et al.*, 2002) was adapted for detection of aqueous phytotoxins using solid phase extraction (Bengtson Nash *et al.*, 2005b; Bengtson Nash *et al.*, 2005c). The ToxY-PAM assay was subsequently applied for detection of herbicide toxicity in Queensland surface waters and the Thames Estuary (Bengtson Nash *et al.*, 2005a; Bengtson Nash *et al.*, 2006). For high throughput screening, the ToxY-PAM was optimised to hold a microplate reader (Schreiber *et al.*, 2007). The new I-PAM method was first applied in combination with passive sampling to assess the phytotoxicity of several fresh and saline surface waters (Escher *et al.*, 2006; Muller *et al.*, 2008b; Shaw *et al.*, 2009). In the next step, the specific photosynthesis assay was complemented by a growth assay in the so-called combined algae test (Escher *et al.*, 2008a). Application of the I-PAM bioassay was further expanded in a battery of tests to assess the toxicity of passive sampler extracts of waters surrounding inshore coral reefs (Shaw *et al.*, 2009). The test battery comprised two *in vivo* early life stage tests, a non-specific assay measuring bioluminescence inhibition of the marine bacterium, *Vibrio fischeri*, and the I-PAM assay.

Lately, Entox researchers have focused on simple cell based bioassays for development of a test battery suitable for screening of environmental waters. The goal was to combine a set of tools targeting various toxic modes of action within the three main MOA categories. Entox first applied such a mode of action based test battery to assess mixture toxicity in two WWTPs (Muller *et al.*, 2007). Passive sampling supplied effluent extracts were tested in the *V. fischeri* assay (non-specific toxicity), the I-PAM phytotoxicity test (specific to herbicides) and the *umu*C assay (reactive toxicity).

The partner group at Eawag commenced with the assessment of estrogenic chemicals in sewage treatment plant effluent and surface water (Rutishauser *et al.*, 2004) but soon moved into the battery type approach (Schweigert *et al.*, 2002) with a study on the removal of micropollutants during urine source separation and treatment that included the ToxY-PAM, the Microtox and the YES assays (Escher *et al.*, 2005b). This battery was validated with individual pharmaceuticals and mixtures prior to validation with environmental samples (Escher *et al.*, 2005a). Subsequently, the battery was expanded by the AChE inhibition assay and the *umuC* test and applied for determination of removal efficiency during classical wastewater treatment (Escher *et al.*, 2008b) and advanced water treatment in a full-scale ozonation plant (Escher *et al.*, 2009).

The joint efforts of Entox and Eawag have culminated in the application of a comprehensive test battery of six bioassays (Table 4) for monitoring of toxicant removal during advanced wastewater treatment in: i) the South Caboolture Water Reclamation Plant (SCWRP) (Macova *et al.*, 2010; Reungoat *et al.*, 2010); ii) the Advanced Water Treatment Plant at Bundamba (Escher *et al.*, 2011); and iii) across a wide range of sampling points across the Western Corridor Recycled Water Scheme (Macova *et al.*, 2010; 2011). The combined application of biological and chemical analysis allowed identification of the most efficient removal processes – coagulation/flocculation/dissolved air flotation and filtration (DAFF), activated carbon filtration and ozonation - within the SCWRP. The study further demonstrated some of the key strengths of bioassay applications: i) the E-SCREEN detected estrogenic activity, where no estrogenic compounds could be detected with chemical analysis; and ii) the bioassay battery made it possible to assess whether or not the total sum of transformation byproducts formed during ozonation were more or less toxic than their precursors combined. In this case, the mixture of transformation byproducts was less toxic than the mixture of parent compounds in the selected assays.

Table 4: Mode of action based test battery applied routinely at Entox (adapted from Macova et al., 2010).

MOA class	Endpoint	Assay	Targeted chemicals	Reference compound	TEQ
Non-specific	Baseline toxicity	Bioluminescence inhibition	All chemicals	Baseline toxicant	Baseline-TEQ
Specific	AChE inhibition	AChE (neurotoxicity)	Organophosphates, carbamate insecticides	Parathion	PTEQ
	Photosynthesis (PSII) inhibition	I-PAM (phytotoxicity) and algal growth	Triazine and phenylurea herbicides	Diuron	DEQ
	Estrogenicity	E-SCREEN	Estrogens, estrogenic industrial chemicals	17 β-Estradiol	EEQ
	Binding to the arylhydrocarbon receptor	AhR CAFLUX	Dioxin-like compounds, PAHs	2,3,7,8-Tetrachloro-dibenzodioxin	TCDDEQ
Reactive	Genotoxicity	*umuC* (genotoxicity)	Chlorinated byproducts, aromatic amines, PAHs	Benzo[a]-pyrene (BaP)	BaPEQ

AChE = acetylcholinesterase, AhR = arylhydrocarbon receptor, PAH = polycyclic aromatic hydrocarbon, PSII = photosystem II, TEQ = toxic equivalent concentration.

At present, the group at Entox is expanding the number of assessed endpoints and modes of action by implementing assays previously used exclusively for chemical risk assessment and validating them for application in water quality assessment. The targeted endpoints include, for example, direct reactivity of soft electrophiles with proteins and glutathione (GSH) and of hard electrophiles with DNA (Harder *et al.*, 2003; Richter and Escher, 2005) as well as oxidative stress (Wang *et al.*, 2006).

Entox is also specialising in comparative modelling for assessment of the proportion of detected toxicity that can be explained by chemical analysis (Rutishauser *et al.*, 2004; Vermeirssen *et al.*, 2009; Vermeirssen *et al.*, 2010).

6.2. Other Australian and New Zealand Laboratories

6.2.1. New South Wales

The University of New South Wales (UNSW) Water Research Centre (WRC) has applied bioanalytical tools for assessing the endocrine disruptive potential of NSW wastewaters. The YES and YAS assays were applied to evaluate the removal efficiencies of several NSW WWTPs with particular emphasis on the usefulness of a membrane bioreactor (Coleman *et al.*, 2008; Coleman *et al.*, 2009).

The University of Technology, Sydney (UTS) conducted a series of investigations to assess current contaminant levels in the wetlands of Sydney Olympic Park, which was exposed to high amounts of commercial, industrial and domestic wastes prior to its remediation for the Sydney Olympics in 2000. Two studies were conducted to assess the residual toxicity of sediments and waters of these wetlands; one applying the rat hepatoma cell (H4IIE) bioassay for detection of dioxin like activity (Rawson *et al.*, 2009) and one applying a luminescent bacterial (*E. coli*) assay for detecting the presence of non-specific toxicity (Ying *et al.*, 2009).

The Department of Primary Industries (DPI), NSW, applies the ER-CALUX and DR-CALUX assays for detection of estrogenicity and dioxin-like chemicals, respectively. NSW DPI performed, for instance, the ER-CALUX for CSIRO Land and Water in an assessment of river water quality in South Australia (Williams *et al.*, 2007, reviewed in section 5.2.3).

6.2.2. Victoria

The Victorian **Centre for Aquatic Pollution Identification and Management (CAPIM)** is an association of scientists from The University of Melbourne, Melbourne Water, Victorian Department of Primary Industries (DPI), The Royal Melbourne Institute of Technology (RMIT) and Victorian Environment Protection Authority (EPA). CAPIM researchers have tested for estrogenic activity in effluents of WWTPs throughout Victoria using the yeast two-hybrid assay (Mispagel *et al.*, 2005;

Allinson *et al.*, 2007; Mispagel *et al.*, 2009; Allinson *et al.*, 2010d). The most recent survey assessed the hormonal activity of the effluents of 45 different WWTPs feeding various water bodies and agricultural land (Allinson *et al.*, 2007; Allinson *et al.*, 2010d). The yeast two-hybrid assay was employed to measure androgenic, retinoic acid (RA) and aryl hydrocarbon (Ah) activity in addition to estrogenicity. Most of the 45 effluents responded positively for estrogenicity in the yeast two hybrid assay (Allinson *et al.*, 2007; Allinson *et al.*, 2010d) while no androgenic activity was detected in any sample (Allinson *et al.*, 2008).

Allinson *et al.* (2010a; 2010b) reported on a pilot survey of AhR and RAR activity of treated effluent from 39 WWTPs located across Victoria. All samples contained compounds that stimulated the AhR receptor in the bioassay (16 - 279 ng/L ß-naphthoflavone equivalents), with almost all samples containing compounds that stimulated the retinoic acid receptor in the RAR bioassay (< limit of detection - 198 ng/L atRA equivalents). Measured concentrations were much less than the concentrations of retinoids reported to affect development in Japanese flounder (14 µg/L RA).

The CAPIM group has also tested for receptor activity in a number of streams and rivers in Victoria. Allinson *et al.*(2009; 2010c) collected water samples from six sites on the main stem of the Yarra River in April 2008, and again in April 2009. No estrogenic or thyroid and little retinoid activity was observed in the Yarra River. AhR activity increased, however, along a downstream gradient (from 10 to 27 ng/L ß-naphthoflavone EQ). AhR activity was higher in April 2009 than at the same time in 2008, perhaps as a result of extensive bush fires in the catchment in the months immediately prior to the 2009 sampling. Additional testing has been undertaken on a further 16 streams and 24 wetlands in and around Melbourne, although this data is not yet published.

6.2.3. South Australia

CSIRO Land and Water has applied *in vitro* assays to examine the quality of Australian surface waters. One CSIRO report documents the estrogenicity of South Australian river waters in rural areas dominated by dairy farming using two different estrogen specific assays (the YES and ER-CALUX assays) (Williams *et al.*, 2007). The study further tested effluents of several WWTPs throughout ACT, QLD and SA for estrogenic activity using the YES assay. Wineries also produce considerable amounts of wastewater of unique composition compared to other wastewaters. CSIRO has applied plant-based assays (garden cress, onion and local macrophyte species) for toxicity assessment of winery effluents (Arienzo *et al.*, 2009; Kumar *et al.*, 2009). Such information is crucial because winery wastewaters are often recycled for irrigation of farmland. CSIRO is now in the process of validating the CALUX bioassay battery (Anu Kumar, personal communication).

United Water is another active member in Australian water quality research and used the YES assay to assess the fate of estrogenic compounds during advanced treatment processes in two Adelaide municipal WWTPs, in a collaborative study with CSIRO Land and Water and UNSW WRC (Holmes *et al.*, 2010).

The Australian Water Quality Centre (AWQC) has made many new developments in the field of naturally occurring cyanotoxins, which are a large group of toxins produced by cyanobacteria. Cyanotoxins can reach hazardous levels in freshwater bodies after cyanobacterial blooms, which have been attributed to extensive livestock deaths in Australia and there is growing concern that drinking water quality could be affected (e.g. Humpage *et al.*, 2007; Humpage *et al.*, 2010). Increasing numbers of cyanotoxins are being identified and it is expected that many more unknown compounds and metabolites exist. In the same way as with anthropogenic contaminants, chemical analysis is therefore insufficient to assess water quality with respect to natural toxins. The neuroblastoma assay is specific for sodium channel blocking in nerve cells caused by neurotoxic cyanotoxins, such as saxitoxins. The assay was validated for screening of natural bloom samples by AWQC researchers, who detected a 35-fold increase in toxicity compared to what could be accounted for by chemical analysis alone (Humpage *et al.*, 2007). Hepatotoxic cyanotoxins such as microcystins and nodularins act via inhibition of protein phosphatases PP1 and PP2A. AWQC validated the PP2A assay for detection of cyanobacterial hepatotoxins below the drinking water guideline (1 µg/L) with no pre-concentration step necessary (Heresztyn and Nicholson, 2001). Recently, it was demonstrated with the PP2A assay that the toxicity of microcystins is reduced by bacterial degradation during tertiary wastewater and drinking water treatment (Ho *et al.*, 2007; Ho *et al.*, 2010). AWQC has also recently developed a flow-

cytometry micronucleus assay to detect genotoxic compounds (Laingam *et al.*, 2008), which is now being used for water quality monitoring in the collaborative project with Griffith University's SWRC (Section 5.1).

Researchers at **Flinders University** are currently developing *in vitro* assays for detection of reproductive toxicity in waters. In one study, human granulosa cells were used to examine the reproductive toxicity of cyanotoxin and cylindrospermopsin (CYN), using a cytotoxicity assay (MTT) together with estrogen and progesterone radioimmunoassays (Young *et al.*, 2008). Ongoing projects address the following potential applications of cell-based assays: i) human spermatozoa cell line for effect assessment of CYN; ii) Jurkat human T-cell (lymphoblast-like cell) line for immunotoxicity assessment of water samples; iii) ELISA assay for detection of EDCs and immunotoxicity; and iv) JAR, OCVAR and KGN human placental cell lines for reproductive toxicity.

6.2.4. New Zealand

Bioanalytical tools are also gaining acceptance for water quality testing in New Zealand, with endocrine disruption being the most frequently evaluated toxic endpoint. **Landcare Research Ltd**, Lincoln, has been active in the field assessing mainly wastewaters for the presence of endocrine active chemicals (e.g. Sarmah *et al.*, 2006). Landcare Research is a collaborator of Griffith University and has been involved in most of the research reviewed for Griffith University in section 5.1 of this report (Leusch *et al.*, 2005; Leusch *et al.*, 2006a; Leusch *et al.*, 2006b; Tan *et al.*, 2007; Leusch *et al.*, 2010). **CENTOX** and **Lincoln University** in Lincoln and **Forest Research** (now Scion Research) in Rotorua were also partners on some of these projects (Leusch *et al.*, 2005; Leusch *et al.*, 2006a; Leusch *et al.*, 2006b). Forest Research further applied a competitive binding assay combined with *in vivo* experiments to evaluate the androgenic potential of pulp and paper mill effluents in New Zealand (Ellis *et al.*, 2003).

In a collaborative study, **Cawthron Institute,** Nelson, surveyed 227 different water bodies in New Zealand for cyanotoxins (Wood *et al.*, 2006). Selected samples were tested for cyanotoxin specific neurotoxicity in the neuroblastoma assay, which was conducted at the **Institute of Environmental Science and Research (ESR)** in Porirua.

6.3. Summary of Australian and New Zealand Cell-Based Assay Applications

The reviewed applications of cell-based assays for water quality monitoring in Australia and New Zealand (including those previously conducted overseas by the partner laboratories) are summarised in Table 5.

Table 5: Cell-based assay applications for water quality monitoring in Australia and New Zealand (including previous overseas work by the partner laboratories).

Target MoA	Assay	Test organism	Application	Specifics	TEQ	Enrichment	Literature reference
NON-SPECIFIC TOXICITY (baseline toxicity)							
Cytotoxicity	Bioluminescence inhibition (Microtox®)	*Vibrio fischeri*	Drinking water	Prim, sec, tert (Cl_2)	*v*	SPE (OASIS®HLB)	(Macova et al. 2011)
			Surface water	River, Switzerland	*v*	SPE (C18, EN)	(Escher et al. 2008b)
				Brisbane rivers, lakes, receiving recycled water	*v*	SPE (OASIS®HLB)	(Macova et al. 2011)
			Wastewater	Prim, sec	*v*	SPE (C18, EN)	(Escher et al. 2008b)
				Prim, sec, tert (O_3, AC)	*v*	SPE (C18, EN)	(Escher et al. 2009)
				Prim, sec. tert (O_3, AC, DAFF)	*v*	SPE (OASIS®HLB)	(Macova et al. 2010)
				Prim, sec, tert (RO, UV, H_2O_2)	*v*	SPE (OASIS®HLB)	(Escher et al. 2011)
				Prim, sec, tert (MF, RO, O_3, BAC)	*v*	SPE (OASIS®HLB)	(Macova et al. 2011)
				Prim, sec, tert (sand filtration, BAC)	*v*	SPE (OASIS®HLB)	(Reungoat, 2011)
		Phosphobacterium phosphoreum	Wastewater	Sec		SPE (C18)	(Allinson et al. 2007)
				Sec	*v*	SPE (C18)	(Mispagel et al. 2009)
				Sec	*v*	SPE (C18)	(Allinson et al. 2010d)
	Yeast growth inhibition	*Saccharomyces cerevisiae*	Wastewater	Unspecified treatment	*v*	SPE (Lichrolut C18, EN)	(Rutishauser et al. 2004)
SPECIFIC TOXICITY							
Endocrine disruption							
Androgen receptor (AR)	Receptor binding	Goldfish testis	Pulp and paper mill effluent, NZ	Sec	*v*	SPE (C18 AR)	(Ellis et al. 2003)
		Rainbow trout brain		Sec	*v*	SPE (XAD-7)	(Bandelj et al. 2006)
			Surface water	Rivers, NZ and Canada	*v*	SPE (XAD-7)	(Bandelj et al. 2006)
			Wastewater	Sec	*v*	SPE (XAD-7)	(Bandelj et al. 2006)
				Raw, prim/sec	*v*	SPE (OASIS®HLB)	(Leusch et al. 2006a)
				Raw	*v*	SPE (comparison of several solid phases)	(Leusch et al. 2006b)
	YAS		Wastewater	Raw, sec, pondage, UV, Cl	*v*	SPE (LC-18, Supelco)	(Coleman et al. 2008)
				Raw + MBR	*v*	SPE (OASIS®HLB)	(Coleman et al. 2009)
	Yeast two-hybrid	*S.cerevisiae* with human AR	Wastewater	Sec	*v*	SPE (C18)	(Allinson et al. 2007; Allinson et al. 2008)
AR indirect	Aromatase	Rainbow trout	Pulp mill effluent,	Sec	*v*	SPE (XAD-7)	(Bandelj et al. 2006)

Target MoA	Assay	Test organism	Application	Specifics	TEQ	Enrichment	Literature reference
Estrogen receptor (ER)	inhibition	ovaries	NZ and Canada				
			Surface water	Rivers, New Zealand and Canada	*v*	SPE (XAD-7)	(Bandelj et al. 2006)
			Wastewater	Sec	*v*	SPE (XAD-7)	(Bandelj et al. 2006)
	ER-CALUX	T47D	Ground water	Queensland, Australia	*v*	SPE (OASIS®HLB)	(Leusch et al. 2010)
			Surface water	Receiving streams, Australia	*v*	SPE Supelco ENVI-18	(Williams et al. 2007)
				River, Queensland, Australia	*v*	SPE (OASIS®HLB)	(Leusch et al. 2010)
			Wastewater	Sec, UV, Cl	*v*	SPE (Supelco ENVI-18)	(Williams et al. 2007)
				Raw + unspecified treatment	*v*	SPE (OASIS®HLB)	(Leusch et al. 2010)
	Other mammalian reporter gene assays	MELN cells	Ground water	Queensland, Australia	*v*	SPE (OASIS®HLB)	(Leusch et al. 2010)
			Surface water	River, Queensland, Australia	*v*	SPE (OASIS®HLB)	(Leusch et al. 2010)
			Wastewater	Raw + unspecified treatment	*v*	SPE (OASIS®HLB)	(Leusch et al. 2010)
		Sheep uterus cells	Wastewater	Raw, sec, tert (Uv, O_3, BAC)	*v*	SPE (RP-C18)	(Leusch et al. 2005)
				Raw, prim/sec	*v*	SPE (OASIS®HLB)	(Leusch et al. 2006a)
				Raw	*v*	SPE (comparison of several solid phases)	(Leusch et al. 2006b)
				Incl. animal waste. Sec	*v*	SPE (OASIS®HLB)	(Sarmah et al. 2006)
		T47D-KBluc	Ground water	Queensland, Australia	*v*	SPE (OASIS®HLB)	(Leusch et al. 2010)
			Surface water	River, Queensland, Australia	*v*	SPE (OASIS®HLB)	(Leusch et al. 2010)
			Wastewater	Raw + unspecified treatment	*v*	SPE (OASIS®HLB)	(Leusch et al. 2010)
	E-SCREEN	MCF-7-BOS	Drinking water	Prim, sec, tert (Cl_2)	*v*	SPE (OASIS®HLB)	(Macova et al. 2011)
			Ground water	Queensland, Australia	*v*	SPE (OASIS®HLB)	(Leusch et al. 2010)
			Surface water	River, Queensland, Australia	*v*	SPE (OASIS®HLB)	(Leusch et al. 2010)
				Brisbane rivers, lakes, receiving recycled water	*v*	SPE (OASIS®HLB)	(Macova et al. 2011)
			Wastewater	Raw, sec, tert (Uv, O_3, BAC)	*v*	SPE (RP-C18)	(Leusch et al. 2005)
				Raw	*v*	SPE (comparison of several solid phases)	(Leusch et al. 2006b)
				Sec	*v*	SPE (OASIS®HLB)	(Tan et al. 2007)
				Raw + unspecified treatment	*v*	SPE (OASIS®HLB)	(Leusch et al. 2010)
				Prim, sec, tert (AC, O_3, DAFF)	*v*	SPE (OASIS®HLB)	(Macova et al. 2010)
				Prim, sec, tert (RO, UV, H_2O_2)	*v*	SPE (OASIS®HLB)	(Escher et al. 2011)

Target MoA	Assay	Test organism	Application	Specifics	TEQ	Enrichment	Literature reference
				Prim, sec, tert (MF, RO, O_3, BAC)	*v*	SPE (OASIS®HLB)	(Macova et al. 2011)
	YES	*S. cerevisiae*	Ground water	Queensland, Australia	*v*	SPE (OASIS®HLB)	(Leusch et al. 2010)
			Surface water	Receiving streams, Australia	*v*	SPE (Supelco ENVI-18)	(Williams et al. 2007)
				River, Switzerland	*v*	SPE (C18, EN)	(Escher et al. 2008b)
				River, Queensland, Australia	*v*	SPE (OASIS®HLB)	(Leusch et al. 2010)
			Wastewater	Treatment unspecified	*v*	SPE (Lichrolut: C18, EN)	(Rutishauser et al. 2004)
				Sec, Cl, UV	*v*	SPE (Supelco ENVI-18)	(Williams et al. 2007)
				Prim, sec	*v*	SPE (C18, EN)	(Escher et al. 2008b)
				Raw, sec, pondage, UV, Cl	*v*	SPE (LC-18, Supelco)	(Coleman et al. 2008)
				Raw + MBR	*v*	SPE (OASIS®HLB)	(Coleman et al. 2009)
				Prim, sec, tert (O_3, AC)	*v*	SPE (C18, EN)	(Escher et al. 2009)
				Sec, Cl, UV, MF/RO, DAFF	*v*	SPE (Supelco ENVI-18)	(Holmes et al. 2010)
				Raw + unspecified treatment	*v*	SPE (OASIS®HLB)	(Leusch et al. 2010)
	Yeast two-hybrid	*S. cerevisiae* Y190 with human and medaka ERα	Wastewater	Sec	*v*	SPE (C18)	(Mispagel et al. 2005)
				Sec	*v*	SPE (C18)	(Allinson et al. 2007)
				Sec	*v*	SPE (C18)	(Mispagel et al. 2009)
				Sec	*v*	SPE (C18)	(Allinson et al. 2010d)
Alternative ER techniques	Receptor competitive binding	Rainbow trout liver	Pulp mill effluent, NZ and Canada	Sec	*v*	SPE (XAD-7)	(Bandelj et al. 2006)
			Surface water	Rivers, New Zealand, Canada	*v*	SPE (XAD-7)	(Bandelj et al. 2006)
			Wastewater	Sec	*v*	SPE (XAD-7)	(Bandelj et al. 2006)
				Raw	*v*	SPE (comparison of several solid phases)	(Leusch et al. 2006b)
	RTG-2 reporter gene assay	RTG-2 cells	Wastewater	Treatment unspecified	*v*	SPE (LiChrolut C18, EN)	(Rutishauser et al. 2004)
Dioxin-like activity							
AhR activation	AhR-CAFLUX	H4IIE	Drinking water	Prim, sec, tert (Cl_2)	*v*	SPE (OASIS®HLB)	(Macova et al. 2011)
	H4IIE bioassay		Surface water	Contaminated wetlands, Sydney	*v*	SPE (Empore C18)	(Rawson et al. 2009)
	AhR-CAFLUX			Brisbane rivers, lakes, receiving recycled water	*v*	SPE (OASIS®HLB)	(Macova et al. 2011)
			Wastewater	Prim, sec, tert (AC, O_3, DAFF)	*v*	SPE (OASIS®HLB)	(Macova et al. 2010)
				Prim, sec, tert (MF, RO, O_3, BAC)	*v*	SPE (OASIS®HLB)	(Macova et al. 2011)

Target MoA	Assay	Test organism	Application	Specifics	TEQ	Enrichment	Literature reference
	Yeast two-hybrid	*S. cerevisiae* Y190 with human and medaka ERα	Wastewater	Prim, sec	*v*	SPE (C18)	(Allinson et al. 2010a)
Developmental toxicity							
Retinoic acid receptor (RAR)	Yeast two-hybrid	*S. cerevisiae* Y190 with human and medaka ERα	Wastewater	Prim, sec	*v*	SPE (C18)	(Allinson et al. 2010b)
Neurotoxicity							
Inhibition of acetylcholin-esterase (AChE)	AChE inhibition	*E. electricus*	Drinking water	Prim, sec, tert (Cl_2)	*v*	SPE (OASIS®HLB)	(Macova et al. 2011)
		Purified beef AChE	Surface water	River, Switzerland	*v*	SPE (C18, EN)	(Escher et al. 2008b)
		E. electricus		Brisbane rivers, lakes, receiving recycled water	*v*	SPE (OASIS®HLB)	(Macova et al. 2011)
		Purified beef AChE	Wastewater	Prim, sec	*v*	SPE (C18, EN)	(Escher et al. 2008b)
				Prim, sec, tert (O_3, AC)	*v*	SPE (C18, EN)	(Escher et al. 2009)
		E. electricus		Prim, sec, tert (AC, O_3, DAFF)	*v*	SPE (OASIS®HLB)	(Macova et al. 2010)
				Prim, sec, tert (MF, RO, O_3, BAC)	*v*	SPE (OASIS®HLB)	(Macova et al. 2011)
Sodium channel blocking	Neuroblastoma assay	Neuro2A ATCC CCL-131	Surface water	Lakes, rivers + oxidation ponds, NZ		WET	(Wood et al. 2006)
REACTIVE TOXICITY							
Genotoxicity: SOS-response	*umu*C	*S.typhimurium* TA1535/pSK1002	Drinking water	Prim, sec, tert (Cl_2)	*v*	SPE (OASIS®HLB)	(Macova et al. 2011)
			Surface water	River, Switzerland	*v*	SPE (C18, EN)	(Escher et al. 2008b)
				Brisbane rivers, lakes, receiving recycled water	*v*	SPE (OASIS®HLB)	(Macova et al. 2011)
			Wastewater	Prim, sec	*v*	SPE (C18, EN)	(Escher et al. 2008b)
				Prim, sec, tert (O_3, AC)	*v*	SPE (C18, EN)	(Escher et al. 2009)
				Prim, sec, tert (AC, O_3, DAFF)	*v*	SPE (OASIS®HLB)	(Macova et al. 2010)
				Prim, sec, tert (MF, RO, O_3, BAC)	*v*	SPE (OASIS®HLB)	(Macova et al. 2011)
LOW-COMPLEXITY *IN VIVO* ASSAYS							
Phytotoxicity (algae)							
PSII derived	ToxY-PAM	*Phaeodactylum*	Surface water	Rivers, Queensland, Australia	*v*	SPE (OASIS®HLB)	(Bengtson Nash et al.

Target MoA	Assay	Test organism	Application	Specifics	TEQ	Enrichment	Literature reference
photosynthesis inhibition		*tricornutum*					2005a)
				Brisbane and Thames Estuaries	***v***	SPE (OASIS®HLB)	(Bengtson Nash et al. 2006)
	IPAM	*P. tricornutum*and *C. vulgaris*	Surface water	Brisbane River, Australia	***v***	SPE (OASIS®HLB)	(Muller et al. 2008b)
		C. vulgaris	Wastewater	Prim, sec, tert (AC, O_3, DAFF)	***v***	SPE (OASIS®HLB)	(Macova et al. 2010)
Algal growth and PSII inhibition	Combined algae test	*P. subcapitata*	Drinking water	Prim, sec, tert (Cl_2)	***v***	SPE (OASIS®HLB)	(Macova et al. 2011)
			Surface water	River, Switzerland	***v***	SPE (C18, EN)	(Escher et al. 2008b)
				Brisbane rivers, lakes, receiving recycled water	***v***	SPE (OASIS®HLB)	(Macova et al. 2011)
			Wastewater	Prim, sec	***v***	SPE (C18, EN)	(Escher et al. 2008b)
				Prim, sec, tert (O_3, AC)	***v***	SPE (C18, EN)	(Escher et al. 2009)
				Prim, sec, tert (RO, UV, H_2O_2)	***v***	SPE (OASIS®HLB)	(Escher et al. 2011)
				Prim, sec, tert (MF, RO, O_3, BAC)	***v***	SPE (OASIS®HLB)	(Macova et al. 2011)

AC = activated carbon, AWWT = advanced wastewater treatment, BAC = biologically activated carbon, Coag = coagulation, DAFF = dissolved air filtration flotation, H4IIE = rat hepatoma cell line, LLE = liquid liquid extraction, MBR = membrane bioreactor, MCF-7 = human breast carcinoma cell line, MELN = stably transfected (for ER-α) MCF-7, MF = membrane filtration, NZ = New Zealand, prim = primary treatment (physical treatment, coagulation/precipitation processes), RO = reverse osmosis, sec = secondary treatment (biological treatment), SPE = solid phase extraction, T47D = human breast adenocarcinoma cell line, tert. = tertiary treatment (advanced oxidation and oxidation processes), TEQ = toxic equivalent concentration (***v*** when applied), WET = whole effluent testing. The table does not include studies that rely entirely on passive sampling.

7. CONCLUSIONS AND OUTLOOK

Bioanalytical tools were introduced in Australia and New Zealand (ANZ) over the last decade and their application in water quality monitoring is increasing. Initial focus was set on estrogenicity in surface water and toxicity induced by natural toxins. The combined capacity throughout the region offers a wide range of assays suitable for water quality assessment and a few laboratories even facilitate in-house comprehensive test batteries. With the growing competence, the application range of bioanalytical tools has also been expanded and now ranges from sewage to drinking water, including all steps of wastewater treatment, advanced water treatment and natural attenuation in surface water and groundwater bodies. ANZ researchers have been particularly active in linking the information provided by bioassays with chemical analysis-derived data through mixture toxicity modelling.

Non-specific toxicity testing has very much focused on simple screening tests such as the bioluminescence inhibition assay with *Vibrio fischeri* (Microtox®). While this assay is practical and useful, it is advised that future research should also include more specific cytotoxicity assays using target organ cell lines, e.g. human liver or gut cell lines. Monitoring of specific toxicity is dominated by recombinant cell bioassays, which have proven particularly valuable for detection of receptor-mediated toxicity including the initial trigger event of binding of micropollutants to nuclear receptors. There are a total of 48 human nuclear receptors, only a fraction of which have been singled out as relevant for water quality assessment. The estrogen, androgen and arylhydrocarbon receptors remain the most widely targeted nuclear receptors but there is a growing interest and application of other nuclear receptor assays in the field. The retinoic acid receptor is relatively new in water quality monitoring but is likely to reach high significance due to its relevance for early life stage (ELS) development and in this way may become a suitable cell-based alternative to traditional ELS testing.

Overall, endpoints applied for reactive toxicity have been restricted to genotoxicity and mutagenicity. Despite the high priority to assess genotoxicity and associated carcinogenicity, future studies should not restrict focus to DNA damage but pay more attention to the assessment of oxidative stress and reactive toxicity towards proteins and lipids. Non-genomic pathways to carcinogenicity and effects on the endocrine system, other than receptor-mediated responses, also require future attention.

The number of test battery applications has increased steadily during the last decade; however the majority of these batteries focus on a particular mode of action class (non-specific, specific or reactive toxicity) or chemical group. Applications of MOA based test batteries are valuable in covering a broader spectrum of toxicant groups within complex mixtures. In order to ensure a balance between specific and general endpoints, it is recommended that future test batteries designed for water quality monitoring include several relevant representatives from each of the three major MOA classes.

Appendix I

Table 6: Overview of worldwide applications of cell-based assays for water quality assessment.

Target MoA	Assay	Species	Application	Specifics	Conc/resp[1]	Enrichment	Literature reference
NON-SPECIFIC TOXICITY (Baseline toxicity)							
Cytotoxicity	Bioluminescence inhibition (Microtox,	*Vibrio fischeri*	Coal gasification process water	Groundwater effluents Wyoming	Y	LLE (DCM) + fractionation	(Timourian et al. 1982)
	Biotox, Lumistox,		Drinking water	Spring and tap water, USA	Y	WET	(Chang et al. 1981)
	Luminotox,			Raw lake, NaClO, ClO_2, PAA	Y	SPE (C18)	(Guzzella et al. 2004)
	ToxAlert®)			Raw lake + ClO_2 and GAC	Y	SPE (C18)	(Zani et al. 2005)
				Treatment plants (ClO_2, O_3, GAC)	Y	SPE (C18)	(Guzzella et al. 2006)
				Bottled, still and carbonated	No	WET	(Ceretti et al. 2010)
				Prim, sec, tert (Cl_2)	Y (TEQ)	SPE (OASIS®HLB)	(Macova et al. 2011)
			Groundwater	Raw + prim	Y	WET	(Gustavson et al. 2000)
			Landfill leachates	Mixed	Y (no response)	WET	(Clement et al. 1996)
				Municipal	Y	WET	(Isidori et al. 2003)
			Oil refinery effluent	USA	Y	WET	(Chang et al. 1981)
				Finland	Y	WET + SPE (OASIS®HLB)	(Pessala et al. 2004)
			Paper/pulp mill	Unspecified treatment	Y	WET + SPE (OASIS®HLB)	(Pessala et al. 2004)
				Prim, sec	Y	WET	(Gartiser et al. 2010)
				Prim, sec	Y	WET	(Rosa et al. 2010)
			Surface water	Mixed USA	Y	WET	(Chang et al. 1981)
				River, Germany	No	SPE + fractionation	(Reineke et al. 2002)
				River + bore hole, UK	Y	WET	(Wadhia et al. 2007)
				River, Switzerland	Y (TEQ)	SPE (C18, EN)	(Escher et al. 2008b)
				River, Turkey	Y (TU)	WET	(Günes et al. 2008)
				Rivers, Poland	No (TU)	WET	(Mankiewicz-Boczek et al. 2008)
				River, Italy	Y (TU)	WET	(Bicchi et al. 2009)
				River, Italy	Y (TU)	WET	(Schilirò et al. 2009)
				Freshwater reservoir, Portugal	Y	WET	(Palma et al. 2010)
				Rivers, lakes receiving recycled water, Australia	Y (TEQ)	SPE (OASIS®HLB)	(Macova et al. 2011)
			Wastewater	Mixed	Y (TU)	WET	(Hao et al. 1996)
				Prim + unspecified treatment, also deep well water	Y (TU)	WET	(Rojícková-Padrtová et al. 1998)
				Mixed	Y (TU)	WET	(Tarkpea et al. 1998)
				Tannery effluents	Y	SPE	(Reemtsma et al. 1999)
	(two tests)			Mixed	Y	SPE (C18)	(Castillo et al. 2001)
				Prim, sec	Y	WET	(Guerra 2001)

			Prim, sec	Y (TU)	WET	(Farré et al. 2002)
			Various TPs, treatment unspecified	Y (TU)	WET	(Manusadzianas et al. 2003)
			Mixed, unspecified treatment	Y	WET + SPE (OASIS®HLB)	(Pessala et al. 2004)
			Prim, sec	Y (TU)	WET	(Schiliró et al. 2004)
			Prim, sec	Y (TU)	WET	(Araujo et al. 2005)
			Hospital effluents	Y (TU)	WET	(Emmanuel et al. 2005)
			Olive mill (raw + fungal treatment)	No	WET	(Dhouib et al. 2006)
			Olive mill (raw + tert (electrochemical))	No	WET	(Khoufi et al. 2006)
			Sec + lab coagulants tests	Y	SPE (C18)	(Petala et al. 2006a)
			Sec + lab coagulation, O_3 tests	No	WET	(Petala et al. 2006b)
			Prim, sec	Y	WET	(Katsoyiannis and Samara 2007)
			Mixed effluents	Y	WET	(Wadhia et al. 2007)
			Prim, sec	Y (TEQ)	SPE (C18, EN)	(Escher et al. 2008b)
			Prim + sec	Y (TU)	WET	(Günes et al. 2008)
			Sec + coag, AC, Cl tests	No	WET	(Kontana et al. 2008)
			Olive mill (prim, tert (electrochemical))	Y	WET, LL (ethyl acetate)	(Mekki et al. 2008)
			Sec, O_3	Y	SPE (C18)	(Petala et al. 2008)
			Raw + bacterial degradation	Y (TU)	WET	(Plaza et al. 2008)
			Sec + PAA	NO	WET	(Antonelli et al. 2009)
			Tert (Cl)	Y (TU)	WET	(Bicchi et al. 2009)
			Tanning effluent. UF, micro- and membrane filtration	Y (TU)	WET	(Catarino et al. 2009)
			Prim, sec, tert (O_3, AC)	Y (TEQ)	SPE (C18, EN)	(Escher et al. 2009)
			Mixed	Y	WET	(Gartiser et al. 2009)
			Prim, sec	Y	WET	(Mendonca et al. 2009)
			Sec, tert (lab coag, Cl, AC, O_3)	No	WET	(Kontana et al. 2009)
			Prim, sec	Y	WET	(Ellouze et al. 2009)
			Municipal, poultry, brewery. Prim, sec	Y	WET	(Ostra et al. 2009)
			From pectin production. Prim, sec	Y	WET and SPE (XAD-8) + fractionation	(Reginatto et al. 2009)
			Prim, tert (MBR)	No	WET	(Saddoud et al. 2009)
			Tert (Cl)	Y (TU)	WET	(Schilirò et al. 2009)
			Fertiliser production (raw + precipitation with hydrated lime)	No	WET	(Gouider et al. 2010)
			Prim, sec. tert (O_3, AC, DAFF)	Y (TEQ)	SPE (OASIS®HLB)	(Macova et al. 2010)
			Sec, tert (biofilm, O_3)	Y	WET	(Lundstrom et al. 2010)
			Raw, tert (anaerobic MBR)	No	WET	(Saddoud et al. 2010)
			Prim, sec, tert (RO, UV, H_2O_2)	Y (TEQ)	SPE (OASIS®HLB)	(Escher et al. 2011)
			Prim, sec, tert (MF, RO, O_3, BAC)	Y (TEQ)	SPE (OASIS®HLB)	(Macova et al. 2011)
			Prim, sec, tert (sand filtration, BAC)	Y (TEQ)	SPE (OASIS®HLB)	(Reungoat, 2011)
	Photobacterium phosphoreum	Surface water	River, Germany	No	WET	(Dizer et al. 2002)
		Wastewater	Sec, Cl, O_3	Y	WET	(Arana et al. 1999)
			Prim, sec	Y (no EC)	WET	(Dizer et al. 2002)
			Sec	Y	SPE (C18) + frac	(Allinson et al. 2007)

				Sec	Y	SPE ((C18) + frac	(Mispagel et al. 2009)
				Sec	Y	SPE (C18) + frac.	(Allinson et al. 2010d)
Inhibition of nitrification		Unspecified bacteria	Wastewater	Mixed effluents	Y	WET	(Wadhia et al. 2007)
Inhibition of respiration		Unspecified bacteria	Wastewater	Mixed effluents	Y	WET	(Wadhia et al. 2007)
		E. coli		TNT effluent	No	WET	(Barreto-Rodrigues et al. 2009)
	Polytox®Test	Unspecified bacteria		Prim, sec	Y	WET	(Mendonca et al. 2009)
Cytotoxicity	Yeast cell viability	*Saccharomyces cerevisiae* (D7)	Drinking water	Raw lake, NaClO, ClO_2, PAA	Y	SPE (C18)	(Guzzella et al. 2004)
				Raw lake + ClO_2 and GAC	Y	SPE (C18)	(Zani et al. 2005)
				Treatment plants (ClO_2, O_3, GAC)	Y	SPE (C18)	(Guzzella et al. 2006)
	Vitotox 10 kit	*Salmonella typhimurium* TA104 *pr*1	Oil refinery	Finland	Y	WET + SPE (OASIS®HLB)	(Pessala et al. 2004)
			Paper/pulp mill	Unspecified treatment	Y	WET + SPE (OASIS®HLB)	(Pessala et al. 2004)
			Surface water	Lake, Italy	Y	SPE (C18)	(Pellacani et al. 2006)
	MARA	Multispecies (10 bacteria, 1 yeast)		River + bore hole, UK	Y (MTC)	WET	(Wadhia et al. 2007)
	Vitotox 10 kit	*S. typhimurium* TA104 *pr*1	Wastewater	Mixed, unspecified treatment	Y	WET + SPE (OASIS®HLB)	(Pessala et al. 2004)
	Yeast growth inhibition	*S. cerevisiae*		Unspecified treatment	Y (TEQ)	SPE (Lichrolut C18, EN)	(Rutishauser et al. 2004)
				Sec	Y (No IC)	WET	(Schmitt et al. 2005)
	Bacterial growth inhibition	Multispecies (3)		Olive mill (prim, tert (electrochemical))	Y	WET, LL (ethyl acetate)	(Mekki et al. 2008)
	Yeast cell yield in GreenScreen EM®	*S. cerevisiae* with reporter gene		O_3 and electrochemical oxidation	Y	WET	(Keenan et al. 2007)
	MARA	Multispecies (10 bacteria, 1 yeast)		Mixed effluents	Y (MTC)	WET	(Wadhia et al. 2007)
				Industrial effluent	Y (MTC)	WET	(Wadhia 2008)
				Industrial effluent	Y (MTC)	WET	(Fai and Grant 2010)
	Yeast growth inhibition	Multispecies (9)		Industrial effluent	Y	WET	(Fai and Grant 2010)
Staining assays	NR/LDH release (Promega®), FDA/EtBr, Tryptan Blue	Human leukocytes, Hep-G2	Drinking water	Advanced treatment	Y	SPE (C18)	(Buschini et al. 2004)
	NR uptake			Raw lake. NaClO, ClO_2, PAA	Y	SPE (C18)	(Marabini et al. 2006)
	NR/LDH release (Promega®), FDA/EtBr	Human lympho-/leukocytes, Hep-G2		Chlorinated tap water, Italy	Y	SPE (C18)	(Marabini et al. 2007)
	FDA/EtBr	Human leukocytes		Prim, ClO_2	Y	SPMD vs. SPE (C18)	(Buschini et al. 2008)
	NR/LDH release (Promega®), FDA/EtBr	Human lympho-/leukocytes, Hep-G2		Prim, Sec, ClO_2, AC, Italy	Y	SPMD vs. SPE (C18)	(Maffei et al. 2009)
	MTT	Hep-G2		River, China (sec, Cl)	Y	SPE (XAD-2)	(Shi et al. 2009b)
				Tap and well, Slovenia	No response	WET	(Zegura et al. 2009)
				River/Lake, Slovenia	Y (No EC)	WET	(Zegura et al. 2009)
	CFDA-AM, AB	Rainbow trout primary hepatocytes	Oil field produced	Offshore effluent, North Sea	Y	SPE (ENV+, C18)	(Farmen et al. 2010)
		RTgill-W1, BB-3	Oil refinery effluent	Ontario, Canada	Y	WET (filtered 0.2 µm) and SPE (C18)	(Schirmer et al. 2001)

	Dehydrogenase	*Arthrobacter globiformis*	Surface water	Rivers, Germany	Y	SPE (XAD-4,7)	(Keiter et al. 2006)
	NR	RTL-W1		Rivers, Germany	Y	SPE (XAD-4,7)	(Keiter et al. 2006)
	FDA/EtBr	Human leukocytes		Lake, Italy	Y	SPE (C18)	(Pellacani et al. 2006)
	NR	RTL-W1, RTG-2		Rivers, Germany	Y	WET and SPE (XAD)	(Wolz et al. 2008)
	MTT	MELN		Rivers, near Paris	No	SPE (OASIS®HLB)	(Miege et al. 2009)
	PI	Rainbow trout hepatocytes	Wastewater	Sec	Y (no EC)	WET	(Gagné and Blaise 1998)
	NR	RTL-W1		Raw, bioreactors (several)	Y	LL, cleanup, fractionation	(Klee et al. 2004)
		MCF-7		Prim, sec, tert (flocc, O_3,biol.)	No	SPE (OASIS®HLB) + column fractionation	(Ma et al. 2005)
	Dehydrogenase	*Arthrobacter globiformis*		Incl. pulp mill effl. Prim, sec	Y	SPE (XAD-4,7)	(Keiter et al. 2006)
	NR	RTL-W1		Incl. pulp mill effl. Prim, sec	Y	SPE (XAD-4,7)	(Keiter et al. 2006)
	AB, CFDA-AM	Rainbow trout (*O. mykiss*) primary hepatocytes		Prim + unspecified treatment	Y	SPE (OASIS®HLB)	(Grung et al. 2007)
	MTT	MELN		Prim, sec	No	SPE (OASIS®HLB)	(Miege et al. 2009)
	NR	MCF7		Incl. hospital waste. Prim + unspecified treatment	Y (No EC)	WET	(Zegura et al. 2009)
	MTT	HG5LN-hPXR		Sec	Y (TEQ)	LLE (DCM)	(Creusot et al. 2010)
		HepG2		Prim + MBR	No	Lyophilisation followed by DCM extraction	(Delgado et al.)
Other	Mitochondrial membrane function (respiration, membrane potential, enzymatic activity, mitochondrial swelling)	Rat liver mitochondria	Wastewater	Olive mill effluent (raw + yeast degradation)	Yes	WET	(Peixoto et al. 2008)
SPECIFIC TOXICITY							
Endocrine disruption							
Androgen receptor (AR)							
AR activity	AR-CALUX	U2OS (human)	Drinking water	Tap	Y (TEQ)	LLE (EtAc)	(Van der Linden et al. 2008)
			Surface water	River, brook, The Netherlands	Y (TEQ)	LLE (EtAc)	(Van der Linden et al. 2008)
			Wastewater	Mixed. Prim, sec	Y (TEQ)	LLE (EtAc)	(Van der Linden et al. 2008)
	AR FP	Rat AR	Wastewater	Prim, sec	Y (TEQ)	WET	(Liu et al. 2009)
	Receptor binding	HeLa (transfected with AR)	Coastal	Singapore	Y	SPE (OASIS®HLB)	(Gong et al. 2003)
		Goldfish testis	Paper/pulp mill	Sec	Y (TEQ)	SPE (C18 AR)	(Ellis et al. 2003)
		Rainbow trout brain		Sec	Y (TEQ)	SPE (XAD-7)	(Bandelj et al. 2006)
			Surface water	Rivers, New Zealand, Canada	Y (TEQ)	SPE (XAD-7)	(Bandelj et al. 2006)
			Wastewater	Sec	Y (TEQ)	SPE (XAD-7)	(Bandelj et al. 2006)
				Raw, prim/sec	Y (TEQ)	SPE (OASIS®HLB)	(Leusch et al. 2006a)
				Raw	Y (TEQ)	SPE (OASIS®HLB, Supelclean LC-18, Isolute C2/C18 (EC))	(Leusch et al. 2006b)
	YAS	*S. cerevisiae*	Oil field produced	Offshore, North Sea	Y (TEQ)	SPE (ENV+)	(Thomas et al. 2004)
			Wastewater	Sec + chlorinated, ponding time	Y (TEQ)	SPE (Empore)	(Conroy et al. 2007)
				Raw, sec, pondage, UV, Cl	TEQ	SPE (LC-18, Supelco)	(Coleman et al. 2008)

				Raw + MBR	No but TEQ	SPE (OASIS®HLB)	(Coleman et al. 2009)
				Incl. pulp mill. Sec	Y (TEQ)	SPE (EN RP18)	(Sousa et al. 2010)
	Yeast two-hybrid	*S. cerevisiae* w human AR		Sec	Y (TEQ)	SPE (C18) + frac	(Allinson et al. 2007; Allinson et al. 2008)
AR (ant)-agonism	AR reporter gene	MDA-kb2		Sec	Y (TEQ)	LLE (DCM)	(Creusot et al. 2010)
		PALM	Wastewater + particulate fraction	Tert (Cl)	Y (TEQ)	SPE (C18) DCM/MeOH extraction (PM)	(Mnif et al. 2010)
	YAS	*S. cerevisiae*	Surface water	Polluted river, Italy	No	SPE (OASIS®HLB)	(Urbatzka et al. 2007)
		Y187	Wastewater	Prim, sec, tert (Cl)	Y (TEQ)	SPE (OASIS®HLB)	(Li et al. 2010a)
AR antagonism	AR Luciferase reporter assay kit (Promega)	CV-1		Industrial (sec)	Y (TEQ)	SPE	(Shi et al. 2009a)
	YAS	*S. cerevisiae*	Oil field produced	Offshore, North Sea	Y (TEQ)	SPE (ENV, C18) + frac.	(Tollefsen et al. 2007)
			Oil field produced	Offshore, North Sea	Y (TEQ)	SPE (ENV, C18) + frac.	(Thomas et al. 2009)
AR indirect	Aromatase inhibition	Rainbow trout ovaries	Pulp mill	Sec	Y (TEQ)	SPE (XAD-7)	(Bandelj et al. 2006)
			Surface water	Rivers, New Zealand, Canada	Y (TEQ)	SPE (XAD-7)	(Bandelj et al. 2006)
			Wastewater	Sec	Y (TEQ)	SPE (XAD-7)	(Bandelj et al. 2006)
Estrogen receptor (ER)							
ER (ant)-agonism	YES	*S. cerevisiae* (RMY326 ER-ERE)	Coastal water	Harbour, open sea, estuarine, river, Italy	No	SPE (XAD-2)	(Pinto et al. 2005)
			Ultrapure water	Commercial and tap water for lab estrogenicity testing	No	SPE (C18, OASIS®HLB, XAD-2)	(Sanfilippo et al. 2010)
ER antagonism	Inhibition of galactose induced activity	W303a	Wastewater	Prim, sec	No	SPE (LiChrolut RP-18)	(Garcia-Reyero et al. 2001)
	Modified YES (inhibition of E2 induced ER activity)			Sec/Cl	Y (%E2 activity)	SPE (C18)	(Buckley 2010)
	Yeast two-hybrid (with ELISA)		Pulp mill effluent	Japan	Y(TEQ)	SPE (C18)	(Terasaki et al. 2009)
ER activity	Yeast luciferase	BMAEREluc/ERα	Wastewater	Treatment unspecified	No but TEQ	SPE (OASIS®HLB)	(Salste et al. 2007)
	ER-CALUX	U2OS (human)	Drinking water	Tap	Y (TEQ)	LLE (EtAc)	(Van der Linden et al. 2008)
		T47D	Ground water	Queensland, Australia	Y (TEQ)	SPE (OASIS®HLB)	(Leusch et al. 2010)
			Surface water	River, estuary, The Netherlands	Y (TEQ)	SPE (SDB-XC)	(Murk et al. 2002)
				Receiving streams, Australia	Y (TEQ)	SPE Supelco ENVI-18	(Williams et al. 2007)
		U2OS (human)		River, brook, The Netherlands	Y (TEQ)	LLE (EtAc)	(Van der Linden et al. 2008)
		T47D		River, Queensland, Australia	Y (TEQ)	SPE (OASIS®HLB)	(Leusch et al. 2010)
			Wastewater	Prim + unspecified treatment	Y (TEQ)	SPE (SDB-XC)	(Murk et al. 2002)
				Sec, UV, Cl	Y (TEQ)	SPE (Supelco ENVI-18)	(Williams et al. 2007)
		U2OS (human)		Mixed. Prim, sec	Y (TEQ)	LLE (EtAc)	(Van der Linden et al. 2008)
		T47D		Prim, sec	Y	WET	(Mendonca et al. 2009)
				Raw + unspecified treatment	Y (TEQ)	SPE (OASIS®HLB)	(Leusch et al. 2010)
	Other mamm. rep. gene/receptor binding assays	CV-1		Industrial (sec)	Y (TEQ)	SPE	(Shi et al. 2009a)
		HEK293	Surface water	Receiving river, Germany	Y (TEQ)	SPE (RP-C18)	(Pawlowski et al. 2003)

	HeLa (tramsfected with ERα or ERβ)	Coastal water	Singapore	Y	SPE (OASIS®HLB)	(Gong et al. 2003)
		Wastewater	Sec	Y (TEQ)	SPE (RP-C18)	(Pawlowski et al. 2003)
	MELN cells	Drinking water	O_3, AC, Cl, e-Cl, flocc, coag	Y (TEQ)	SPE (OASIS®HLB)	(Jugan et al. 2009)
		Ground water	Queensland, Australia	Y (TEQ)	SPE (OASIS®HLB)	(Leusch et al. 2010)
		Surface water	Rivers, Paris	No but TEQ	SPE (C18)	(Cargouet et al. 2004)
			River, Paris	Y (TEQ)	SPE (OASIS®HLB)	(Jugan et al. 2009)
			Rivers near Paris	No but TEQ	SPE (OASIS®HLB)	(Miege et al. 2009)
			River, Queensland, Australia	Y (TEQ)	SPE (OASIS®HLB)	(Leusch et al. 2010)
		Wastewater	Prim, sec	No	WET	(Balaguer et al. 1999)
			Prim, sec	No but TEQ	SPE (C18)	(Cargouet et al. 2004)
			Prim, sec	Y (TEQ)	SPE (OASIS®HLB)	(Muller et al. 2008a)
			Sec	Y (TEQ)	SPE (C18)	(Mahjoub et al. 2009)
			Dom (Paris), prim, sec	Y (TEQ)	SPE (OASIS®HLB)	(Jugan et al. 2009)
			Prim, sec	No but TEQ	SPE (OASIS®HLB)	(Miege et al. 2009)
			Sec	Y (TEQ)	LLE (DCM)	(Creusot et al. 2010)
			Sec (dissolved phase, suspended solids	Y (TEQ)	SPE (GX-271 ASPEC™) EtAc/MeOH extraction (PM)	(Dagnino et al. 2010)
			Raw + unspecified treatment	Y (TEQ)	SPE (OASIS®HLB)	(Leusch et al. 2010)
	MVLN		Sec	Y (TEQ)	SPE (Empore disks)	(Snyder et al. 2001)
			Prim + sec	Y (TEQ)	SPE (EDS-1)	(Furuichi et al. 2006)
	Rat uterus cytosol	Surface water	River, estuary, The Netherlands	Y (TEQ)	SPE (SDB-XC)	(Murk et al. 2002)
		Wastewater	Prim + unspecified treatment	Y (TEQ)	SPE (SDB-XC)	(Murk et al. 2002)
	Sheep uterus cells		Raw, sec, tert (Uv, O_3, BAC)	Y (TEQ)	SPE (RP-C18) + frac	(Leusch et al. 2005)
			Raw, sec	Y (TEQ)	SPE (OASIS®HLB)	(Leusch et al. 2006a)
			Raw	Y (TEQ)	SPE (OASIS®HLB, Supelclean LC-18, Isolute C2/C18 (EC))	(Leusch et al. 2006b)
			Incl. animal waste. Sec	Y (TEQ)	SPE (OASIS®HLB)	(Sarmah et al. 2006)
	Transfected MCF-7		Prim + O_3	NO	WET	(Bertanza et al. 2010)
	T47D-KBluc	Ground water	Queensland, Australia	Y (TEQ)	SPE (OASIS®HLB)	(Leusch et al. 2010)
		Surface water	River, Queensland, Australia	Y (TEQ)	SPE (OASIS®HLB)	(Leusch et al. 2010)
		Wastewater	Raw + unspecified treatment	Y (TEQ)	SPE (OASIS®HLB)	(Leusch et al. 2010)
MELN with inhibition assay	MELN cells	Surface water	River, France + sed. extracts	Y (TEQ)	SPE (C18)	(Pillon et al. 2005)
		Wastewater + particulate fraction	Tert (Cl)	Y (TEQ)	SPE (C18) DCM/MeOH extraction (PM)	(Mnif et al. 2010)
E-SCREEN	MCF-7-BOS	Drinking water	Prim, sec, tert (Cl_2)	Y (TEQ)	SPE (OASIS®HLB)	(Macova et al. 2011)
		Ground water	Queensland, Australia	Y (TEQ)	SPE (OASIS®HLB)	(Leusch et al. 2010)
	T47D	Surface water	River, Japan	No but TEQ	SPE (Sep-pak C18)	(Matsuoka et al. 2005)
	MCF-7 BOS		Rivers, wetlands, ponds, USA	Y (TEQ)	SPE (OASIS®HLB)	(Shappell 2006)
	MCF-7 BUS		River, Italy	Y (TEQ)	SPE (low vol)	(Bicchi et al. 2009)
			River, Korea	Y (TEQ)	LLE (DCM) + fractionation	(Oh et al. 2009)
			River, Italy	Y (TEQ)	SPE (low vol)	(Schilirò et al. 2009)
	MCF-7-BOS		River, Queensland, Australia	Y (TEQ)	SPE (OASIS®HLB)	(Leusch et al. 2010)

	MVLN		Kaoping River, Taiwan	Y (TEQ)	SPE (EN)	(Shue et al. 2009)
	MCF-BUS		Creek and tile drainage near dairy, USA	Y (TEQ)	SPE (OASIS®HLB)	(Shappell et al. 2010)
	MVLN		Donggan River, Taiwan	Y (TEQ)	SPE (C18, EN)	(Shue et al. 2010)
E-SCREEN (modified)	MCF-7		Rivers, South Africa	No	SPE (C18)	(Swart et al. 2011)
E-SCREEN			Rivers, lakes, receiving recycled water, Australia	Y (TEQ)	SPE (OASIS®HLB)	(Macova et al. 2011)
	MCF-7	Wastewater	Prim, sec, (one sample UF)	Y (TEQ)	SPE (C18, ENV+)	(Körner et al. 1999)
			Prim, sec	Y (TEQ)	SPE (C18, ENV+)	(Körner et al. 2000)
			Sec	Y (TEQ)	SPE (RP-C18)	(Körner et al. 2001)
			Prim, sec	Y (TEQ)	SPE (low vol)	(Schiliró et al. 2004)
			Raw, sec, tert (Uv, O_3, BAC)	Y (TEQ)	SPE (RP-C18) + frac	(Leusch et al. 2005)
			Raw	Y (TEQ)	SPE (OASIS®HLB, Supelclean LC-18, Isolute C2/C18 (EC))	(Leusch et al. 2006b)
	MCF-7 BOS		Sec	Y (TEQ)	SPE (OASIS®HLB)	(Shappell 2006)
	MCF-7		Swine farm. Prim, sec.	Y (TEQ)	SPE (OASIS®HLB)	(Shappell et al. 2007)
			Sec	Y (TEQ)	SPE (OASIS®HLB)	(Tan et al. 2007)
	MCF-7 BUS		Prim, MBR, NF, RO	No but TEQ	SPE (Sep-pak®)	(Lee et al. 2008)
			Tert (Cl)	Y (TEQ)	SPE (low vol)	(Bicchi et al. 2009)
			Tert (Cl)	Y (TEQ)	SPE (low vol)	(Schilirò et al. 2009)
	MCF-7-BOS		Raw + unspecified treatment	Y (TEQ)	SPE (OASIS®HLB)	(Leusch et al. 2010)
			Prim, sec, tert (AC, O_3, DAFF)	Y (TEQ)	SPE (OASIS®HLB)	(Macova et al. 2010)
E-SCREEN (modified)	MCF-7		Unspecified treatment	No	SPE (C18)	(Swart et al. 2011)
E-SCREEN			Prim, sec, tert (RO, UV, H_2O_2)	Y (TEQ)	SPE (OASIS®HLB)	(Escher et al. 2011)
			Prim, sec, tert (MF, RO, O_3, BAC)	Y (TEQ)	SPE (OASIS®HLB)	(Macova et al. 2011)
Flow cytometric E-SCREEN	MCF-7		Prim, sec, UV	Y (TEQ)	SPE (OASIS®HLB)	(Vanparys et al. 2010)
YES	*S. cerevisiae*	Coastal waters	Baltic Sea, Germany	Y (TEQ)	SPE (OASIS®HLB)	(Beck et al. 2006a; Beck et al. 2006b)
	S. cerevisiae (RMY326)		Seafood farm water, Italy	No	LLE (DCM)	(Pinto et al. 2008)
	S. cerevisiae		Gulf of Mexico, USA	Y (TEQ)	LLE (DCM)	(Weston et al. 2010)
		Drinking water	Mixed rural sources	Y (TEQ)	SPE (C18)	(Aneck-Hahn et al. 2009)
			Bottled water, PET packaging	Y (TEQ)	SPE (C18)	(Pinto and Reali 2009)
	S. cerevisiae (RMY326)		Bottled, mixed packaging incl. PET	Y (TEQ)	WET	(Wagner and Oehlmann 2009)
	S. cerevisiae	Ground water	Queensland, Australia	Y (TEQ)	SPE (OASIS®HLB)	(Leusch et al. 2010)
		Oil field produced	Offshore, North Sea	Y (TEQ)	SPE (ENV+)	(Thomas et al. 2004)
			Offshore, North Sea	Y (TEQ)	SPE (ENV+, C18) + frac.	(Tollefsen et al. 2007)
			Offshore, North Sea	Y (TEQ)	SPE (ENV+, C8)	(Balaam et al. 2009)
			Offshore, North Sea	Y (TEQ)	SPE (ENV, C18) + frac.	(Thomas et al. 2009)
		Pulp mill	Chile(?)	Y (TEQ)	SPE (C18)	(Chamorro et al. 2010)
		Surface water	River, Japan	Y (TEQ)	SPE (Sep-pak®)	(Matsui et al. 2000)
			River, estuary, The Netherlands	Y (TEQ)	SPE (SDB-XC)	(Murk et al. 2002)
			Receiving river, Germany	Y (TEQ)	SPE (RP-C18)	(Pawlowski et al. 2003)
			Receiving river, lake, Switzerland	Y (TEQ)	SPE (EN, RP18)	(Aerni et al. 2004)
			Receiving river, Germany	Y (TEQ)	SPE (RP-C18)	(Pawlowski et al. 2004)

			River, Japan	No but TEQ	SPE (Sep-pak C18)	(Matsuoka et al. 2005)
			Rivers, Switzerland	Y (TEQ)	SPE (Carbopack X120/400)	(Vermeirssen et al. 2005)
			Rivers, Germany	Y (TEQ)	SPE (XAD-4, 7)	(Keiter et al. 2006)
			Rivers, Switzerland	Y (TEQ)	SPE (Carbopack X 120/400)	(Vermeirssen et al. 2006)
			Receiving rivers, Beijing	Y (TEQ)	SPE (OASIS®HLB)	(Ma et al. 2007)
			Receiving streams, Australia	Y (TEQ)	SPE (Supelco ENVI-18)	(Williams et al. 2007)
			Nature reserve, South Africa	Y (TEQ)	SPE (C18)	(Aneck-Hahn et al. 2008)
			River, Switzerland	Y (TEQ)	SPE (C18, EN)	(Escher et al. 2008b)
			Receiving rivers, USA	Y (TEQ)	SPE (OASIS®HLB)	(Yu and Chu 2009)
			River, Queensland, Australia	Y (TEQ)	SPE (OASIS®HLB)	(Leusch et al. 2010)
		Wastewater	Dom (UK), mixed treatment	Y (TEQ)	SPE (C18)	(Desbrow et al. 1998)
			Prim, sec, Cl	Y (TEQ)	SPE (Sep-pak®)	(Matsui et al. 2000)
			Prim + unspecified treatment	Y (TEQ)	SPE (SDB-XC)	(Murk et al. 2002)
			Prim, sec	Y (TEQ)	SPE (C18)	(Tilton et al. 2002)
			Dom (NYC), Sec	Y (TEQ)	SPE (SDB-XC, C18)	(Huggett et al. 2003)
			Sec	Y (TEQ)	SPE (RP-C18)	(Pawlowski et al. 2003)
			Sec	Y (TEQ)	SPE (EN, RP18)	(Aerni et al. 2004)
			Sec	Y (TEQ)	SPE (C18)	(Aguayo et al. 2004)
			Sec	Y (TEQ)	SPE (RP-C18)	(Pawlowski et al. 2004)
			Treatment unspecified	Y (TEQ)	SPE (Lichrolut: C18, EN)	(Rutishauser et al. 2004)
			Prim, sec, tert (flocc, O_3,biol.)	Y (TEQ)	SPE (OASIS®HLB) + column fractionation	(Ma et al. 2005)
			Incl. pulp mill effl. Prim, sec.	Y (TEQ)	SPE (XAD-4, 7)	(Keiter et al. 2006)
			Various effluents UK, treatment unspecified	Y (TEQ)	SPE (not specified)	(Thorpe et al. 2006)
			Sec	Y (TEQ)	SPE (Carbopack X120/400)	(Vermeirssen et al. 2006)
			Sec+chlorination, ponding time	Y (TEQ)	SPE (Empore)	(Conroy et al. 2007)
			Prim, sec	Y (RIE)	WET	(Isidori et al. 2007)
			Prim, sec. Mixed sources	Y (TEQ)	SPE (OASIS®HLB)	(Ma et al. 2007)
			Sec, Cl, UV	Y (TEQ)	SPE (Supelco ENVI-18)	(Williams et al. 2007)
			Prim, sec	Y (TEQ)	SPE (C18, EN)	(Escher et al. 2008b)
			Pri, sec + flocc.	Y (TEQ)	SPE (OASIS®HLB)	(Sun et al. 2008)
			Raw, sec, pondage, UV, Cl	TEQ	SPE (LC-18, Supelco)	(Coleman et al. 2008)
			Raw + MBR	No but TEQ	SPE (OASIS®HLB)	(Coleman et al. 2009)
			Prim, sec, tert (O_3, AC)	Y (TEQ)	SPE (C18, EN)	(Escher et al. 2009)
			Prim, sec	Y (TEQ)	SPE (OASIS®HLB)	(Yu and Chu 2009)
			Sec/Cl	Y (%E2 max)	SPE (C18)	(Buckley 2010)
			Sec, Cl, UV, MF/RO, DAFF	Y (TEQ)	SPE (Supelco ENVI-18)	(Holmes et al. 2010)
			Raw + unspecified treatment	Y (TEQ)	SPE (OASIS®HLB)	(Leusch et al. 2010)
			Prim, sec, tert (Cl)	Y (TEQ)	SPE (OASIS®HLB)	(Li et al. 2010a)
			Incl. pulp mill. Sec	Y (TEQ)	SPE (EN RP18)	(Sousa et al. 2010)
Yeast two-hybrid	*S. cerevisiae* Y190 with human and medaka ERα	Wastewater	Sec	Y (TEQ)	SPE (C18) + fractionation	(Mispagel et al. 2005)
			Sec	Y (TEQ)	SPE (C18) + fractionation	(Allinson et al. 2007)

				Treatment unspecified	No	SPE (PLS-3)	(Takahashi et al. 2007)
				Sec	Y (TEQ)	SPE (C18)	(Mispagel et al. 2009)
				Sec	Y (TEQ)	SPE (C18) + fractionation.	(Allinson et al. 2010d)
				Sec, dairy and municipal	Y (TEQ)	WET	(Oishi and Moriuchi 2010)
Alternative ER methods	Vtg ELISA	Juvenile brown trout hepatocytes	Oil refinery	Finland	Y	WET	(Pessala et al. 2004)
			Paper/pulp mill	Unspecified treatment	Y	WET	(Pessala et al. 2004)
	Receptor competitive binding	Rainbow trout liver		Sec	Y (TEQ)	SPE (XAD-7)	(Bandelj et al. 2006)
	Vtg dot blot/RNAse assay	Rainbow trout primary hepatocytes	Surface water	Receiving river, Germany	Y (TEQ)	SPE (RP-C18)	(Pawlowski et al. 2003)
		Rainbow trout hepatocytes		Rivers, mixed locations, Germany	No	SPE (XAD)	(Hollert et al. 2005)
	Ishikawa cell ALP	3H-12 cell		River, Japan	No but TEQ	SPE (Sep-pak C18)	(Matsuoka et al. 2005)
	Receptor competitive binding	Rainbow trout liver		Rivers, New Zealand, Canada	Y (TEQ)	SPE (XAD-7)	(Bandelj et al. 2006)
	Vtg/Western blot	S. cerevisiae BMA 64-1A		Rivers, CA, USA	Y (TEQ)	SPE (C18)	(Lavado et al. 2009)
	MCF-7 ERα ELISA	MCF-7		Rivers, South Africa	No	SPE (C18)	(Swart and Pool 2009)
		MCF-7		Rivers, South Africa	No	SPE (C18)	(Swart et al. 2011)
	Vtg alkali labile phosphate method	Rainbow trout hepatocytes	Wastewater	Sec	Y (no EC)	WET	(Gagné and Blaise 1998)
	Vtg CISH assay	Rainbow trout hepatocytes		Sec	Y (no EC)	WET	(Gagné and Blaise 1998)
	RYA (recombinant yeast assay)	BJ559, BY4741		Prim, sec	Y ('TEQ')	SPE (LiChrolut RP-18)	(Garcia-Reyero et al. 2001)
	Vtg dot blot/RNAse assay	Rainbow trout primary hepatocytes		Sec	Y (TEQ)	SPE (RP-C18)	(Pawlowski et al. 2003)
	Vtg ELISA	Juvenile brown trout hepatocytes		Mixed, unspecified treatment	Y	WET	(Pessala et al. 2004)
	RTG-2 reporter gene assay	RTG-2 cells		Treatment unspecified	Y (TEQ)	SPE (LiChrolut C18, EN)	(Rutishauser et al. 2004)
	Receptor competitive binding	Rainbow trout liver		Sec	Y (TEQ)	SPE (XAD-7)	(Bandelj et al. 2006)
		Rainbow trout liver		Raw	Y (TEQ)	SPE (OASIS®HLB, Supelclean LC-18, Isolute C2/C18 (EC))	(Leusch et al. 2006b)
	RYA (recombinant yeast assay)	S. cerevisiae BMA 64-1A		Prim, sec	Y (TEQ)	SPE (RP, C18)	(Roda et al. 2006)
	ER-α FP	hrERα		Ozonation of prim and sec	Y (TEQ)	SPE (OASIS®HLB)	(Zhang et al. 2006)
	NRL ER-α	Human TIF2/*E. Coli*		Ozonation of prim and sec	Y (TEQ)	SPE (OASIS®HLB)	(Zhang et al. 2006)
	ELISA (Vtg)	*O. mykiss* hepatocytes		Prim + unspecified treatment	Y (TEQ)	SPE (OASIS®HLB)	(Grung et al. 2007)
	ER-α FP	Cell line unspecified		Sec + O_3	Y (TEQ)	SPE (OASIS®HLB)	(Kim et al. 2007)
	A. thaliana assay	Transgenic *A. thaliana*		Treatment unspecified	No	WET and SPE (PLS-3)	(Takahashi et al. 2007)
	ELISA (Vtg)	Three-spined stickleback hepatocytes		Treatment unspecified	Y (TEQ)	SPE (OASIS®HLB)	(Björkblom et al. 2008)
	Recombinant yeast	W303-1A (with hER, 2ERE)		Farm, prim, sec	Y (TEQ)	SPE (HZ-802)	(Li et al. 2008b)

	rtER	Rainbow trout liver		Dom/paper mill, sec. Daily variation.	Y (TEQ)	SPE (C18)	(Martinovic et al. 2008)
	ER	Cell line unspecified		Tert (O_3)	No but TEQ	SPE (several, ref not clear)	(Tsuno et al. 2008)
	NRL ER-α	Cell line unspecified		Tert (O_3)	No but TEQ	SPE (several, ref not clear)	(Tsuno et al. 2008)
	NRL ER-α	Human TIF2/*E. Coli*		Prim, tert (O_3)	Y (TEQ)	SPE (OASIS®HLB)	(Zhang et al. 2008)
	ER	Cell line unspecified		Prim, sec	Y (TEQ)	WET	(Liu et al. 2009)
	Four-hr yeast assay	Yeast (W303α)		Prim, sec +Cl	Y (TEQ)	WET + SBSE	(Balsiger et al. 2010)
	nAES (novel *Arxula adeninivorans* yeast estrogen screen)	*A. adeninivorans* G1212		Mixed	Y (TEQ)	SPE (C18)	(Kaiser et al. 2010)
	MCF-7 ERα ELISA	MCF-7		Unspecified treatment	No	SPE (C18)	(Swart et al. 2011)
Glucocorticoid receptor (GR) activity	GR-CALUX	U2OS (human)	Drinking water	Tap	Y (TEQ)	LLE (EtAc)	(Van der Linden et al. 2008)
			Surface water	River, brook, The Netherlands	Y (TEQ)	LLE (EtAc)	(Van der Linden et al. 2008)
			Wastewater	Mixed. Prim, sec	Y (TEQ)	LLE (EtAc)	(Van der Linden et al. 2008)
Pregnane X receptor (PXR) activity	PXR activity via reporter cell line	HeLa cells	Wastewater	Sec	Y (TEQ)	SPE (C18)	(Mahjoub et al. 2009)
		HG5LN-hPXR		Sec	Y (TEQ)	LLE (DCM)	(Creusot et al. 2010)
		HG_5LNGal-4-PXR	Wastewater incl. particulate fraction	Tert (Cl)	Y (TEQ)	SPE (C18) DCM/MeOH extraction (PM)	(Mnif et al. 2010)
Progesterone receptor (PR)							
PR (ant)-agonism	PR-CALUX	U2OS (human)	Drinking water	Tap	Y (TEQ)	LLE (EtAc)	(Van der Linden et al. 2008)
			Surface water	River, brook, The Netherlands	Y (TEQ)	LLE (EtAc)	(Van der Linden et al. 2008)
			Wastewater	Mixed. Prim, sec	Y (TEQ)	LLE (EtAc)	(Van der Linden et al. 2008)
		HG_5LNGal-4-PR (HeLa cells)	Wastewater incl. particulate fraction	Tert (Cl)	Y (TEQ)	SPE (C18) DCM/MeOH extraction (PM)	(Mnif et al. 2010)
	RYA (recomb. yeast assay)	BJ559, BY4741	Wastewater	Prim, sec	Y ('TEQ')	SPE (LiChrolut RP-18)	(Garcia-Reyero et al. 2001)
PR antagonism	Recombinant yeast assay	*S. cerevisiae*	Wastewater	Prim, sec, tert (Cl)	Y (TEQ)	SPE (OASIS®HLB)	(Li et al. 2010a)
Thyroid receptor (TR)							
TR antagonism	TR reporter gene assay (luciferase reporter assay kit, Promega)	CV-1	Wastewater	Industrial (sec)	Y (TEQ)	SPE	(Shi et al. 2009a)
TR activity	TR-reporter gene assay	PC-DR-LUC	Drinking water	O_3, AC, Cl, e-Cl, flocc, coag	Y (TEQ)	SPE (OASIS®HLB)	(Jugan et al. 2009)
			Surface water	River, Paris	Y (TEQ)	SPE (OASIS®HLB)	(Jugan et al. 2009)
			Wastewater	Dom (Paris), prim, sec	Y (TEQ)	SPE (OASIS®HLB)	(Jugan et al. 2009)
	Yeast two-hybrid	*S. cerevisiae*	Wastewater	Prim, sec, tert (Cl)	Y (TEQ)	SPE (OASIS®HLB)	(Li et al. 2010a)
TR (ant)agonism		Y-187 hTR-GRIP1	Drinking water	Source and treated (incl. Cl_2 and AC)	Y (TEQ)	SPE (OASIS®HLB)	(Li et al. 2010b)
	Reporter gene	CV-1	Surface water	Yangtze river, China	Y (TEQ)	SPE (C18)	(Shi et al. 2011)

Dioxin-like activity							
AhR activation	AhR-CAFLUX	H4IIE	Drinking water	Prim, sec, tert (Cl_2)	Y (TEQ)	SPE (OASIS®HLB)	(Macova et al. 2011)
	DR-CALUX		Oil field produced	Offshore effluent, North Sea	No but TEQ	SPE (C18)	(Hurst et al. 2005)
				Offshore effluent, North Sea	Y (TEQ)	SPE (C8, ENV+)	(Balaam et al. 2009)
	DRE-CALUX	Hepa 1c1c7	Surface water	Rivers, Korea	Y (TEQ)	LL (DCM)	(Joung et al. 2007)
	H4IIE bioassay	H4IIE		Contaminated wetlands, Sydney	Y (TEQ)	SPE (Empore C18)	(Rawson et al. 2009)
	DR-CALUX			Seawater, Japan	Y (TEQ)	SPE (Empore C18)	(Sato et al. 2010)
	AhR-CAFLUX			Rivers, lakes receiving recycled water, Australia	Y (TEQ)	SPE (OASIS®HLB)	(Macova et al. 2011)
	DR-CALUX		Wastewater	Prim + reed bed material	Y (TEQ)	Soxhlet, toluene + fractionation	(Gustavsson et al. 2007)
	AhR-CAFLUX			Prim, sec, tert (AC, O_3, DAFF)	Y (TEQ)	SPE (OASIS®HLB)	(Macova et al. 2010)
				Prim, sec, tert (MF, RO, O_3, BAC)	Y (TEQ)	SPE (OASIS®HLB)	(Macova et al. 2011)
	AhR via reporter cell line	HahLP cells	Surface water	River, France + sed. extracts	Y (TEQ)	SPE (C18)	(Pillon et al. 2005)
		HeLa	Wastewater	Sec	Y (TEQ)	SPE (C18)	(Mahjoub et al. 2009)
		HahLP cells		Sec (dissolved phase) suspended solids	Y (TEQ)	SPE (GX-271 ASPEC™)	(Dagnino et al. 2010)
			Wastewater + particulate fraction	Tert (Cl)	Y (TEQ)	SPE (C18) DCM/MeOH extraction (PM)	(Mnif et al. 2010)
	AhR yeast two-hybrid	*S. cerevisiae* Y190 with human and medaka ERα	Wastewater	Prim, sec	Y (TEQ)	SPE (C18)	(Allinson et al. 2010a)
	EROD	H4IIE	Coastal water	Gulf of Mexico, USA	Y (TEQ)	LLE (DCM)	(Weston et al. 2010)
		RTL-W1	Groundwater	Germany	Y (TEQ)	WET	(Schirmer et al. 2004)
			Oil field produced + receiving river	Land-based, China	Y (TEQ)	SPE (OASIS®HLB)	(Li et al. 2008a)
			Oil refinery	Ontario, Canada	Y	WET (filtered 0.2 µm) and SPE (C18)	(Schirmer et al. 2001)
		Juvenile brown trout hepatocytes		Finland	Y	WET	(Pessala et al. 2004)
			Paper/pulp mill	Unspecified treatment	Y	WET	(Pessala et al. 2004)
		RTL-W1		Non-bleached, Germany	Y (TEQ)	WET	(Schirmer et al. 2004)
		PLHC-1	Surface water	Polluted lake, Finland	Y	LL (DCM, diethylether)	(Huuskonen et al. 1998)
		Hepa 1c1c7		Rivers, Korea	Y (TEQ)	LL (DCM)	(Joung et al. 2007)
		RTL-W1		Rivers, Germany	Y (TEQ) but no response	WET and SPE (XAD)	(Wolz et al. 2008)
		Juvenile brown trout hepatocytes	Wastewater	Mixed, unspecified treatment	Y	WET	(Pessala et al. 2004)
		RTL-W1		Prim, sec, tert (flocc, O_3,biol.)	Y (TEQ)	SPE (OASIS®HLB) + column fractionation	(Ma et al. 2005)
		O. mykiss, prim. hepatocytes		Prim, sec (no info)	Y (TEQ)	SPE (OASIS®HLB)	(Grung et al. 2007)
		PLHC-1		Sec	Y (TEQ)	LLE (DCM)	(Creusot et al. 2010)
	EROD for PAHs			Sec	Y (TEQ)	LLE (DCM)	(Creusot et al. 2010)

Developmental toxicity							
Retinoic acid receptor (RAR)/RXR agonist activity	RAR/RXR assay	F9S:1 (mouse embryonic carcinoma cells?)	Paper mill effluent/surface	River/paper mill influent/effluent, Florida	No	SPE (C18)	(Schoff and Ankley 2002)
	RAR yeast two hybrid assay	*S. cerevisiae*Y190	Surface water	Rivers, Japan	Y	SPE (OASIS®HLB)	(Inoue et al. 2009b)
				Rivers, China	Y (TEQ)	SPE (OASIS®HLB)	(Zhen et al. 2009)
				Rivers, Japan	Y (TEQ)	SPE (OASIS®HLB)	(Inoue et al. 2010)
			Wastewater	Sec, tert (O_3, MF, UV, Cl, RO, coag)	Y (TEQ)	SPE (OASIS®HLB)	(Cao et al. 2009b)
				Prim, sec, tert (O_3, UV, Cl, coag, MF)	Y	SPE (OASIS®HLB)	(Inoue et al. 2009a)
				Prim, sec	Y (TEQ)	SPE (OASIS®HLB)	(Zhen et al. 2009)
				Prim, sec	Y (TEQ)	SPE (C18)	(Allinson et al. 2010b)
Neurotoxicity							
Inhibition of acetylcholine esterase (AChE)		*Drosophila melanogaster*	Drinking water	Tap water, Greece	Y	WET	(Vamvakaki and Chaniotakis 2007)
		E. electricus		Prim, sec, tert (Cl_2)	Y (TEQ)	SPE (OASIS®HLB)	(Macova et al. 2011)
		Gold fish brain	Pulp/paper mill effluent	Prim, sec	No	LLE (several solvents)	(Basu et al. 2009)
		E. electricus and *Apis mellifera*	Rain water	Conservation area, Holland	Y (TEQ)	SPE (SDVB)	(Hamers et al. 2000)
		Purified beef AChE	Surface water	River, Switzerland	Y (TEQ)	SPE (C18, EN)	(Escher et al. 2008b)
		E. electricus		Rivers, lakes receiving recycled water, Australia	Y (TEQ)	SPE (OASIS®HLB)	(Macova et al. 2011)
		Purified beef AChE	Wastewater	Prim, sec	Y (TEQ)	SPE (C18, EN)	(Escher et al. 2008b)
				Prim, sec, tert (O_3, AC)	Y (TEQ)	SPE (C18, EN)	(Escher et al. 2009)
		E. electricus		Prim, sec, tert (AC, O_3, DAFF)	Y (TEQ)	SPE (OASIS®HLB)	(Macova et al. 2010)
				Prim, sec, tert (MF, RO, O_3, BAC)	Y (TEQ)	SPE (OASIS®HLB)	(Macova et al. 2011)
	Other enzyme biomarkers						
	GABA-T	Gold fish brain	Pulp/paper mill effluent	Prim, sec	No	LLE (several solvents)	(Basu et al. 2009)
	GAD	Gold fish brain		Prim, sec	No	LLE (several solvents)	(Basu et al. 2009)
	MAO	Gold fish brain		Prim, sec	No	LLE (several solvents)	(Basu et al. 2009)
	Receptor binding						
	Dopamine-2	Gold fish brain		Prim, sec	No	LLE (several solvents)	(Basu et al. 2009)
	GABA (A)	Gold fish brain		Prim, sec	No	LLE (several solvents)	(Basu et al. 2009)
	mACh	Gold fish brain		Prim, sec	No	LLE (several solvents)	(Basu et al. 2009)
	NMDA	Gold fish brain		Prim, sec	No	LLE (several solvents)	(Basu et al. 2009)
Sodium channel blocking	Neuroblastoma assay	Neuro2A ATCC CCL-131	Cyanobacterial blooms	Lakes, rivers + oxidation ponds, NZ	No	WET	(Wood et al. 2006)
		Neuro-2A, MRC-5		Lakes, rivers, reservoirs, channels	No	Filtration (0.2 µm)	(Cetojevic-Simin et al. 2009)
		Neuro2A ATCC CCL-		Open ocean aquaculture cages.	No	Filtration (0.25 µm)	(Campora et al. 2010)

		131		Macroalgae/water			
		N2A		Marine, New Caledonia, freeze dried samples	Yes	Methanol, diethylether extraction	(Kerbrat et al. 2010)
	Receptor binding	Rat brain synaptosomes	Cyanobacterial blooms	Marine, New Caledonia, freeze dried samples	Yes	Methanol, diethylether extraction	(Kerbrat et al. 2010)
Immunotoxicity							
Cytotoxicity	LDH leakage	Whole blood	Surface water	River South Africa	No	SPE (C18)	(Pool and Magcwebeba 2009)
Immunity (cell-mediated	IFN-γ activity			River, South Africa	No	SPE (C18)	(Pool and Magcwebeba 2009)
Immunity (humoral)	IL-10 activity			River, South Africa	No	SPE (C18)	(Pool and Magcwebeba 2009)
Inflammatory activity	IL-6 activity			River, South Africa	No	SPE (C18)	(Pool and Magcwebeba 2009)
Cytotoxicity	Proliferation of IL1/IL2	Mouse splenocytes	Wastewater	Sec + coag, AC, Cl tests	Y	WET	(Kontana et al. 2008)
Lympho-proliferation	Lympho-proliferation bioassay + IL1/IL2 characterisation			Sec, tert (Cl, O_3,coag, AC)	Y	WET	(Kontana et al. 2009)
Reproductive toxicity							
Rat testicular cell quality, androgenicity	MTT assay (cytotoxicity), plasma membrane integrity, lactic dehydroge-nase (LDH) leakage, apoptotic/necrotic cells measurement, testosterone secretion	Sprague-Dawley rat sertoli, spermatogenic and leydig cells	Petrochemical plant effluent	Treatment unspecified	No	SPE (Supelco)	(Wang et al. 2010)
REACTIVE TOXICITY							
Genotoxicity	GreenScreen EM®	*S. cerevisiae* with γEGFP3	Wastewater	O_3 and electrochemical oxidation	Y	WET	(Keenan et al. 2007)
	PI/sub-G1	Human lympo/leuko-cytes and hep-G2	Drinking water	Prim, Sec, ClO_2, AC	Y	SPE (C18)	(Maffei et al. 2009)
	Bacillus subtilis DNA repair assay	*B. subtilis* – five strains	Petrochemical plant	Runoff	Y	SPE (XAD-2, 7)	(Brown and Donnelly 1984a)
		B. subtilis - six strains			Y	LLE (DCM)	(Brown and Donnelly 1984b)
(SOS-response)	SOS/Chromo	*E. coli* (PQ37)	Drinking water	Raw lake, NaClO, ClO_2, PAA	Y	SPE (C18)	(Guzzella et al. 2004)
				Raw lake + ClO_2 and GAC	Y	SPE (C18)	(Zani et al. 2005)
				Disinfection (ClO_2, O_3, GAC)	Y	SPE (C18)	(Guzzella et al. 2006)
	umu	*S. typhimurium* NM2009		From the entire length of Japan	Y	SPE (CSP-800)	(Takanashi et al. 2009)
	*umu*C	*S. typhimurium* TA1535/pSK1002		Tap and well, Slovenia	No	WET	(Zegura et al. 2009)
				Prim, sec, tert (Cl_2)	Y (TEQ)	SPE (OASIS®HLB)	(Macova et al. 2011)
	SOS/*umu*		Oil field produced	Land-based, China	Y	SPE (OASIS®HLB)	(Li et al. 2008a)
	Vitotox 10 kit	TA104 *rec*N2-4	Oil refinery	Finland	Y	WET + SPE (OASIS®HLB)	(Pessala et al. 2004)
			Paper/pulp mill	Unspecified treatment	Y	WET + SPE (OASIS®HLB)	(Pessala et al. 2004)
	umu			Prim, sec	Y	WET	(Gartiser et al. 2010)
			Surface water	Rhine river, Germany	Y	SPE (XAD-7)	(Reifferscheid et al. 1991)

	SOS/Chromo	*E. coli*		Rivers, lakes, St Lawrence, Canada	Y	LLE (DCM)	(Langevin et al. 1992)
	umu also for cytotoxicity	*S. typhimurium* TA1535/pSK1002		River, Germany	No	WET	(Dizer et al. 2002)
	Survival of SOS defective *E. coli*	Several *E. coli* K-12 strains		Polluted river, India	No	SPE (XAD-4, 8), LLE (n-hexane)	(Aleem and Malik 2005)
	*umu*C	*S. typhimurium* TA1535/pSK1002		River, Switzerland	Y	SPE (C18, EN)	(Escher et al. 2008b)
	SOS/*umu*			River, China	Y	SPE (OASIS®HLB)	(Li et al. 2008a)
	*umu*C			River and lake, Slovenia	No	WET	(Zegura et al. 2009)
				Rivers, lakes receiving recycled water, Australia	Y (TEQ)	SPE (OASIS®HLB)	(Macova et al. 2011
	umu		Wastewater	Mixed	Y	SPE (C18)	(Castillo et al. 2001)
	umu alsofor cytotoxicity			Prim, sec	Y (no EC)	WET	(Dizer et al. 2002)
	SOS/Chromo	*E. coli* PQ37		Prim, hospital	No	WET	(Jolibois et al. 2003)
				Various TPs, unspecified treatment	Y (TU)	WET	(Manusadzianas et al. 2003)
	Vitotox 10 kit	TA104 *rec*N2-4		Mixed, unspecified treatment	Y	WET + SPE (OASIS®HLB)	(Pessala et al. 2004)
	umuC	*S. typhimurium* TA1535/pSK1002		Prim, sec (anaerobic, aerobic biodegradation)	Y	WET (sonication, centrifugation)	(Gustavsson and Engwall 2006)
	umu			Sec, Cl (tests effect of [NH_4^+]	Y (TEQ)	SPE (CHP20P)	(Wang et al. 2007)
	umuC			Prim + after reed bed treatment	Y	Soxhlet, toluene	(Gustavsson et al. 2007)
	SOS/Chromo	*E. coli* PQ37		Prim, sec	Y	WET	(Isidori et al. 2007)
	umu – 2 hr auto	*S. typhimurium* TA1535/pSK1002		Prim	Y	WET	(Brinkmann and Eisentraeger 2008)
	*umu*C	*S. typhimurium* TA1535/pSK1002		Prim, sec	Y	SPE (C18, EN)	(Escher et al. 2008b)
	SOS/*umu*			Sec, tert (coag, O_3, Cl_2)	Y (TEQ)	SPE (OASIS®HLB)	(Cao et al. 2009a)
				Sec, tert (O_3, MF, UV, Cl, RO, coag)	No	SPE (OASIS®HLB)	(Cao et al. 2009b)
	*umu*C			Prim, sec, tert (O_3, AC)	Y (TEQ)	SPE (C18, EN)	(Escher et al. 2009)
	umu			Mixed effluents	Y	WET	(Gartiser et al. 2009)
				Prim + unspecified treatment	No	WET	(Zegura et al. 2009)
				Prim, sec, tert (AC, O_3, DAFF)	Y (TEQ)	SPE (OASIS®HLB)	(Macova et al. 2010)
	SOS/*umu*			Sec, Cl (+- Br)	Y (TEQ)	Frac (XAD-8) + SPE (CHP20P)	(Wu et al. 2010b)
				Tert: Cl, UF (test influence of NH4+)	No but TEQ	SPE (XAD-8)	(Wu et al. 2010a)
	Survival of SOS defective *E.coli*	*E. coli* K-12, several AB and KL strains		Prim	No	SPE (XAD-4/8 mix), LLE (hexane)	(Ansari and Malik 2009)
		E. coli K-12, several AB strains		Sec. tannery effluents	Yes but no EC	SPE (XAD-4, 8)	(Alam et al. 2010)
	umuC	*S. typhimurium* TA1535/pSK1002		Prim, sec, tert (MF, RO, O_3, BAC)	Y (TEQ)	SPE (OASIS®HLB)	(Macova et al. 2011)
(Genomic DNA damage)	Comet	Human leukocytes	Drinking water	Bottled, still and carbonated	No	Lyophilisation and solvent extraction (several)	(Biscardi et al. 2003)
	SCGE	Human leukocytes, Hep-G2		Advanced treatment	Y	SPE (C18)	(Buschini et al. 2004)
		Caco2, HepG2		Tap + charcoal filtration	No	SPE (XAD4)	(Lah et al. 2005)
				Chlorinated tap water	Y	SPE (C18)	(Marabini et al. 2007)

	Comet + SCGE	PR109		Tap water, India	No	WET	(Banerjee et al. 2008)
		Human leucocytes		Prim, ClO_2	Y	SPMD vs. SPE (C18)	(Buschini et al. 2008)
		Hep-G2		Prim, Sec, ClO_2, AC	Y	SPMD vs. SPE (C18)	(Maffei et al. 2009)
				River, China (Sec + Cl)	Y	SPE (XAD-2)	(Shi et al. 2009b)
	Comet			Tap and well, Slovenia	No	WET	(Zegura et al. 2009)
		Human leukocytes		Bottled, still and carbonated	No	SPE (C18)	(Ceretti et al. 2010)
	SCGE	L-02		River, China (sec, Cl)	Y	SPE (XAD-7)	(Xie et al. 2010)
	Comet	Rat hepatoma cells	Oil refinery effluent	Sec	Y	WET	(Rodrigues et al. 2010)
	Yeast SCGE	*S. cerevisiae* MTCC 36	Pulp mill effluent	Before and after treatment	No	WET	(Singhal and Thakur 2009)
	SCGE/micro-plate comet	CHO AS52	Recycled water	US indoor/outdoor pools, hot tubs	Y	LLE (methyl tert butyl ether)	(Liviac et al. 2010)
	Comet/SCGE	Zebrafish hepato-cytes	Surface water	Rivers, Germany	No	WET	(Schnurstein and Braunbeck 2001)
	Comet	*O. mykiss* hepatoma cell line RTH-149		River, Israel	No	WET	(Avishai et al. 2002)
		Human lymphocytes		Rivers, China	Y	SPE (XAD-2)	(Zhong et al. 2001)
	SCGE	Human leukocytes		Lake, Italy (raw + ClO_2 for drinking)	Y	SPE (C18)	(Maffei et al. 2005)
				Lake, Italy	Y	SPE (C18)	(Pellacani et al. 2006)
	Comet	RTL-W1		Rivers, Germany	Y	SPE (XAD-4,7)	(Keiter et al. 2006)
		CHO-K1		River, Argentina	No	WET	(Caffetti et al. 2008)
		Human lymphocytes		River, Korea	Y	LLE (DCM), SPE (XAD-2)	(Kwon et al. 2008)
		Human leukocytes		River and lake, Slovenia	Y (no EC)	WET	(Zegura et al. 2009)
		CHO-K1		Streams, lakes, Brazil	no	WET	(Rigonato et al. 2010)
		RTL-W1	Wastewater	Raw, bioreactors (several)	Y	LL, cleanup, fractionation	(Klee et al. 2004)
				Incl. pulp mill effl. Prim, sec	Y	SPE (XAD-4,7)	(Keiter et al. 2006)
		Hep-G2		Prim + unspecified treatment	Y (no EC)	WET	(Zegura et al. 2009)
	Micronucleus (MN) assay	Hep-G2	Drinking water	Advanced treatment	Y	SPE (C18)	(Buschini et al. 2004)
		Human lymphocytes, Hep-G2		Chlorinated tap water	Y	SPE (C18)	(Marabini et al. 2007)
	MN + cell proliferation	Human lymphocytes		Tap water	Y (No EC)	WET	(Asslouj et al. 2009)
			Groundwater	Well water	Y (No EC)	WET	(Asslouj et al. 2009)
	MN			Prim, Sec, ClO_2, AC	Y (No EC)	WET	(Maffei et al. 2009)
		Hep-G2		River, China (sec + Cl)	Y	SPE (XAD-2)	(Shi et al. 2009b)
		Human lymphocytes	Surface water	Lake, Italy, (raw +ClO_2 for drinking)	Y	SPE (XAD-2)	(Maffei et al. 2005)
	MN	V79 cells		Rivers receiving tannery effluents, Brazil.	No	WET	(Lemos et al. in press)
	Cytokinesis-block MN (CBMN)	Human lymphocytes		Rivers receiving tannery effluents, Brazil.	No	WET	(Lemos et al. in press)
	MN + cell proliferation	V79	Wastewater	Treatment unspecified	No	WET	(Reifferscheid et al. 2008)
				Treatment unspecified	Y (No EC)	WET	(Asslouj et al. 2009)
Alternative genotoxicity tests	Survival of bacteriophage γ	Bacteriophage γ	Surface water	Polluted river, India	No	SPE (XAD-4, 8), LLE (n-hexane)	(Aleem and Malik 2005)
	Chromosomal aberration	PBMC	Wastewater	Incl. petrochem. Prim.	Y (no EC)	LLE (DCM)	(Krishnamurthi et al. 2008)
	DNA fragmentation			Incl. petrochem. Prim.	Y (no EC)	LLE (DCM)	(Krishnamurthi et al. 2008)

	DNA unwinding			Incl. petrochem. Prim.	Y (no EC)	LLE (DCM)	(Krishnamurthi et al. 2008)
	P53 protein accumulation (Western blot)			Incl. petrochem. Prim.	Y (no EC)	LLE (DCM)	(Krishnamurthi et al. 2008)
	Yeast reporter assay			Sec	Y (no EC)	WET	(Schmitt et al. 2005)
Mutagenicity	Alternative yeast mutagenicity assay						
		S. cerevisiae (D7)	Drinking water	Raw lake, NaClO, ClO_2, PAA	Y	SPE (C18)	(Guzzella et al. 2004)
				Raw lake + ClO_2 and GAC	Y	SPE (C18)	(Zani et al. 2005)
			Surface water	Lake, Italy	Y	SPE (C18)	(Pellacani et al. 2006)
	Ames test + cytotoxicity	*S. typhimurium* TA98 and 100	Coal gasification process water	Condensate obtained from Pittsburgh Energy Research Centre	Y	Stedman extraction and fractionation (Swain et al. 1969)	(Epler et al. 1978)
	Ames test			Groundwater effluents Wyoming	No	LLE (DCM) + fractionation	(Timourian et al. 1982)
		TA98, 100	Coal process water	Hydrothermally treated coal	Yes	SPE (Sep-Pak Plus CSP-800)	(Nakajima et al. in press)
		TA98, 100, 1537		Coke plant effluent, raw and filtered	Y	LLE (DCM)	(Schaeffer and Kerster 1985)
		TA100	Drinking water	Tap water, Cincinnati	No	Dialysis, lyophilisation, solvent extraction	(Simmon and Tardiff 1976)
		TA98, 100		Raw and treated	Y	SPE (XAD2)	(Nestmann et al. 1979)
		TA98, 100, 1535, 1537, 1538		Raw and lab treated: Cl2, chloramine	Y	SPE (XAD4)	(Cheh et al. 1980)
		TA98, 100		Bottled, PET	No	WET and SPE (C18)	(de Fusco et al. 1990)
		TA98, 100		Raw lake, NaClO, ClO_2, PAA	Y	SPE (C18)	(Guzzella et al. 2004)
		TA97a, 100, TA1535		Tap + charcoal filtration	No	SPE (XAD4)	(Lah et al. 2005)
		TA98, 100		Raw lake + ClO_2 and GAC	Y	SPE (C18)	(Zani et al. 2005)
		TA100	Marine water	North Adriatic, 50, 500 m offshore	No	LLE (hexane)	(Kurelec et al. 1979)
		TA98, 100	Petrochem. plant	Runoff	Y	SPE (XAD-2,7)	(Brown and Donnelly 1984a)
		TA98, 100		-	Y	LLE (DCM)	(Brown and Donnelly 1984b)
		TA98, 100, 1535, 1537	Pulp mill effluent	Various stages of bleaching	No	LLE (unsure of solvent used)	(Bjorseth et al. 1979)
		TA98, 100, 1535		Sulphite plant	No	LLE (methyl-isobutyl-ketone)	(Carlberg et al. 1980)
		TA98, 100, 1535, 1537, 1538		Prim, sec effluent.	No	WET	(Nestmann et al. 1984)
		TA100, RSJ100	Recreational water	Swimming pools, Spain, Cl, Br	Y	SPE (XAD)	(Richardson et al. 2010)
	Ames test + cytotoxicity	TA98, 100	Recycled water	High ground water receiving secondary treated waste water. Effect of O_3.	Y	Low temp. (37-38°C) distillation	(Gruener 1978)
	Ames test	TA98,100, 1535, 1536, 1537, 1538	Surface water	Mississippi River	Y	WET	(Pelon et al. 1977)
		TA98, 100, 1535, 1538		Rivers Rhine and Meuse, The Netherlands	Y	SPE (XAD-4, 8)	(Vankreijl et al. 1980)
		TA98, 100		River, Spain, raw + coag/GAC	Y	SPE (GAC)	(Romero et al. 1991)
		TA98, 100		Receiving lake, Beijing. Advanced treatment: O_3, coag, sedimentation	Y	SPE (XAD2)	(Zhang and Wang 2000)
		Various TA strains		Polluted river, India	Y	SPE (XAD-4, 8), LLE (n-hexane)	(Aleem and Malik 2005)
		TA98,100		Treatment plants (ClO_2, O_3, GAC)	Y	SPE (C18)	(Guzzella et al. 2006)
		TA98		Rivers, Germany	Y	SPE (XAD-4, 7)	(Keiter et al. 2006)

	TA98, 100		Receiving river, Argentina	Y	WET	(Gana et al. 2008)
	TA98		River, Korea	Y	LLE (DCM), SPE (XAD-2)	(Kwon et al. 2008)
	TA98, 100		From the entire length of Japan	Y	SPE (CSP-800)	(Takanashi et al. 2009)
	TA98, 100, 1537, 1537	Wastewater	Mixed domestic/industrial sources and tertiary treatment including O_3, Cl_2	Y	SPE (XAD-2, 7)	(Rappaport et al. 1979)
	TA100, 1535		Municipal. Sec, tert incl. AC, Cl_2	No	WET	(Saxena and Schwartz 1979)
	TA98, 100, 1535, 1538, 98NR, 100NR		Industrial production of nitrobenzoic acids, nitrotoluenes.	Y	LLE (diethylether)	(Sundvall et al. 1984)
	TA98, 1535		Industrial, pharmaceutical and paper mill effluents (treatment unspecified) + surface water recipients	No	LLE (DCM)	(Sanchez et al. 1988)
	TA100		Dom, industr, landfill leachate, mould extract. Cl-treatment	Y (MFP)	SPE (CSP-800)	(Takanashi et al. 2001)
	TA98, 100		Sec	Y (no EC)	SPE (C18)	(Aguayo et al. 2004)
	TA98, 98R, 100		Raw, bioreactors (several)	Y	LL, cleanup, fractionation	(Klee et al. 2004)
	Various TA strains		Unsure of treatment if any	Y (no EC)	WET	(Fatima and Ahmad 2006)
	TA98, 100		Sec + coag (lab jar test)	Y	SPE (C18)	(Petala et al. 2006a)
	TA98, 100		Prim, sec	Y	WET	(Isidori et al. 2007)
	TA98		Incl. pulp mill effl. Prim, sec	Y	SPE (XAD-4, 7)	(Keiter et al. 2006)
	TA98, 100		Sec + O_3	Y	SPE (C18)	(Petala et al. 2008)
	TA97a, 100, 102, 104		Prim	Y (induction factor, mutagenic potential)	SPE (XAD-4, 8 mix), LLE (hexane)	(Ansari and Malik 2009)
	TA98, 100		Mixed	Y	WET	(Gartiser et al. 2009)
	TA98, 100, 102		Hospital. Prim, sec, Cl	Y	WET	(Gupta et al. 2009)
	Various TA strains		Sec. tannery effluents	Yes (no EC)	SPE (XAD-4, 8)	(Alam et al. 2010)
	TA100, 104		Sec (varying pH in act. sludge)	Y (no EC)	SPME + fractionation	(Caffaro-Filho et al. 2010)
Ames fluctuation test	TA98	Surface water	Rivers Rhine and Meuse, The Netherlands	Y	SPE (XAD-4, 8)	(Vankreijl et al. 1980)
	TA98, 100	Wastewater	Prim, hospital	No	WET	(Jolibois et al. 2003)
	Various TA strains		Unsure of treatment if any	Y (no EC)	WET	(Fatima and Ahmad 2006)
Ames/micro-suspension	TA98, 100, YG1041, 1042	Groundwater	Sao Paulo state, Brazil	Y	MF, LLE, SPE (XAD-4)	(Valente-Campos et al. 2009)
	TA100	Surface water	River, Japan, effect of Cl	Y (mutagen formation potential, MFP)	SPE (CSP-800)	(Takanashi et al. 2001)
	TA97a, 98, 100, 1535		Polluted river, Brazil	No	SPE (XAD-4)	(Lemos et al. 2009)
	TA98, 100		River, Brazil. Prim, flocc/filtration/Cl for public supply.	Y (mutagenic index)	SPE (XAD4) + fractionation	(da Silva Pereira et al. 2007)
	YG1024				SPE (Supelpak2)	(Ohe et al. 2009)
Ames test (+ cytotoxicity)	*S. typhimurium* TA98, 100,1535, 1537	Wastewater	Prim, sec	Y	LLE (DCM)	(Meier and Bishop 1985)
Other reverse mutation assays	*E. coli* WP_2	Wastewater	Industrial, pharmaceutical and paper mill effluents (treatment unspecified) + surface water recipients	No	LLE (DCM)	(Sanchez et al. 1988)
	S. cerevisiae S211,		Industrial, pharmaceutical and paper mill	No	LLE (DCM)	(Sanchez et al. 1988)

		S318		effluents (treatment unspecified) + surface water recipients			
	Mutatox assay	*V. fischeri*	Drinking water	Raw lake, NaClO, ClO_2, PAA	Y	SPE (C18)	(Guzzella et al. 2004)
				Raw lake + ClO_2 and GAC	Y	SPE (C18)	(Zani et al. 2005)
	V. harveyi mutagenicity assay	Genetically modified *V. harveyi*	Marine water	Europe, USA	No	WET	(Czyz et al. 2003)
	V. harveyi luminescent mutagenicity assay	*V. harveyi A16*		Baltic sea	No	WET	(Podgorska et al. 2007)
Sister chromatid exchange (SCE) induction	Giemsa	Human lymphocytes	Drinking water	Bottled, PET	No	WET	(Ergene et al. 2008)
		Chinese hamster lung (CHL) cells	Surface water	Waka River, Japan. Near industrial + domestic sewage effluent	Y (mitotic index)	SPE (Supelpak2)	(Ohe et al. 2009)
Oxidative stress (OS) and reactive oxygen species (ROS)							
OS	GSH	Hep-G2 cells	Drinking water	Raw lake, NaClO, ClO_2, PAA	No	SPE (C18)	(Marabini et al. 2006)
	GSH-Px, SOD, MDA			River, China (sec + Cl)	Y	SPE (XAD-2)	(Shi et al. 2009b)
	GSH, MDA	L-02 cells			-	SPE (XAD-7)	(Xie et al. 2010)
	GSH	Rainbow trout primary hepatocytes	Oil field produced	Offshore effluent, North Sea	Y	SPE (ENV+, C18)	(Farmen et al. 2010)
ROS	DCFH-DA			Offshore effluent, North Sea	Y	SPE (ENV+, C18)	(Farmen et al. 2010)
		Hep-G2 cells	Surface water	Raw lake. NaClO, ClO_2, PAA	No	SPE (C18)	(Marabini et al. 2006)
				River, China (sec + Cl)	Y	SPE (XAD-2)	(Shi et al. 2009b)
	Change in free radical scavengers (SOD, CAT, mannitol, ascorbate) and production of free radicals (O_2^- and H_2O_2)	*S. typhimurium* and *Allium cepa*	Wastewater	Treatment unspecified	Y	WET	(Fatima and Ahmad 2006)
LOW COMPLEXITY *IN VIVO* ASSAYS							
Phytotoxicity							
Algae							
Algal growth inhibition (AGI)	AGI	*Scenedesmus subspicatus*	Landfill leachates	Mixed	Y (no response)	WET	(Clement et al. 1996)
		P. subcapitata	Oil refinery	Finland	Y	WET + SPE (OASIS®HLB)	(Pessala et al. 2004)
			Paper/pulp mill	Unspecified treatment	Y	WET + SPE (OASIS®HLB)	(Pessala et al. 2004)
		S. subspicatus		Prim, sec	Y	WET	(Gartiser et al. 2010)
	AGI/microscope	*P. subcapitata*		Prim, sec	Y	WET	(Rosa et al. 2010)
	AGI/OD		Surface water	Rivers, North Estonia	No	WET	(Blinova 2000)
				Receiving river, Austria	No	WET	(Latif and Licek 2004)
	AGI/OD (observe stimulation)	*Scenedesmus quadricauda*		Rivers (various types), Argentina	No but TU	WET	(Di Marzio et al. 2005)

	AGI/OD	*P. subcapitata*		Sava river, Southeast Europe	Y (TU)	SPE (ENV+ and C18)	(Källqvist et al. 2008)
				Rivers, Poland	No	WET	(Mankiewicz-Boczek et al. 2008)
			Wastewater	Prim + unspecified treatment, also deep well water	Y (TU)	WET	(Rojícková-Padrtová et al. 1998)
		Selenastrum capricornutum		Mixed	Y (TU)	WET	(Tarkpea et al. 1998)
		P. subcapitata		Prim + unspecified treatment	Y	WET	(Blinova 2000)
				Various TPs (treatment unspecified)	Y (TU)	WET	(Manusadzianas et al. 2003)
		C. vulgaris		Sec	Y (no EC)	SPE (C18)	(Aguayo et al. 2004)
				Effluent (treatment unspecified)	No	WET	(Latif and Licek 2004)
		P. subcapitata		Mixed, unspecified treatment	Y	WET + SPE (OASIS®HLB)	(Pessala et al. 2004)
	AGI/microscope			Hospital effluents	Y (TU)	WET	(Emmanuel et al. 2005)
	AGI/OD	*S. capricornutum*		Sec	Y (TU)	WET	(Ra et al. 2007)
				Various TPs (treatment unspecified)	Y	WET	(Paixão et al. 2008)
				Sec + PAA	No	WET	(Antonelli et al. 2009)
	AGI/microscope	*S. subspicatus*		Mixed effluents	Y	WET	(Gartiser et al. 2009)
	AGI/OD	*P. subcapitata*		Prim, sec	Y	WET	(Mendonca et al. 2009)
		S. subspicatus		From pectin production. Prim, sec	Y	WET and SPE (XAD-8) + fractionation	(Reginatto et al. 2009)
		P. subcapitata		Sec + tert (biofilm, O_3)	Y	WET	(Lundstrom et al. 2010)
PSII derived photosynthe-sis inhibition	ToxY-PAM	*Phaeodactylum tricornutum*	Surface water	Rivers, Queensland, Australia	Y (TEQ)	SPE (OASIS®HLB)	(Bengtson Nash et al. 2005a)
				Brisbane and Thames Estuaries	Y (TEQ)	SPE (OASIS®HLB)	(Bengtson Nash et al. 2006)
	IPAM	*P. tricornutum* and *C. vulgaris*		Brisbane River, Australia	Y (TEQ)	SPE (OASIS®HLB)	(Muller et al. 2008b)
		C. vulgaris	Wastewater	Prim, sec, tert (AC, O_3, DAFF)	Y (TEQ)	SPE (OASIS®HLB)	(Macova et al. 2010)
AGI + PSII	Combined algae test	*P. subcapitata*	Drinking water	Prim, sec, tert (Cl_2)	Y (TEQ)	SPE (OASIS®HLB)	(Macova et al. 2011))
			Surface water	River, Switzerland	Y (TEQ)	SPE (C18, EN)	(Escher et al. 2008b)
				Rivers, lakes receiving recycled water, Australia	Y (TEQ)	SPE (OASIS®HLB)	(Macova et al. 2011))
			Wastewater	Prim, sec	Y (TEQ)	SPE (C18, EN)	(Escher et al. 2008b)
				Prim, sec, tert (O_3, AC)	Y (TEQ)	SPE (C18, EN)	(Escher et al. 2009)
				Prim, sec, tert (RO, UV, H_2O_2)	Y (TEQ)	SPE (OASIS®HLB)	(Escher et al. 2011)
				Prim, sec, tert (MF, RO, O_3, BAC)	Y (TEQ)	SPE (OASIS®HLB)	(Macova et al. 2011))
Plants							
Cytotoxicity, growth inhibition	Root growth	*Allium cepa* (onion)	Drinking water	Bottled, glass, PET, sunlight exposure	No	WET	(Evandri et al. 2000)
	Growth inhibition	*Lemna minor*	Landfill leachates	Mixed	Y (no response)	WET	(Clement et al. 1996)
		A. cepa		Municipal	Y	WET	(Bortolotto et al. 2009)
	Root growth		Oil refinery effluent	Finland	Y	WET	(Pessala et al. 2004)
	Mitotic index			Sec	Y	WET	(Rodrigues et al. 2010)
	Cell growth		Paper/pulp mill	India	Y	WET	(Shanthamurthy and

							Rangaswamy 1979)
	Root growth			Unspecified treatment	Y	WET	(Pessala et al. 2004)
	Mitotic index			Raw, sec	No	WET	(Chaparro et al. 2010)
	Growth inhibition	*L. minor*		Prim, sec	Y	WET	(Gartiser et al. 2010)
		L. minor L		Prim, sec	Y	WET	(Rosa et al. 2010)
		Ornithogalum virens, *A. cepa*	Surface water	River, India, receiving effluents from Rayon factory	No	WET	(Ravindran and Ravindran 1978)
		L. minor		Rivers, North Estonia	No	WET	(Blinova 2000)
				Rivers, Poland	No	WET	(Mankiewicz-Boczek et al. 2008)
	Growth inhibition and chl. A content	*Lemna minor L*		River Sava, Croatia. Polluted. Prim/sec	Y (No EC)	WET	(Radić et al. 2010a)
	Growth inhibition	*A. cepa*		River, various sites/types, Croatia	No	WET	(Radić et al. 2010b)
	Cell death	*A. cepa*		River incl. sites receiving raw sewage, Brazil	No	WET	(Bianchi et al. in press)
	Seed viability	*Oryza sativa* (rice)	Wastewater	Molasses effluent	Y	WET	(Behera and Misra 1982)
	Growth inhibition	*L. minor*		Prim + unspecified treatment	Y	WET	(Blinova 2000)
	Root growth	*A. cepa*		Mixed, unspecified treatment	Y	WET	(Pessala et al. 2004)
	Mitotic index	*A. cepa* seeds		Textile effluent	Y	WET	(Carilta and Marin-Morales 2008)
				Various TPs (treatment unspecified)	Y	WET	(Paixão et al. 2008)
		Garden cress, A. cepa and various local plant species incl. three macrophytes		Winery waste water, Australia	Y	WET	(Arienzo et al. 2009)
		L. minor		Prim, sec	Y	WET	(Mendonca et al. 2009)
				Municipal, poultry, brewery. Prim, sec	Y	WET	(Ostra et al. 2009)
	Growth inhibition	Multispecies (4)		Fertiliser production (raw + precipitation with hydrated lime)	No	WET	(Gouider et al. 2010)
	Root growth	*A. cepa*		Textile effl. (raw + bacterial treatment)	Y	WET	(Jadhav et al. 2010)
	Growth inhibition	*L. minor*		Sec + O_3, AC	No	WET	(Stalter et al. 2010)
Seed germination and root growth	Germination, growth and more	*O. sativa*	Paper mill effluent	Unspecified treatment	Y	WET	(Misra and Behera 1991)
	Phytotoxkit	*Lepidium sativum*	Surface water	Receiving river, Austria	No	WET	(Latif and Licek 2004)
	Root growth and mitotic index	*A. cepa*		Receiving streams, Brazil	No	WET	(Mitteregger et al. 2007)
	Growth inhibition and mitotic index	*A. cepa*		River, Brazil	Y	WET	(Barberio et al. 2009)
	Germination, growth	*O. sativa*	Wastewater	Molasses effluent	Y	WET	(Behera and Misra 1982)
	Growth and germination index	*L. sativum*		Olive mill effluent (prim, sec)	Y	WET	(Filidei et al. 2003)
	Phytotoxkit	*A. cepa*		Effluent (treatment unspecified)	No	WET	(Latif and Licek 2004)
	Germination index	*Lycopersicon esculentum* (tomato) and *Chicorium intybus* seeds		Olive mill (raw + prim)	No	WET	(Komilis et al. 2005)
	Germination index	*L. esculentum* seeds		Olive mill (raw + fungal treatment)	No	WET	(Dhouib et al. 2006)
				Olive mill (raw + tert (electrochemical))	No	WET	(Khoufi et al. 2006)

		L. sativum		Raw, tert (UF, MF)	No	WET	(Saddoud et al. 2007)
	Phytotoxkit	*Alba sinapis, Shorgum saccharatum, L. sativum*		Sec + coag, AC, Cl tests	No	WET	(Kontana et al. 2008)
	Root growth	*A. cepa*		Raw pharmaceutical effluent Nigeria	Y	WET	(Bakare et al. 2009)
	Seed germination	*L. sativum*		Prim, sec	No	WET	(Ellouze et al. 2009)
	Phytotoxkit	*Sinapis alba, S. saccharatum, L. sativum*		Sec, tert (Cl, O_3,coag, AC)	No	WET	(Kontana et al. 2009)
	Growth inhibition	*S. alba*		Municipal, poultry, brewery. Prim, sec	Y	WET	(Ostra et al. 2009)
	Germination index	*L. sativum*		Prim, tert (MBR)	No	WET	(Saddoud et al. 2009)
		Multispecies (6)		Fertiliser production (raw + precipitation with hydrated lime)	Yes	WET	(Gouider et al. 2010)
	Seed + root	*T. aestivum, P. mungo*			Y	WET	(Jadhav et al. 2010)
	Growth inhibition	*S. alba*		Dom + pharmaceutical, prim/sec	No	WET	(Radić et al. 2010b)
	Germination index	*L. sativum*		Raw, tert (anaerobic MBR)	No	WET	(Saddoud et al. 2010)
	Phytotoxkit	*S. saccharatum, L. sativum, S. alba*		Sec, tert (UV, Cl)	No	WET	(Bakopoulou et al. 2011)
Genotoxicity	Mitotic index, chromosome aberrations	*A. cepa*	Drinking water	Bottled, glass, PET, sunlight exposure	No	WET	(Evandri et al. 2000)
	MN	*Tradescantia* (clone #4430)		Bottled, still and carbonated	No	Lyophilised concentrates	(Biscardi et al. 2003)
	MN, mitotic index	*A. cepa* and *Tradescantia* (clone #4430)		Bottled, still and carbonated	No	WET	(Ceretti et al. 2010)
	Comet	*A. cepa*	Landfill leachates	Municipal	Y	WET	(Bortolotto et al. 2009)
	MN		Oil refinery effluent	Sec	Y	WET	(Rodrigues et al. 2010)
	Chromosome aberrations, MN		Pulp mill effluent	Raw, sec	No	WET	(Chaparro et al. 2010)
	MN	*Vicia faba (*bean)	Surface water	Rivers China	No	SPE (XAD-2)	(Zhong et al. 2001)
	Mitotic index, MN	*A. cepa*		Receiving streams, Brazil	No	WET	(Mitteregger et al. 2007)
				Receiving river, Argentina	Y	WET	(Gana et al. 2008)
	Chromosome ab.	*A. cepa* seeds		Oil contaminated river, Brazil	No	WET	(Leme et al. 2008)
		A. cepa		River, Brazil	Y	WET	(Barberio et al. 2009)
	Mitotic index, MN and more			Creek, Brazil	No	WET	(Santos et al. 2009)
	Comet	*Lemna minor L*		Receiving river, Croatia. Prim/sec	No	WET	(Radić et al. 2010a)
	Allium root assay	*A. cepa*		River, various sites/types, Croatia	No	WET	(Radić et al. 2010b)
	Chromosome ab.			River incl. sites receiving raw sewage, Brazil	No	WET	(Bianchi et al. in press)
	Allium root assay		Wastewater	Unspecified treatment	Not specified	WET	(Fatima and Ahmad 2006)
	Chromosome ab., MN	*A. cepa* seeds		Textile effluent	Y	WET	(Carilta and Marin-Morales 2008)
		A. cepa		Raw pharmaceutical effluent Nigeria	Y	WET	(Bakare et al. 2009)
	Chromosome abb. + Comet assay			Textile effl. (raw + bacterial treatment)	Y	WET	(Jadhav et al. 2010)
	Allium root assay			Dom + pharmaceutical, prim/sec	No	WET	(Radić et al. 2010b)
OS	MDA	*Lemna minor L*	Receiving waters	River Sava, Croatia. Prim/sec	Y (no EC)	WET	(Radić et al. 2010a)
	POD						(Radić et al. 2010a)

Protozoan toxicity							
Cytotoxicity	Growth inhibition, Protoxkit F^{TM}	*T. thermophila*	Surface water	Rivers, North Estonia	No	WET	(Blinova 2000)
				Receiving river, Austria	No	WET	(Latif and Licek 2004)
				Rivers, Poland	No	WET	(Mankiewicz-Boczek et al. 2008)
			Wastewater	Prim + unspecified treatment	Y	WET	(Blinova 2000)
				Various TPs (treatment unspecified)	Y (TU)	WET	(Manusadzianas et al. 2003)
				Effluent (treatment unspecified)	No	WET	(Latif and Licek 2004)
				Sec + coag, AC, Cl tests	No	WET	(Kontana et al. 2008)
				Sec, tert (Cl, O_3,coag, AC)	No	WET	(Kontana et al. 2009)
	Mortality, Microbiotest	*Spirostomum ambiguum*	Landfill leachates	Mixed	Y	WET	(Clement et al. 1996)
	Mortality, deformities		Surface water	Rivers, Poland	No	WET	(Mankiewicz-Boczek et al. 2008)
	Mortality		Wastewater	Prim + unspecified treatment, also deep well water	Y (TU)	WET	(Rojícková-Padrtová et al. 1998)
Genotoxicity	Comet/SCGE	*T. thermophila*	Drinking water	Tap + charcoal filtration	No	SPE (XAD-4)	(Lah et al. 2005)

[1]Conc/resp = concentration/response, Y indicates yes, TEQ = toxic equivalent concentration, TU = toxic unit. 3H-12 (Ishikawa cell) = human endometrial cancer cell line, AB = alamar blue, AC = activated carbon, BAC = biologically activated carbon, CFDA-AM = 5-carboxyfluorescein diacetate acetoxymethyl ester, CHO = Chinese hamster ovarian cells, CISH = chemoluminescent in situ hybridisation assay, coag = coagulation, CV-1 = African green monkey kidney cell line, DAFF = dissolved air filtration flotation, DCFH-DA = 2',7'-dichloro-fluorescein diacetate, FDA/EtBr = fluoresceine diacetate/ethidium bromide assay(Merk and Speit 1999 and unpublished work), GABA = gamma-aminobutyric acid, GAD = glutamic acid decarboxylase, GSH = glutathione, GSH-Px = glutathione peroxidase, H4IIE = rat hepatoma cell line, HahLP = transfected HeLa cells, HEK 293 = human embryonic kidney cell line, HeLa = human cervical cancer cell line, Hep-G2 = human hepatocytes, HG_5LNGal-4-PXR = transfected HeLa cells, HG5LN-hPXR = transfected HeLa cells, IFN-γ = interferon-gamma, IL = interleukin, L-02 = human liver cell line, LDH = lactate dehydrogenase, LLE = liquid liquid extraction, mACh = muscarinic acetylcholine, MAO = monoamine oxidase, MBR = membrane bioreactor, MARA = microbial assay for risk assessment, MCF-7 = human breast carcinoma cell line, MDA = malondialdehyde, MDA-kb2 = transfected MDA-MB-453 (human breast cancer cell line), MELN = stably transfected (for ER-α) MCF-7, MN = micronucleus assay, MTC = microbial toxic concentration, MVLN – transfected MCF-7 cells, NF = nano filtration, NMDA = N-methyl-D-aspartic acid, NR = neutral red, OD = optical density, PAA = peracetic acid, PALM = PC-3 human prostate cancer cell line, PBMC = (human) peripheral mononuclear blood cells, PC-DR-LUC = rat cell line with avian coding, PET = polyethylene terephthalate, PI = propidium iodide, PHC-1 = fish hepatic cell line, PM = particulate matter, POD = peroxidase, PR109 = fission yeast strain (*S. Pombe*), prim = primary treatment (physical treatment, coagulation/precipitation processes), RO = reverse osmosis, RTG-2 = rainbow trout gonad cell line, RTL-W1 = rainbow trout liver cell line, sec = secondary treatment (biological treatment), SOD = superoxidase dismutase, SPE = solid phase extraction, SPME = solid phase micro extraction, T47D = human breast adenocarcinoma cell line, tert = tertiary treatment (advanced oxidation and oxidation processes), TP = treatment plant, TU = toxic unit, V79 = Chinese hamster lung cells.

GLOSSARY

Adverse outcome pathways (AOP) – conceptual framework that leads from the initiating event of interaction between a toxicant and a receptor in an organism over cellular and organ response to an adverse outcome at organism - or population level (Ankley *et al.*, 2010). Related to the term toxicity pathway but goes beyond cellular (individual) level effects in targeting effects at the environmental (population) level.

AhR – arylhydrocarbon receptor (dioxin receptor)

AR – androgen receptor

Baseline or non-specific toxicity – minimal toxicity that any compound exhibits by partitioning into biological cell membranes causing non-specific disturbance of the integrity and functioning of cell membranes.

Biologically effective dose - the biologically effective dose (BED), or the amount that actually reaches cells, sites, or membranes where adverse effects occur may represent only a fraction of the delivered dose, but it is obviously the best one for predicting adverse effects (Paustenbach 2000).

Cytochrome P450 (CYP450) – an enzyme superfamily involved in the metabolism of endogenous and xenobiotic compounds.

EDC – endocrine disrupting chemical; a chemical capable of modifying natural hormone function.

ELISA – enzyme-linked immunosorbent assay. The ELISA quantifies the amount of e.g. a hormone through its binding to an antibody linked to an enzyme. The amount of bound enzyme is then measured via a colorimetric response.

ER – estrogen receptor

In vitro – refers to tests performed outside the organism, i.e. using immortal cell lines or tissue/enzymes isolated from a living organism.

In vivo – refers to tests performed with whole organisms and populations.

GR – glucorticoid receptor.

Mechanism of toxic action – crucial biochemical processes and/or xenobiotic-biological interactions underlying a given mode of action (Rand 1995).

Mode of toxic action or mode of action (MOA) – a common set of physiological and behavioural signs that characterize a type of adverse biological response (Rand, 1995).

Narcosis mode of action – physiological and behavioural responses elicited by baseline toxicants, sub-categories include non-polar and polar narcosis and ester narcosis. Narcosis in this context refers to minimum toxicity that any compound exhibits and is not related to narcosis/anaesthesia in clinical medicine.

Non-specific mode of action – physiological and behavioural responses elicited by baseline toxicants, often used synonymous to “narcosis mode of action”.

Nuclear receptor – a protein receptor that senses hormones and is capable of binding directly to DNA, thereby regulating gene expression (also see receptor).

Passive sampling – time-integrated sampling of water through deployment of sampling devices (passive samplers) containing sorbent material with affinity for groups of chemicals with similar physicochemical properties (e.g. polar, non-polar chemicals).

PR – progesterone receptor.

Primary mechanism, primary effects – the type and degree of interaction of a toxicant with biomolecules at the target site triggers the toxic determines the primary mechanism of toxic action.

PXR – pregnane receptor.

RAR – retinoic acid receptor.

Reactive toxicity – mode of toxic action that is associated with chemical reactions where covalent bonds are formed. Can be either direct reactivity of electrophilic chemicals with biological nucleophiles, like DNA bases or proteins, or indirect reactivity via reactive oxygen species that are formed indirectly from chemical pollutants.

Receptor – a protein to which certain ligands (e.g. hormones and hormone-mimicking chemicals) can bind. Each receptor is specific to binding of ligands with particular structure(s) (also see nuclear receptor).

Recombinant cell – recombinant cell lines are created by insertion of a reporter plasmid, which carries a responsive element for a particular receptor (e.g. ER) followed by a reporter gene encoding a measurable marker (e.g. green fluorescent protein).

Reporter gene – a gene with a particular characteristic that can be utilised through insertion into a gene that would not otherwise express this feature. An example of a reporter gene is the green fluorescent protein, which is introduced to cells in order to encode a measurable marker (also see recombinant cell).

Reporter plasmid – a transferable form of DNA that is separate and independent of chromosomal DNA.

Responsive element – a short sequence of DNA capable of binding to certain receptors (e.g. ER), thereby regulating transcription.

RXR – retinoid X receptor.

Specific mode of toxic action – a mode of toxic action that causes higher toxicity than baseline toxicity, either caused by specific interaction with receptors or enzymes or by reactive toxicity.

Stable transfection – stable transfer of genetic material into a cell as opposed to transient transfection, which is unstable after reproduction.

Toxic equivalent concentration (TEQ) – concentration of a reference chemical that would elicit the same effect as the unresolved mixture of micropollutants in a water sample.

Toxicity pathway– "the cellular response pathways after chemical exposure expected to ultimately result in adverse health effects" (Collins et al., 2008).

TR – thyroid receptor.

Yeast two-hybrid assay – applies a recombinant yeast, which has been transfected with two different reporter plasmids (also see reporter plasmid and recombinant cell).

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