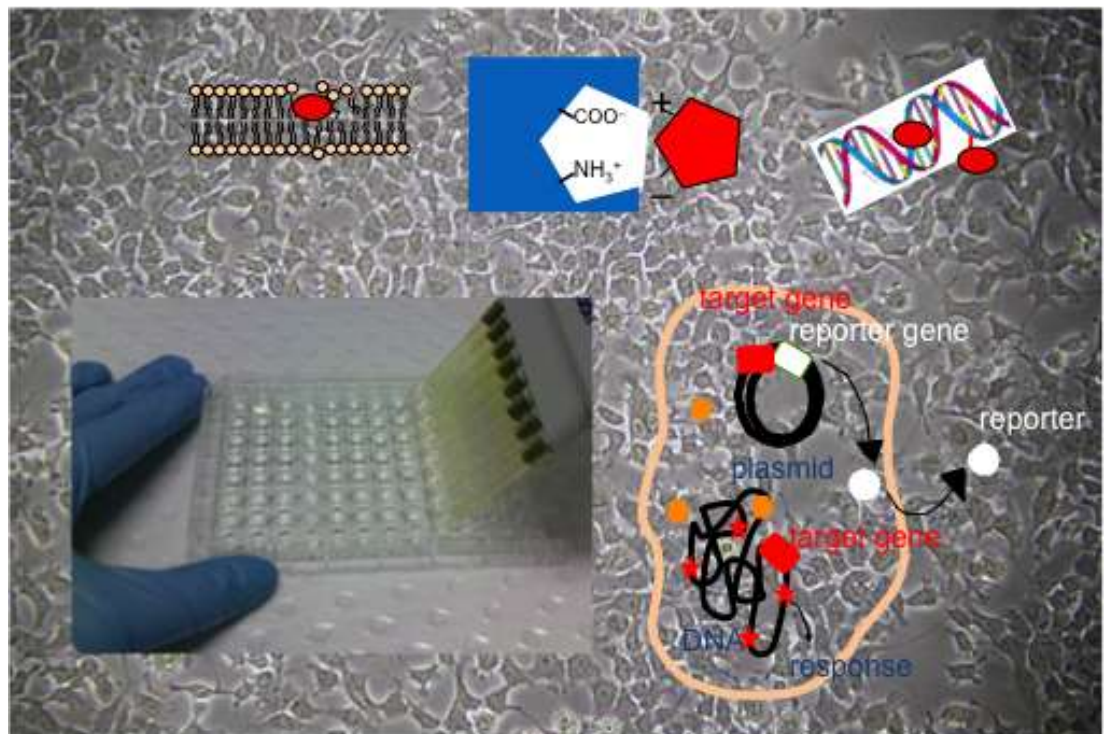


# Application of Bioanalytical Tools for Water Quality Assessment

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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.

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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.  $\beta$ -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 17 $\beta$ -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- $\alpha$ , which inhibits subsequent ER activation in MELN cells by free estrogens as these can also bind to the recombinant ER- $\alpha$ . 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- $\alpha$  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., ER $\alpha$ , ER $\beta$ ) 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), glucocorticoid (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).

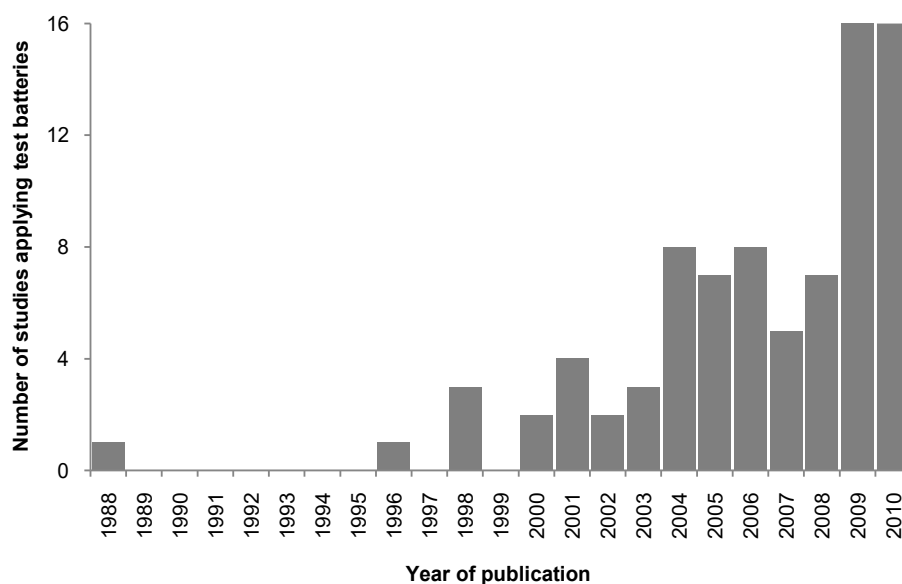
While most research in the field has focused on surface water (those of rivers and lakes) and wastewater, chemical disinfection of drinking water (using e.g. chlorine, chlorine dioxide and ozone) can form potentially harmful disinfection by-products (DBPs) from natural and synthetic organic matter. As 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 (Liviak *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

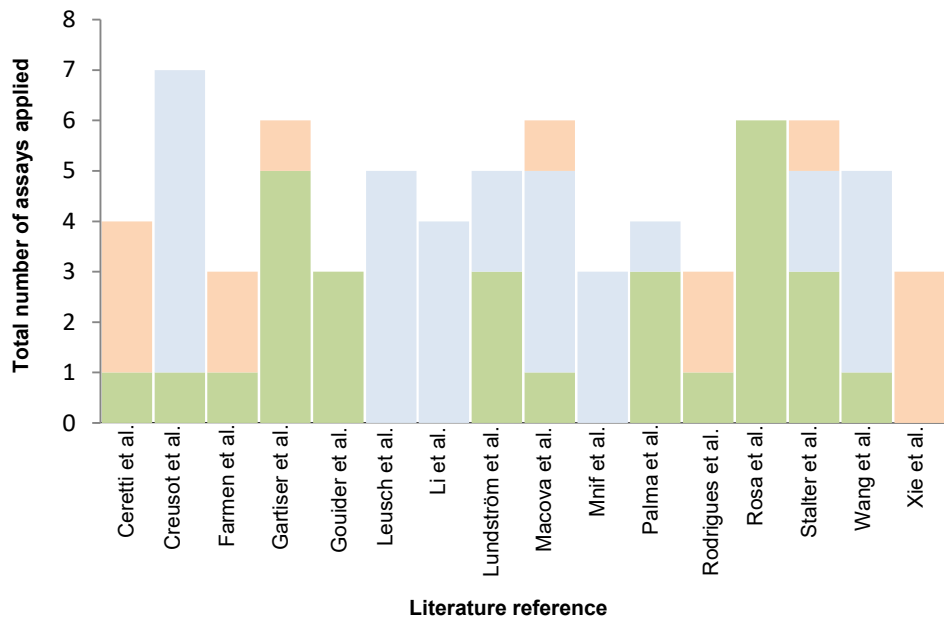
Researchers have applied single and multiple assays for water quality assessment for decades but battery applications have dramatically increased over the last decade and testing was expanded from contaminated sites and effluents to surface waters and highly treated waters, including drinking water.

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 ( $\geq 3$  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	other	Imm	Neuro	PAH/Dioxin	PSII	Rep	Genotox	OS		ROS
Mixed	xxxxx									xxx			(Sanchez et al. 1988)
LF	xxxxxxx												(Clement et al. 1996)
WW	x		xx										(Gagné and Blaise 1998)
Mixed	xxxxxxx												(Rojicková-Padrťová 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									xx			(Dizer et al. 2002)
R, WW			xxx										(Murk et al. 2002)
LF	xxxx												(Isidori et al. 2003)
WW (mix)	xxxxxxx									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	xxxxxx		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			(Escher et al. 2008b)
WW									xxxx			(Krishnamurthi et al. 2008)
AWWT	xxx					x						(Kontana et al. 2008)
OPFW	x						X		x			(Li et al. 2008a)
R	xxxxxxx	x										(Mankiewicz-Boczek et al. 2008)
DW, R, WW			x	xxx								(Van der Linden et al. 2008)
AWWT	xxx											(Antonelli et al. 2009)
PME							xxxxxxxx					(Basu et al. 2009)
AWWT	x	xx							x			(Cao et al. 2009b)
AWWT	xx		x			x			x			(Escher et al. 2009)
WW (mix)	xxx								xx			(Gartiser et al. 2009)
R		xx	x	xx								(Inoue et al. 2009b)
AWWT	xxxx					xx						(Kontana et al. 2009)
DWT	xxx								xxx			(Maffei et al. 2009)
WW			x	x		x						(Mahjoub et al. 2009)
WW	xxxx		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)
OPFW	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, OPFW = 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: progesteric	PR-mediated reporter gene	PR-CALUX
	ED: thyroidal	TR $\beta$ -mediated reporter gene	TR $\beta$ -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 = progesteric receptor, T47D-kBluc = stably transfected human breast cancer cell line, THP1 = human acute monocytic leukemia cell line, TR $\beta$  = thyroid receptor  $\beta$ .

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 *umuC* 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 $\beta$ -Estradiol	EEQ
	Binding to the arylhydrocarbon receptor	AhR CAFLUX	Dioxin-like compounds, PAHs	2,3,7,8-Tetrachloro-dibenzodioxin	TCDDAQ
Reactive	Genotoxicity	<i>umuC</i> (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  $\beta$ -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  $\mu$ g/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  $\beta$ -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  $\mu$ g/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	
<b>NON-SPECIFIC TOXICITY (baseline toxicity)</b>								
<b>Cytotoxicity</b>	Bioluminescence inhibition (Microtox®)	<i>Vibrio fischeri</i>	Drinking water	Prim, sec, tert (Cl <sub>2</sub> )	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)
			<i>Phosphobacterium phosphoreum</i>	Wastewater	Sec	v	SPE (C18, EN)	(Escher et al. 2009)
					Prim, sec, tert (O <sub>3</sub> , AC)	v	SPE (OASIS®HLB)	(Macova et al. 2010)
					Prim, sec, tert (O <sub>3</sub> , AC, DAFF)	v	SPE (OASIS®HLB)	(Escher et al. 2011)
					Prim, sec, tert (RO, UV, H <sub>2</sub> O <sub>2</sub> )	v	SPE (OASIS®HLB)	(Macova et al. 2011)
	Prim, sec, tert (MF, RO, O <sub>3</sub> , BAC)	v			SPE (OASIS®HLB)	(Reungoat, 2011)		
	Prim, sec, tert (sand filtration, BAC)	v			SPE (OASIS®HLB)	(Allinson et al. 2007)		
	<i>Saccharomyces cerevisiae</i>	Wastewater	Sec	v	SPE (C18)	(Mispagel et al. 2009)		
			Sec	v	SPE (C18)	(Allinson et al. 2010d)		
Yeast growth inhibition	Wastewater	Unspecified treatment	v	SPE (Lichrolut C18, EN)	(Rutishauser et al. 2004)			
<b>SPECIFIC TOXICITY</b>								
<b>Endocrine disruption</b>								
<b>Androgen receptor (AR)</b>	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			<i>S.cerevisiae</i> with human AR	Sec	v	SPE (C18)	(Allinson et al. 2007; Allinson et al. 2008)
	<b>AR indirect</b>	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	✓	SPE (XAD-7)	(Bandelj et al. 2006)		
			Wastewater	Sec	✓	SPE (XAD-7)	(Bandelj et al. 2006)		
	ER-CALUX	T47D	Ground water	Queensland, Australia	✓	SPE (OASIS®HLB)	(Leusch et al. 2010)		
				Surface water	Receiving streams, Australia	✓	SPE Supelco ENVI-18	(Williams et al. 2007)	
			Wastewater	River, Queensland, Australia	✓	SPE (OASIS®HLB)	(Leusch et al. 2010)		
				Sec, UV, Cl	✓	SPE (Supelco ENVI-18)	(Williams et al. 2007)		
			Raw + unspecified treatment	✓	SPE (OASIS®HLB)	(Leusch et al. 2010)			
				Other mammalian reporter gene assays	MELN cells	Ground water	Queensland, Australia	✓	SPE (OASIS®HLB)
			Surface water	River, Queensland, Australia	✓	SPE (OASIS®HLB)	(Leusch et al. 2010)		
				Wastewater	Raw + unspecified treatment	✓	SPE (OASIS®HLB)	(Leusch et al. 2010)	
			Sheep uterus cells	Wastewater	Raw, sec, tert (UV, O <sub>3</sub> , BAC)	✓	SPE (RP-C18)	(Leusch et al. 2005)	
					Raw, prim/sec	✓	SPE (OASIS®HLB)	(Leusch et al. 2006a)	
	Raw	✓			SPE (comparison of several solid phases)	(Leusch et al. 2006b)			
	Incl. animal waste. Sec	✓			SPE (OASIS®HLB)	(Sarmah et al. 2006)			
	T47D-KBluc	Ground water	Queensland, Australia	✓	SPE (OASIS®HLB)	(Leusch et al. 2010)			
			Surface water	River, Queensland, Australia	✓	SPE (OASIS®HLB)	(Leusch et al. 2010)		
			Wastewater	Raw + unspecified treatment	✓	SPE (OASIS®HLB)	(Leusch et al. 2010)		
	E-SCREEN	MCF-7-BOS	Drinking water	Prim, sec, tert (Cl <sub>2</sub> )	✓	SPE (OASIS®HLB)	(Macova et al. 2011)		
				Ground water	Queensland, Australia	✓	SPE (OASIS®HLB)	(Leusch et al. 2010)	
			Surface water	River, Queensland, Australia	✓	SPE (OASIS®HLB)	(Leusch et al. 2010)		
				Brisbane rivers, lakes, receiving recycled water	✓	SPE (OASIS®HLB)	(Macova et al. 2011)		
			Wastewater	Raw, sec, tert (UV, O <sub>3</sub> , BAC)	✓	SPE (RP-C18)	(Leusch et al. 2005)		
Raw				✓	SPE (comparison of several solid phases)	(Leusch et al. 2006b)			
Sec			✓	SPE (OASIS®HLB)	(Tan et al. 2007)				
			Raw + unspecified treatment	✓	SPE (OASIS®HLB)	(Leusch et al. 2010)			
Prim, sec, tert (AC, O <sub>3</sub> , DAFF)			✓	SPE (OASIS®HLB)	(Macova et al. 2010)				
Prim, sec, tert (RO, UV, H <sub>2</sub> O <sub>2</sub> )			✓	SPE (OASIS®HLB)	(Escher et al. 2011)				

Target MoA	Assay	Test organism	Application	Specifics	TEQ	Enrichment	Literature reference	
<b>Alternative ER techniques</b>	YES	<i>S. cerevisiae</i>	Ground water	Prim, sec, tert (MF, RO, O <sub>3</sub> , BAC)	v	SPE (OASIS®HLB)	(Macova et al. 2011)	
				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)	
			Wastewater	River, Queensland, Australia	v	SPE (OASIS®HLB)	(Leusch et al. 2010)	
				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)	
	Yeast two-hybrid	<i>S. cerevisiae</i> Y190 with human and medaka ERα	Wastewater	Prim, sec, tert (O <sub>3</sub> , 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)	
				Sec	v	SPE (C18)	(Mispagel et al. 2005)	
	Receptor competitive binding	Rainbow trout liver	Pulp mill effluent, NZ and Canada	Sec	v	SPE (C18)	(Allinson et al. 2007)	
				Sec	v	SPE (C18)	(Mispagel et al. 2009)	
				Sec	v	SPE (C18)	(Allinson et al. 2010d)	
				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)		
<b>Dioxin-like activity</b>								
<b>AhR activation</b>	AhR-CAFLUX	H4IIE	Drinking water	Prim, sec, tert (Cl <sub>2</sub> )	v	SPE (OASIS®HLB)	(Macova et al. 2011)	
			Surface water	Contaminated wetlands, Sydney	v	SPE (Empore C18)	(Rawson et al. 2009)	
	AhR-CAFLUX	Wastewater	Brisbane rivers, lakes, receiving recycled water	v	SPE (OASIS®HLB)	(Macova et al. 2011)		
			Prim, sec, tert (AC, O <sub>3</sub> , DAFF)	v	SPE (OASIS®HLB)	(Macova et al. 2010)		
			Prim, sec, tert (MF, RO, O <sub>3</sub> , BAC)	v	SPE (OASIS®HLB)	(Macova et al. 2011)		

Target MoA	Assay	Test organism	Application	Specifics	TEQ	Enrichment	Literature reference
	Yeast two-hybrid	<i>S. cerevisiae</i> Y190 with human and medaka ER $\alpha$	Wastewater	Prim, sec	v	SPE (C18)	(Allinson et al. 2010a)
<b>Developmental toxicity</b>							
<b>Retinoic acid receptor (RAR)</b>	Yeast two-hybrid	<i>S. cerevisiae</i> Y190 with human and medaka ER $\alpha$	Wastewater	Prim, sec	v	SPE (C18)	(Allinson et al. 2010b)
<b>Neurotoxicity</b>							
<b>Inhibition of acetylcholin-esterase (AChE)</b>	AChE inhibition	<i>E. electricus</i>	Drinking water	Prim, sec, tert (Cl <sub>2</sub> )	v	SPE (OASIS®HLB)	(Macova et al. 2011)
		Purified beef AChE	Surface water	River, Switzerland	v	SPE (C18, EN)	(Escher et al. 2008b)
		<i>E. electricus</i>		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 <sub>3</sub> , AC)	v	SPE (C18, EN)	(Escher et al. 2009)
		<i>E. electricus</i>		Prim, sec, tert (AC, O <sub>3</sub> , DAFF)	v	SPE (OASIS®HLB)	(Macova et al. 2010)
		Prim, sec, tert (MF, RO, O <sub>3</sub> , BAC)	v	SPE (OASIS®HLB)	(Macova et al. 2011)		
<b>Sodium channel blocking</b>	Neuroblastoma assay	Neuro2A ATCC CCL-131	Surface water	Lakes, rivers + oxidation ponds, NZ		WET	(Wood et al. 2006)
<b>REACTIVE TOXICITY</b>							
<b>Genotoxicity: SOS-response</b>	<i>umuC</i>	<i>S. typhimurium</i> TA1535/pSK1002	Drinking water	Prim, sec, tert (Cl <sub>2</sub> )	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)
				Prim, sec, tert (O <sub>3</sub> , AC)	v	SPE (C18, EN)	(Escher et al. 2009)
				Prim, sec, tert (AC, O <sub>3</sub> , DAFF)	v	SPE (OASIS®HLB)	(Macova et al. 2010)
				Prim, sec, tert (MF, RO, O <sub>3</sub> , BAC)	v	SPE (OASIS®HLB)	(Macova et al. 2011)
<b>LOW-COMPLEXITY IN VIVO ASSAYS</b>							
<b>Phytotoxicity (algae)</b>							
<b>PSII derived</b>	ToxY-PAM	<i>Phaeodactylum</i>	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		<i>tricomutum</i>		Brisbane and Thames Estuaries	v	SPE (OASIS®HLB)	2005a) (Bengtson Nash et al. 2006)
	IPAM	<i>P. tricomutum</i> and <i>C. vulgaris</i>	Surface water	Brisbane River, Australia	v	SPE (OASIS®HLB)	(Muller et al. 2008b)
Algal growth and PSII inhibition		<i>C. vulgaris</i>	Wastewater	Prim, sec, tert (AC, O <sub>3</sub> , DAFF)	v	SPE (OASIS®HLB)	(Macova et al. 2010)
	Combined algae test	<i>P. subcapitata</i>	Drinking water	Prim, sec, tert (Cl <sub>2</sub> )	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 <sub>3</sub> , AC)	v	SPE (C18, EN)	(Escher et al. 2009)
				Prim, sec, tert (RO, UV, H <sub>2</sub> O <sub>2</sub> )	v	SPE (OASIS®HLB)	(Escher et al. 2011)
			Prim, sec, tert (MF, RO, O <sub>3</sub> , 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- $\alpha$ ) 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 <sup>1</sup>	Enrichment	Literature reference		
<b>NON-SPECIFIC TOXICITY (Baseline toxicity)</b>									
<b>Cytotoxicity</b>	Bioluminescence inhibition (Microtox, Biotox, Lumistox, Luminotox, ToxAlert®)	<i>Vibrio fischeri</i>	Coal gasification process water	Groundwater effluents Wyoming	Y	LLE (DCM) + fractionation	(Timourian et al. 1982)		
			Drinking water	Spring and tap water, USA	Y	WET	(Chang et al. 1981)		
				Raw lake, NaClO, ClO <sub>2</sub> , PAA	Y	SPE (C18)	(Guzzella et al. 2004)		
				Raw lake + ClO <sub>2</sub> and GAC	Y	SPE (C18)	(Zani et al. 2005)		
				Treatment plants (ClO <sub>2</sub> , O <sub>3</sub> , GAC)	Y	SPE (C18)	(Guzzella et al. 2006)		
				Bottled, still and carbonated	No	WET	(Ceretti et al. 2010)		
				Prim, sec, tert (Cl <sub>2</sub> )	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)	
					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	(Rojicková-Padrťová 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 <sub>3</sub> 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 <sub>3</sub>	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 <sub>3</sub> , 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 <sub>3</sub> )	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	(Schiliró et al. 2009)
		Fertiliser production (raw + precipitation with hydrated lime)	No	WET	(Gouider et al. 2010)
		Prim, sec, tert (O <sub>3</sub> , AC, DAFF)	Y (TEQ)	SPE (OASIS®HLB)	(Macova et al. 2010)
		Sec, tert (biofilm, O <sub>3</sub> )	Y	WET	(Lundstrom et al. 2010)
		Raw, tert (anaerobic MBR)	No	WET	(Saddoud et al. 2010)
		Prim, sec, tert (RO, UV, H <sub>2</sub> O <sub>2</sub> )	Y (TEQ)	SPE (OASIS®HLB)	(Escher et al. 2011)
		Prim, sec, tert (MF, RO, O <sub>3</sub> , BAC)	Y (TEQ)	SPE (OASIS®HLB)	(Macova et al. 2011)
		Prim, sec, tert (sand filtration, BAC)	Y (TEQ)	SPE (OASIS®HLB)	(Reungoat, 2011)
<i>Photobacterium phosphoreum</i>	Surface water	River, Germany	No	WET	(Dizer et al. 2002)
	Wastewater	Sec, Cl, O <sub>3</sub>	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)
<b>Inhibition of nitrification</b>	Unspecified bacteria	Wastewater	Mixed effluents		Y	WET	(Wadhia et al. 2007)
<b>Inhibition of respiration</b>	Unspecified bacteria	Wastewater	Mixed effluents		Y	WET	(Wadhia et al. 2007)
	<i>E. coli</i>		TNT effluent		No	WET	(Barreto-Rodrigues et al. 2009)
	Polytox®Test	Unspecified bacteria		Prim, sec	Y	WET	(Mendonca et al. 2009)
<b>Cytotoxicity</b>	Yeast cell viability	<i>Saccharomyces cerevisiae</i> (D7)	Drinking water	Raw lake, NaClO, ClO <sub>2</sub> , PAA	Y	SPE (C18)	(Guzzella et al. 2004)
				Raw lake + ClO <sub>2</sub> and GAC	Y	SPE (C18)	(Zani et al. 2005)
	Vitotox 10 kit	<i>Salmonella typhimurium</i> TA104 <i>pr1</i>	Oil refinery	Treatment plants (ClO <sub>2</sub> , O <sub>3</sub> , GAC) Finland	Y	SPE (C18)	(Guzzella et al. 2006)
			Paper/pulp mill	Unspecified treatment	Y	WET + SPE (OASIS®HLB)	(Pessala et al. 2004)
	MARA	Multispecies (10 bacteria, 1 yeast)	Surface water	Lake, Italy	Y	SPE (C18)	(Pellacani et al. 2006)
	Vitotox 10 kit	<i>S. typhimurium</i> TA104 <i>pr1</i>	Wastewater	River + bore hole, UK	Y (MTC)	WET	(Wadhia et al. 2007)
	Yeast growth inhibition	<i>S. cerevisiae</i>		Mixed, unspecified treatment	Y	WET + SPE (OASIS®HLB)	(Pessala et al. 2004)
	Bacterial growth inhibition	Multispecies (3)		Unspecified treatment	Y (TEQ)	SPE (Lichrolut C18, EN)	(Rutishauser et al. 2004)
	Yeast cell yield in GreenScreen EM® MARA	<i>S. cerevisiae</i> with reporter gene		Sec	Y (No IC)	WET	(Schmitt et al. 2005)
		Multispecies (10 bacteria, 1 yeast)		Olive mill (prim, tert (electrochemical))	Y	WET, LL (ethyl acetate)	(Mekki et al. 2008)
				O <sub>3</sub> and electrochemical oxidation	Y	WET	(Keenan et al. 2007)
				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)
<b>Staining assays</b>	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 <sub>2</sub> , 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 <sub>2</sub>	Y	SPMD vs. SPE (C18)	(Buschini et al. 2008)
	NR/LDH release (Promega®), FDA/EtBr	Human lympho-/leukocytes, Hep-G2		Prim, Sec, ClO <sub>2</sub> , 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	<i>Arthrobacter globiformis</i>	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 <sub>3</sub> ,biol.)	No	SPE (OASIS®HLB) + column fractionation	(Ma et al. 2005)
	Dehydrogenase	<i>Arthrobacter globiformis</i>		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 ( <i>O. mykiss</i> ) 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 HepG2		Sec Prim + MBR	Y (TEQ) No	LLE (DCM) Lyophilisation followed by DCM extraction	(Creusot et al. 2010) (Delgado et al.)
<b>Other</b>	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)

<b>AR activity</b>	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	<i>S. cerevisiae</i>	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)

	Yeast two-hybrid	<i>S. cerevisiae</i> w human AR		Raw + MBR Incl. pulp mill. Sec	No but TEQ Y (TEQ) Y (TEQ)	SPE (OASIS®HLB) SPE (EN RP18) SPE (C18) + frac	(Coleman et al. 2009) (Sousa et al. 2010) (Allinson et al. 2007; Allinson et al. 2008)
<b>AR (ant)-agonism</b>	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	<i>S. cerevisiae</i> Y187	Surface water Wastewater	Polluted river, Italy Prim, sec, tert (Cl)	No Y (TEQ)	SPE (OASIS®HLB) SPE (OASIS®HLB)	(Urbatzka et al. 2007) (Li et al. 2010a)
<b>AR antagonism</b>	AR Luciferase reporter assay kit (Promega)	CV-1		Industrial (sec)	Y (TEQ)	SPE	(Shi et al. 2009a)
	YAS	<i>S. cerevisiae</i>	Oil field produced Oil field produced	Offshore, North Sea Offshore, North Sea	Y (TEQ) Y (TEQ)	SPE (ENV, C18) + frac. SPE (ENV, C18) + frac.	(Tollefsen et al. 2007) (Thomas et al. 2009)
<b>AR indirect</b>	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)
<b>Estrogen receptor (ER)</b>							
<b>ER (ant)-agonism</b>	YES	<i>S. cerevisiae</i> (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)
<b>ER antagonism</b>	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)
<b>ER activity</b>	Yeast luciferase	BMAEREluc/ERα	Wastewater	Treatment unspecified	No but TEQ	SPE (OASIS®HLB)	(Salste et al. 2007)
	ER-CALUX	U2OS (human) T47D	Drinking water	Tap	Y (TEQ)	LLE (EtAc)	(Van der Linden et al. 2008)
			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) T47D		River, brook, The Netherlands	Y (TEQ)	LLE (EtAc)	(Van der Linden et al. 2008)
				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) T47D		Mixed. Prim, sec	Y (TEQ)	LLE (EtAc)	(Van der Linden et al. 2008)
				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 (transfected with ER $\alpha$ or ER $\beta$ )	Coastal water	Singapore	Y	SPE (OASIS®HLB)	(Gong et al. 2003)
	MELN cells	Wastewater	Sec	Y (TEQ)	SPE (RP-C18)	(Pawlowski et al. 2003)
		Drinking water	O <sub>3</sub> , 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™)	(Dagnino et al. 2010)
			Raw + unspecified treatment	Y (TEQ)	EtAc/MeOH extraction (PM)	
	MVLN		Sec	Y (TEQ)	SPE (OASIS®HLB)	(Leusch et al. 2010)
			Prim + sec	Y (TEQ)	SPE (Empore disks)	(Snyder et al. 2001)
	Rat uterus cytosol	Surface water	River, estuary, The Netherlands	Y (TEQ)	SPE (EDS-1)	(Furuichi et al. 2006)
		Wastewater	Prim + unspecified treatment	Y (TEQ)	SPE (SDB-XC)	(Murk et al. 2002)
	Sheep uterus cells		Raw, sec, tert (Uv, O <sub>3</sub> , BAC)	Y (TEQ)	SPE (SDB-XC)	(Murk et al. 2002)
			Raw, sec	Y (TEQ)	SPE (RP-C18) + frac	(Leusch et al. 2005)
			Raw	Y (TEQ)	SPE (OASIS®HLB)	(Leusch et al. 2006a)
					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 T47D-KBluc		Prim + O <sub>3</sub>	NO	WET	(Bertanza et al. 2010)
		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)	(Mnif et al. 2010)
					DCM/MeOH extraction (PM)	
E-SCREEN	MCF-7-BOS	Drinking water	Prim, sec, tert (Cl <sub>2</sub> )	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)	(Schilirò et al. 2004)
			Raw, sec, tert (Uv, O <sub>3</sub> , 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 <sub>3</sub> , 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 <sub>2</sub> O <sub>2</sub> )	Y (TEQ)	SPE (OASIS®HLB)	(Escher et al. 2011)
			Prim, sec, tert (MF, RO, O <sub>3</sub> , 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	<i>S. cerevisiae</i>	Coastal waters	Baltic Sea, Germany	Y (TEQ)	SPE (OASIS®HLB)	(Beck et al. 2006a; Beck et al. 2006b)
	<i>S. cerevisiae</i> (RMY326)		Seafood farm water, Italy	No	LLE (DCM)	(Pinto et al. 2008)
	<i>S. cerevisiae</i>	Drinking water	Gulf of Mexico, USA	Y (TEQ)	LLE (DCM)	(Weston et al. 2010)
			Mixed rural sources	Y (TEQ)	SPE (C18)	(Aneck-Hahn et al. 2009)
			Bottled water, PET packaging	Y (TEQ)	SPE (C18)	(Pinto and Reali 2009)
	<i>S. cerevisiae</i> (RMY326)		Bottled, mixed packaging incl. PET	Y (TEQ)	WET	(Wagner and Oehlmann 2009)
	<i>S. cerevisiae</i>	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 <sub>3</sub> ,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 <sub>3</sub> , 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	<i>S. cerevisiae</i> 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)
<b>Alternative ER methods</b>	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	<i>S. cerevisiae</i> BMA 64-1A		Rivers, CA, USA	Y (TEQ)	SPE (C18)	(Lavado et al. 2009)
	MCF-7 ER $\alpha$ 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)	<i>S. cerevisiae</i> BMA 64-1A		Prim, sec	Y (TEQ)	SPE (RP, C18)	(Roda et al. 2006)
	ER- $\alpha$ FP	hrER $\alpha$		Ozonation of prim and sec	Y (TEQ)	SPE (OASIS®HLB)	(Zhang et al. 2006)
	NRL ER- $\alpha$ ELISA (Vtg)	Human TIF2/ <i>E. Coli</i>		Ozonation of prim and sec	Y (TEQ)	SPE (OASIS®HLB)	(Zhang et al. 2006)
	ER- $\alpha$ FP	<i>O. mykiss</i> hepatocytes		Prim + unspecified treatment	Y (TEQ)	SPE (OASIS®HLB)	(Grung et al. 2007)
	<i>A. thaliana</i> assay	Cell line unspecified		Sec + O <sub>3</sub>	Y (TEQ)	SPE (OASIS®HLB)	(Kim et al. 2007)
	ELISA (Vtg)	Transgenic <i>A. thaliana</i>		Treatment unspecified	No	WET and SPE (PLS-3)	(Takahashi et al. 2007)
	Recombinant yeast	Three-spined stickleback hepatocytes		Treatment unspecified	Y (TEQ)	SPE (OASIS®HLB)	(Björkblom et al. 2008)
		W303-1A (with hER, ZERE)		Farm, prim, sec	Y (TEQ)	SPE (HZ-802)	(Li et al. 2008b)

	rER	Rainbow trout liver		Dom/paper mill, sec. Daily variation.	Y (TEQ)	SPE (C18)	(Martinovic et al. 2008)
	ER	Cell line unspecified		Tert (O <sub>3</sub> )	No but TEQ	SPE (several, ref not clear)	(Tsuno et al. 2008)
	NRL ER- $\alpha$	Cell line unspecified		Tert (O <sub>3</sub> )	No but TEQ	SPE (several, ref not clear)	(Tsuno et al. 2008)
	NRL ER- $\alpha$	Human TIF2/ <i>E. Coli</i>		Prim, tert (O <sub>3</sub> )	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 (W303 $\alpha$ )		Prim, sec +Cl	Y (TEQ)	WET + SBSE	(Balsiger et al. 2010)
	nAES (novel <i>Arxula adenivorans</i> yeast estrogen screen)	<i>A. adenivorans</i> G1212		Mixed	Y (TEQ)	SPE (C18)	(Kaiser et al. 2010)
	MCF-7 ER $\alpha$ ELISA	MCF-7		Unspecified treatment	No	SPE (C18)	(Swart et al. 2011)
<b>Glucocorticoid receptor (GR) activity</b>	GR-CALUX	U2OS (human)	Drinking water	Tap	Y (TEQ)	LLE (EtAc)	(Van der Linden et al. 2008)
			Surface water Wastewater	River, brook, The Netherlands Mixed. Prim, sec	Y (TEQ) Y (TEQ)	LLE (EtAc) LLE (EtAc)	(Van der Linden et al. 2008) (Van der Linden et al. 2008)
<b>Pregnane X receptor (PXR) activity</b>	PXR activity via reporter cell line	HeLa cells	Wastewater	Sec	Y (TEQ)	SPE (C18)	(Mahjoub et al. 2009)
		HG5LN-hPXR HG <sub>5</sub> LNGal-4-PXR	Wastewater incl. particulate fraction	Sec Tert (Cl)	Y (TEQ) Y (TEQ)	LLE (DCM) SPE (C18) DCM/MeOH extraction (PM)	(Creusot et al. 2010) (Mnif et al. 2010)
<b>Progesterone receptor (PR) PR (ant)-agonism</b>	PR-CALUX	U2OS (human)	Drinking water	Tap	Y (TEQ)	LLE (EtAc)	(Van der Linden et al. 2008)
			Surface water Wastewater	River, brook, The Netherlands Mixed. Prim, sec	Y (TEQ) Y (TEQ)	LLE (EtAc) LLE (EtAc)	(Van der Linden et al. 2008) (Van der Linden et al. 2008)
			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)
<b>PR antagonism</b>	Recombinant yeast assay	<i>S. cerevisiae</i>	Wastewater	Prim, sec, tert (Cl)	Y (TEQ)	SPE (OASIS®HLB)	(Li et al. 2010a)
<b>Thyroid receptor (TR) TR antagonism</b>	TR reporter gene assay (luciferase reporter assay kit, Promega)	CV-1	Wastewater	Industrial (sec)	Y (TEQ)	SPE	(Shi et al. 2009a)
<b>TR activity</b>	TR-reporter gene assay	PC-DR-LUC	Drinking water	O <sub>3</sub> , AC, Cl, e-Cl, flocc, coag	Y (TEQ)	SPE (OASIS®HLB)	(Jugan et al. 2009)
			Surface water Wastewater	River, Paris Dom (Paris), prim, sec	Y (TEQ) Y (TEQ)	SPE (OASIS®HLB) SPE (OASIS®HLB)	(Jugan et al. 2009) (Jugan et al. 2009)
	Yeast two-hybrid	<i>S. cerevisiae</i>	Wastewater	Prim, sec, tert (Cl)	Y (TEQ)	SPE (OASIS®HLB)	(Li et al. 2010a)
<b>TR (ant)agonism</b>	Y-187 hTR-GRIP1	CV-1	Drinking water	Source and treated (incl. Cl <sub>2</sub> 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**

<b>AhR activation</b>	AhR-CAFLUX	H4IIE	Drinking water	Prim, sec, tert (Cl <sub>2</sub> )	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)
	DRE-CALUX	Hepa 1c1c7	Surface water	Offshore effluent, North Sea	Y (TEQ)	SPE (C8, ENV+)	(Balaam et al. 2009)
	H4IIE bioassay	H4IIE		Rivers, Korea	Y (TEQ)	LL (DCM)	(Joung et al. 2007)
	DR-CALUX			Contaminated wetlands, Sydney	Y (TEQ)	SPE (Empore C18)	(Rawson et al. 2009)
	AhR-CAFLUX			Seawater, Japan	Y (TEQ)	SPE (Empore C18)	(Sato et al. 2010)
	DR-CALUX		Wastewater	Rivers, lakes receiving recycled water, Australia	Y (TEQ)	SPE (OASIS®HLB)	(Macova et al. 2011)
	AhR-CAFLUX			Prim + reed bed material	Y (TEQ)	Soxhlet, toluene + fractionation	(Gustavsson et al. 2007)
				Prim, sec, tert (AC, O <sub>3</sub> , DAFF)	Y (TEQ)	SPE (OASIS®HLB)	(Macova et al. 2010)
				Prim, sec, tert (MF, RO, O <sub>3</sub> , 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)	Y (TEQ)	SPE (GX-271 ASPEC™)	(Dagnino et al. 2010)	
			suspended solids				
		Wastewater + particulate fraction	Tert (Cl)	Y (TEQ)	SPE (C18)	(Mnif et al. 2010)	
AhR yeast two-hybrid	<i>S. cerevisiae</i> Y190 with human and medaka ERα	Wastewater	Prim, sec	Y (TEQ)	DCM/MeOH extraction (PM) 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 <sub>3</sub> , biol.)	Y (TEQ)	SPE (OASIS®HLB) + column fractionation	(Ma et al. 2005)
		<i>O. mykiss</i> , 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	<i>S. cerevisiae</i> Y190	Surface water	Rivers, Japan	Y	SPE (OASIS®HLB)	(Inoue et al. 2009b)	
				Rivers, China	Y (TEQ)	SPE (OASIS®HLB)	(Zhen et al. 2009)	
			Wastewater	Rivers, Japan	Y (TEQ)	SPE (OASIS®HLB)	(Inoue et al. 2010)	
				Sec, tert (O <sub>3</sub> , MF, UV, Cl, RO, coag)	Y (TEQ)	SPE (OASIS®HLB)	(Cao et al. 2009b)	
				Prim, sec, tert (O <sub>3</sub> , 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)	<i>Drosophila melanogaster</i>		Drinking water	Tap water, Greece	Y	WET	(Vamvakaki and Chaniotakis 2007)	
				Prim, sec, tert (Cl <sub>2</sub> )	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)
	<i>E. electricus</i> and <i>Apis mellifera</i>		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)
	<i>E. electricus</i>		Wastewater	Rivers, lakes receiving recycled water, Australia	Y (TEQ)	SPE (OASIS®HLB)	(Macova et al. 2011)	
				Purified beef AChE	Prim, sec	Y (TEQ)	SPE (C18, EN)	(Escher et al. 2008b)
	<i>E. electricus</i>		Wastewater	Prim, sec, tert (O <sub>3</sub> , AC)	Y (TEQ)	SPE (C18, EN)	(Escher et al. 2009)	
				Prim, sec, tert (AC, O <sub>3</sub> , DAFF)	Y (TEQ)	SPE (OASIS®HLB)	(Macova et al. 2010)	
				Prim, sec, tert (MF, RO, O <sub>3</sub> , 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)
					Prim, sec	No	LLE (several solvents)	(Basu et al. 2009)
					Prim, sec	No	LLE (several solvents)	(Basu et al. 2009)
Prim, sec					No	LLE (several solvents)	(Basu et al. 2009)	
Prim, sec					No	LLE (several solvents)	(Basu et al. 2009)	
Prim, sec					No	LLE (several solvents)	(Basu et al. 2009)	
Prim, sec					No	LLE (several solvents)	(Basu et al. 2009)	
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)
<b>Immunotoxicity</b>							
<b>Cytotoxicity</b>	LDH leakage	Whole blood	Surface water	River South Africa	No	SPE (C18)	(Pool and Magcwebeba 2009)
<b>Immunity (cell-mediated)</b>	IFN- $\gamma$ activity			River, South Africa	No	SPE (C18)	(Pool and Magcwebeba 2009)
<b>Immunity (humoral)</b>	IL-10 activity			River, South Africa	No	SPE (C18)	(Pool and Magcwebeba 2009)
<b>Inflammatory activity</b>	IL-6 activity			River, South Africa	No	SPE (C18)	(Pool and Magcwebeba 2009)
<b>Cytotoxicity</b>	Proliferation of IL1/IL2	Mouse splenocytes	Wastewater	Sec + coag, AC, Cl tests	Y	WET	(Kontana et al. 2008)
<b>Lympho-proliferation</b>	Lympho-proliferation bioassay + IL1/IL2 characterisation			Sec, tert (Cl, O <sub>3</sub> ,coag, AC)	Y	WET	(Kontana et al. 2009)
<b>Reproductive toxicity</b>							
<b>Rat testicular cell quality, androgenicity</b>	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)
<b>REACTIVE TOXICITY</b>							
<b>Genotoxicity</b>	GreenScreen EM®	<i>S. cerevisiae</i> with $\gamma$ EGFP3	Wastewater	O <sub>3</sub> and electrochemical oxidation	Y	WET	(Keenan et al. 2007)
	PI/sub-G1	Human lympho/leuko-cytes and hep-G2	Drinking water	Prim, Sec, ClO <sub>2</sub> , AC	Y	SPE (C18)	(Maffei et al. 2009)
	<i>Bacillus subtilis</i> DNA repair assay	<i>B. subtilis</i> – five strains	Petrochemical plant	Runoff	Y	SPE (XAD-2, 7)	(Brown and Donnelly 1984a)
		<i>B. subtilis</i> - six strains			Y	LLE (DCM)	(Brown and Donnelly 1984b)
<b>(SOS-response)</b>	SOS/Chromo	<i>E. coli</i> (PQ37)	Drinking water	Raw lake, NaClO, ClO <sub>2</sub> , PAA	Y	SPE (C18)	(Guzzella et al. 2004)
				Raw lake + ClO <sub>2</sub> and GAC	Y	SPE (C18)	(Zani et al. 2005)
				Disinfection (ClO <sub>2</sub> , O <sub>3</sub> , GAC)	Y	SPE (C18)	(Guzzella et al. 2006)
	<i>umu</i>	<i>S. typhimurium</i> NM2009		From the entire length of Japan	Y	SPE (CSP-800)	(Takanashi et al. 2009)
	<i>umuC</i>	<i>S. typhimurium</i> TA1535/pSK1002		Tap and well, Slovenia	No	WET	(Zegura et al. 2009)
	SOS/ <i>umu</i>		Oil field produced	Prim, sec, tert (Cl <sub>2</sub> )	Y (TEQ)	SPE (OASIS®HLB)	(Macova et al. 2011)
	Vitotox 10 kit	TA104 <i>recN2-4</i>	Oil refinery	Land-based, China	Y	SPE (OASIS®HLB)	(Li et al. 2008a)
			Paper/pulp mill	Finland	Y	WET + SPE (OASIS®HLB)	(Pessala et al. 2004)
				Unspecified treatment	Y	WET + SPE (OASIS®HLB)	(Pessala et al. 2004)
	<i>umu</i>			Prim, sec	Y	WET	(Gartiser et al. 2010)
			Surface water	Rhine river, Germany	Y	SPE (XAD-7)	(Reifferscheid et al. 1991)

SOS/Chromo	<i>E. coli</i>		Rivers, lakes, St Lawrence, Canada	Y	LLE (DCM)	(Langevin et al. 1992)	
<i>umu</i> also for cytotoxicity	<i>S. typhimurium</i> TA1535/pSK1002		River, Germany	No	WET	(Dizer et al. 2002)	
Survival of SOS defective <i>E. coli</i>	Several <i>E. coli</i> K-12 strains		Polluted river, India	No	SPE (XAD-4, 8), LLE (n-hexane)	(Aleem and Malik 2005)	
<i>umuC</i>	<i>S. typhimurium</i> TA1535/pSK1002		River, Switzerland	Y	SPE (C18, EN)	(Escher et al. 2008b)	
SOS/ <i>umu</i>			River, China	Y	SPE (OASIS®HLB)	(Li et al. 2008a)	
<i>umuC</i>			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)	
<i>umu</i>		Wastewater	Mixed	Y	SPE (C18)	(Castillo et al. 2001)	
<i>umu</i> also for cytotoxicity			Prim, sec	Y (no EC)	WET	(Dizer et al. 2002)	
SOS/Chromo	<i>E. coli</i> PQ37		Prim, hospital	No	WET	(Jolibois et al. 2003)	
			Various TPs, unspecified treatment	Y (TU)	WET	(Manusadzianas et al. 2003)	
Vitotox 10 kit	TA104 <i>recN2-4</i>		Mixed, unspecified treatment	Y	WET + SPE (OASIS®HLB)	(Pessala et al. 2004)	
<i>umuC</i>	<i>S. typhimurium</i> TA1535/pSK1002		Prim, sec (anaerobic, aerobic biodegradation)	Y	WET (sonication, centrifugation)	(Gustavsson and Engwall 2006)	
<i>umu</i>			Sec, Cl (tests effect of [NH <sub>4</sub> <sup>+</sup> ])	Y (TEQ)	SPE (CHP20P)	(Wang et al. 2007)	
<i>umuC</i>			Prim + after reed bed treatment	Y	Soxhlet, toluene	(Gustavsson et al. 2007)	
SOS/Chromo	<i>E. coli</i> PQ37		Prim, sec	Y	WET	(Isidori et al. 2007)	
<i>umu</i> – 2 hr auto	<i>S. typhimurium</i> TA1535/pSK1002		Prim	Y	WET	(Brinkmann and Eisentraeger 2008)	
<i>umuC</i>	<i>S. typhimurium</i> TA1535/pSK1002		Prim, sec	Y	SPE (C18, EN)	(Escher et al. 2008b)	
SOS/ <i>umu</i>			Sec, tert (coag, O <sub>3</sub> , Cl <sub>2</sub> )	Y (TEQ)	SPE (OASIS®HLB)	(Cao et al. 2009a)	
			Sec, tert (O <sub>3</sub> , MF, UV, Cl, RO, coag)	No	SPE (OASIS®HLB)	(Cao et al. 2009b)	
<i>umuC</i>			Prim, sec, tert (O <sub>3</sub> , AC)	Y (TEQ)	SPE (C18, EN)	(Escher et al. 2009)	
<i>umu</i>			Mixed effluents	Y	WET	(Gartiser et al. 2009)	
			Prim + unspecified treatment	No	WET	(Zegura et al. 2009)	
			Prim, sec, tert (AC, O <sub>3</sub> , DAFF)	Y (TEQ)	SPE (OASIS®HLB)	(Macova et al. 2010)	
SOS/ <i>umu</i>			Sec, Cl (+- Br)	Y (TEQ)	Frac (XAD-8) + SPE (CHP20P)	(Wu et al. 2010b)	
			Tert: Cl, UF (test influence of NH <sub>4</sub> <sup>+</sup> )	No but TEQ	SPE (XAD-8)	(Wu et al. 2010a)	
Survival of SOS defective <i>E. coli</i>	<i>E. coli</i> K-12, several AB and KL strains		Prim	No	SPE (XAD-4/8 mix), LLE (hexane)	(Ansari and Malik 2009)	
	<i>E. coli</i> K-12, several AB strains		Sec. tannery effluents	Yes but no EC	SPE (XAD-4, 8)	(Alam et al. 2010)	
<i>umuC</i>	<i>S. typhimurium</i> TA1535/pSK1002		Prim, sec, tert (MF, RO, O <sub>3</sub> , BAC)	Y (TEQ)	SPE (OASIS®HLB)	(Macova et al. 2011)	
<b>(Genomic DNA damage)</b>	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 leukocytes		Prim, ClO <sub>2</sub>	Y	SPMD vs. SPE (C18)	(Buschini et al. 2008)	
Comet	Hep-G2		Prim, Sec, ClO <sub>2</sub> , AC	Y	SPMD vs. SPE (C18)	(Maffei et al. 2009)	
			River, China (Sec + Cl)	Y	SPE (XAD-2)	(Shi et al. 2009b)	
			Tap and well, Slovenia	No	WET	(Zegura et al. 2009)	
SCGE	Human leukocytes		Bottled, still and carbonated	No	SPE (C18)	(Ceretti et al. 2010)	
Comet	L-02		River, China (sec, Cl)	Y	SPE (XAD-7)	(Xie et al. 2010)	
Yeast SCGE	Rat hepatoma cells	Oil refinery effluent	Sec	Y	WET	(Rodrigues et al. 2010)	
SCGE/micro-plate comet	<i>S. cerevisiae</i> MTCC 36	Pulp mill effluent	Before and after treatment	No	WET	(Singhal and Thakur 2009)	
Comet/SCGE	CHO AS52	Recycled water	US indoor/outdoor pools, hot tubs	Y	LLE (methyl tert butyl ether)	(Liviatic et al. 2010)	
Comet	Zebrafish hepato-cytes	Surface water	Rivers, Germany	No	WET	(Schnurstein and Braunbeck 2001)	
Comet	<i>O. mykiss</i> hepatoma cell line RTH-149		River, Israel	No	WET	(Avishai et al. 2002)	
SCGE	Human lymphocytes		Rivers, China	Y	SPE (XAD-2)	(Zhong et al. 2001)	
	Human leukocytes		Lake, Italy (raw + ClO <sub>2</sub> for drinking)	Y	SPE (C18)	(Maffei et al. 2005)	
Comet			Lake, Italy	Y	SPE (C18)	(Pellacani et al. 2006)	
	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)	
MN + cell proliferation	Human lymphocytes, Hep-G2		Chlorinated tap water	Y	SPE (C18)	(Marabini et al. 2007)	
	Human lymphocytes		Tap water	Y (No EC)	WET	(Asslouj et al. 2009)	
MN		Groundwater	Well water	Y (No EC)	WET	(Asslouj et al. 2009)	
			Prim, Sec, ClO <sub>2</sub> , AC	Y (No EC)	WET	(Maffei et al. 2009)	
MN	Hep-G2		River, China (sec + Cl)	Y	SPE (XAD-2)	(Shi et al. 2009b)	
	Human lymphocytes	Surface water	Lake, Italy, (raw + ClO <sub>2</sub> for drinking)	Y	SPE (XAD-2)	(Maffei et al. 2005)	
Cytokinesis-block MN (CBMN)	V79 cells		Rivers receiving tannery effluents, Brazil.	No	WET	(Lemos et al. in press)	
MN + cell proliferation	Human lymphocytes		Rivers receiving tannery effluents, Brazil.	No	WET	(Lemos et al. in press)	
	V79	Wastewater	Treatment unspecified	No	WET	(Reifferscheid et al. 2008)	
			Treatment unspecified	Y (No EC)	WET	(Asslouj et al. 2009)	
<b>Alternative genotoxicity tests</b>	Survival of bacteriophage $\gamma$	Bacteriophage $\gamma$	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)
<b>Mutagenicity</b>	Alternative yeast mutagenicity assay					
		<i>S. cerevisiae</i> (D7)	Drinking water			
			Raw lake, NaClO, ClO <sub>2</sub> , PAA	Y	SPE (C18)	(Guzzella et al. 2004)
			Raw lake + ClO <sub>2</sub> and GAC	Y	SPE (C18)	(Zani et al. 2005)
			Surface water	Y	SPE (C18)	(Pellacani et al. 2006)
			Lake, Italy			
	Ames test + cytotoxicity	<i>S. typhimurium</i> TA98 and 100	Coal gasification process water	Y	Stedman extraction and fractionation (Swain et al. 1969)	(Epler et al. 1978)
	Ames test					
		TA98, 100	Coal process water	No	LLE (DCM) + fractionation	(Timourian et al. 1982)
			Groundwater effluents Wyoming	Yes	SPE (Sep-Pak Plus CSP-800)	(Nakajima et al. in press)
			Hydrothermally treated coal			
		TA98, 100, 1537	Coke plant effluent, raw and filtered	Y	LLE (DCM)	(Schaeffer and Kerster 1985)
		TA100	Drinking water	No	Dialysis, lyophilisation, solvent extraction	(Simmon and Tardiff 1976)
			Tap water, Cincinnati			
		TA98, 100	Raw and treated	Y	SPE (XAD2)	(Nestmann et al. 1979)
		TA98, 100, 1535, 1537, 1538	Raw and lab treated: Cl <sub>2</sub> , 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 <sub>2</sub> , 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 <sub>2</sub> and GAC	Y	SPE (C18)	(Zani et al. 2005)
		TA100	Marine water	No	LLE (hexane)	(Kurelec et al. 1979)
			North Adriatic, 50, 500 m offshore			
		TA98, 100	Petrochem. plant	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	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	Y	SPE (XAD)	(Richardson et al. 2010)
	Ames test + cytotoxicity	TA98, 100	Recycled water	Y	Low temp. (37-38°C) distillation	(Gruener 1978)
			High ground water receiving secondary treated waste water. Effect of O <sub>3</sub> .			
	Ames test	TA98, 100, 1535, 1536, 1537, 1538	Surface water	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 <sub>3</sub> , 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 <sub>2</sub> , O <sub>3</sub> , 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 <sub>3</sub> , Cl <sub>2</sub>	Y	SPE (XAD-2, 7)	(Rappaport et al. 1979)
	TA100, 1535		Municipal. Sec, tert incl. AC, Cl <sub>2</sub>	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 <sub>3</sub>	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)	<i>S. typhimurium</i> TA98, 100, 1535, 1537	Wastewater	Prim, sec	Y	LLE (DCM)	(Meier and Bishop 1985)
Other reverse mutation assays	<i>E. coli</i> WP <sub>2</sub>	Wastewater	Industrial, pharmaceutical and paper mill effluents (treatment unspecified) + surface water recipients	No	LLE (DCM)	(Sanchez et al. 1988)
	<i>S. cerevisiae</i> S211,		Industrial, pharmaceutical and paper mill	No	LLE (DCM)	(Sanchez et al. 1988)

		S318		effluents (treatment unspecified) + surface water recipients			
	Mutatox assay	<i>V. fischeri</i>	Drinking water	Raw lake, NaClO, ClO <sub>2</sub> , PAA	Y	SPE (C18)	(Guzzella et al. 2004)
	<i>V. harveyi</i> mutagenicity assay	Genetically modified <i>V. harveyi</i>	Marine water	Raw lake + ClO <sub>2</sub> and GAC	Y	SPE (C18)	(Zani et al. 2005)
	<i>V. harveyi</i> luminescent mutagenicity assay	<i>V. harveyi</i> A16		Europe, USA	No	WET	(Czyz et al. 2003)
				Baltic sea	No	WET	(Podgorska et al. 2007)
<b>Sister chromatid exchange (SCE) induction</b>	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)
<b>Oxidative stress (OS) and reactive oxygen species (ROS)</b>							
<b>OS</b>	GSH	Hep-G2 cells	Drinking water	Raw lake, NaClO, ClO <sub>2</sub> , 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)
<b>ROS</b>	DCFH-DA			Offshore effluent, North Sea	Y	SPE (ENV+, C18)	(Farmen et al. 2010)
		Hep-G2 cells	Surface water	Raw lake. NaClO, ClO <sub>2</sub> , 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 <sub>2</sub> <sup>-</sup> and H <sub>2</sub> O <sub>2</sub> )	<i>S. typhimurium</i> and <i>Allium cepa</i>	Wastewater	Treatment unspecified	Y	WET	(Fatima and Ahmad 2006)
<b>LOW COMPLEXITY IN VIVO ASSAYS</b>							
<b>Phytotoxicity</b>							
<b>Algae</b>							
<b>Algal growth inhibition (AGI)</b>	AGI	<i>Scenedesmus subspicatus</i>	Landfill leachates	Mixed	Y (no response)	WET	(Clement et al. 1996)
		<i>P. subcapitata</i>	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)
		<i>S. subspicatus</i>		Prim, sec	Y	WET	(Gartiser et al. 2010)
	AGI/microscope	<i>P. subcapitata</i>		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)	<i>Scenedesmus quadricauda</i>		Rivers (various types), Argentina	No but TU	WET	(Di Marzio et al. 2005)

	AGI/OD	<i>P. subcapitata</i>		Sava river, Southeast Europe Rivers, Poland	Y (TU) No	SPE (ENV+ and C18) WET	(Källqvist et al. 2008) (Mankiewicz-Boczek et al. 2008)
			Wastewater	Prim + unspecified treatment, also deep well water	Y (TU)	WET	(Rojčková-Padrťová et al. 1998)
		<i>Selenastrum capricornutum</i>		Mixed	Y (TU)	WET	(Tarkpea et al. 1998)
		<i>P. subcapitata</i>		Prim + unspecified treatment	Y	WET	(Blinova 2000)
				Various TPs (treatment unspecified)	Y (TU)	WET	(Manusadzianas et al. 2003)
		<i>C. vulgaris</i>		Sec	Y (no EC)	SPE (C18)	(Aguayo et al. 2004)
				Effluent (treatment unspecified)	No	WET	(Latif and Licek 2004)
	AGI/microscope	<i>P. subcapitata</i>		Mixed, unspecified treatment	Y	WET + SPE (OASIS®HLB)	(Pessala et al. 2004)
	AGI/OD	<i>S. capricornutum</i>		Hospital effluents	Y (TU)	WET	(Emmanuel et al. 2005)
				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	<i>S. subspicatus</i>		Mixed effluents	Y	WET	(Gartiser et al. 2009)
	AGI/OD	<i>P. subcapitata</i>		Prim, sec	Y	WET	(Mendonca et al. 2009)
		<i>S. subspicatus</i>		From pectin production. Prim, sec	Y	WET and SPE (XAD-8) + fractionation	(Reginatto et al. 2009)
		<i>P. subcapitata</i>		Sec + tert (biofilm, O <sub>3</sub> )	Y	WET	(Lundstrom et al. 2010)
<b>PSII derived photosynthesis inhibition</b>	ToxY-PAM	<i>Phaeodactylum tricornutum</i>	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	<i>P. tricornutum</i> and <i>C. vulgaris</i>		Brisbane River, Australia	Y (TEQ)	SPE (OASIS®HLB)	(Muller et al. 2008b)
		<i>C. vulgaris</i>	Wastewater	Prim, sec, tert (AC, O <sub>3</sub> , DAFF)	Y (TEQ)	SPE (OASIS®HLB)	(Macova et al. 2010)
<b>AGI + PSII</b>	Combined algae test	<i>P. subcapitata</i>	Drinking water	Prim, sec, tert (Cl <sub>2</sub> )	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 <sub>3</sub> , AC)	Y (TEQ)	SPE (C18, EN)	(Escher et al. 2009)
				Prim, sec, tert (RO, UV, H <sub>2</sub> O <sub>2</sub> )	Y (TEQ)	SPE (OASIS®HLB)	(Escher et al. 2011)
				Prim, sec, tert (MF, RO, O <sub>3</sub> , BAC)	Y (TEQ)	SPE (OASIS®HLB)	(Macova et al. 2011))
<b>Plants</b>							
Cytotoxicity, growth inhibition	Root growth	<i>Allium cepa</i> (onion)	Drinking water	Bottled, glass, PET, sunlight exposure	No	WET	(Evandri et al. 2000)
	Growth inhibition	<i>Lemna minor</i>	Landfill leachates	Mixed	Y (no response)	WET	(Clement et al. 1996)
		<i>A. cepa</i>		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

	Root growth			Unspecified treatment	Y	WET	Rangaswamy 1979)
	Mitotic index			Raw, sec	No	WET	(Pessala et al. 2004)
	Growth inhibition	<i>L. minor</i>		Prim, sec	Y	WET	(Chaparro et al. 2010)
		<i>L. minor L</i>		Prim, sec	Y	WET	(Gartiser et al. 2010)
		<i>Ornithogalum virens, A. cepa</i>	Surface water	River, India, receiving effluents from Rayon factory	No	WET	(Rosa et al. 2010)
		<i>L. minor</i>		Rivers, North Estonia	No	WET	(Ravindran and Ravindran 1978)
				Rivers, Poland	No	WET	(Blinova 2000)
	Growth inhibition and chl. A content	<i>Lemna minor L</i>		River Sava, Croatia. Polluted. Prim/sec	Y (No EC)	WET	(Mankiewicz-Boczek et al. 2008)
	Growth inhibition	<i>A. cepa</i>		River, various sites/types, Croatia	No	WET	(Radić et al. 2010a)
	Cell death	<i>A. cepa</i>		River incl. sites receiving raw sewage, Brazil	No	WET	(Radić et al. 2010b)
	Seed viability	<i>Oryza sativa</i> (rice)	Wastewater	Molasses effluent	Y	WET	(Bianchi et al. in press)
	Growth inhibition	<i>L. minor</i>		Prim + unspecified treatment	Y	WET	(Behera and Misra 1982)
	Root growth	<i>A. cepa</i>		Mixed, unspecified treatment	Y	WET	(Blinova 2000)
	Mitotic index	<i>A. cepa</i> seeds		Textile effluent	Y	WET	(Pessala et al. 2004)
				Various TPs (treatment unspecified)	Y	WET	(Carilta and Marin-Morales 2008)
		<i>Garden cress, A. cepa</i> and various local plant species incl. three macrophytes		Winery waste water, Australia	Y	WET	(Paixão et al. 2008)
		<i>L. minor</i>		Prim, sec	Y	WET	(Arienzo et al. 2009)
	Growth inhibition	Multispecies (4)		Municipal, poultry, brewery. Prim, sec	Y	WET	(Mendonca et al. 2009)
	Root growth	<i>A. cepa</i>		Fertiliser production (raw + precipitation with hydrated lime)	No	WET	(Ostra et al. 2009)
	Growth inhibition	<i>L. minor</i>		Textile effl. (raw + bacterial treatment)	Y	WET	(Gouider et al. 2010)
				Sec + O <sub>3</sub> , AC	No	WET	(Jadhav et al. 2010)
<b>Seed germination and root growth</b>	Germination, growth and more	<i>O. sativa</i>	Paper mill effluent	Unspecified treatment	Y	WET	(Stalter et al. 2010)
	Phytotoxkit	<i>Lepidium sativum</i>	Surface water	Receiving river, Austria	No	WET	(Misra and Behera 1991)
	Root growth and mitotic index	<i>A. cepa</i>		Receiving streams, Brazil	No	WET	(Latif and Licek 2004)
	Growth inhibition and mitotic index	<i>A. cepa</i>		River, Brazil	Y	WET	(Mitteregger et al. 2007)
	Germination, growth	<i>O. sativa</i>	Wastewater	Molasses effluent	Y	WET	(Barberio et al. 2009)
	Growth and germination index	<i>L. sativum</i>		Olive mill effluent (prim, sec)	Y	WET	(Behera and Misra 1982)
	Phytotoxkit	<i>A. cepa</i>		Effluent (treatment unspecified)	No	WET	(Filidei et al. 2003)
	Germination index	<i>Lycopersicon esculentum</i> (tomato) and <i>Chicorium intybus</i> seeds		Olive mill (raw + prim)	No	WET	(Latif and Licek 2004)
	Germination index	<i>L. esculentum</i> seeds		Olive mill (raw + fungal treatment)	No	WET	(Komilis et al. 2005)
				Olive mill (raw + tert (electrochemical))	No	WET	(Dhouib et al. 2006)
						WET	(Khoufi et al. 2006)

	Phytotoxkit	<i>L. sativum</i> <i>Alba sinapis, Shorgum saccharatum, L. sativum</i>		Raw, tert (UF, MF) Sec + coag, AC, Cl tests	No No	WET WET	(Saddoud et al. 2007) (Kontana et al. 2008)
	Root growth	<i>A. cepa</i>		Raw pharmaceutical effluent Nigeria	Y	WET	(Bakare et al. 2009)
	Seed germination	<i>L. sativum</i>		Prim, sec	No	WET	(Ellouze et al. 2009)
	Phytotoxkit	<i>Sinapis alba, S. saccharatum, L. sativum</i>		Sec, tert (Cl, O <sub>3</sub> ,coag, AC)	No	WET	(Kontana et al. 2009)
	Growth inhibition	<i>S. alba</i>		Municipal, poultry, brewery. Prim, sec	Y	WET	(Ostra et al. 2009)
	Germination index	<i>L. sativum</i> Multispecies (6)		Prim, tert (MBR) Fertiliser production (raw + precipitation with hydrated lime)	No Yes	WET WET	(Saddoud et al. 2009) (Gouider et al. 2010)
	Seed + root	<i>T. aestivum, P. mungo</i>			Y	WET	(Jadhav et al. 2010)
	Growth inhibition	<i>S. alba</i>		Dom + pharmaceutical, prim/sec	No	WET	(Radić et al. 2010b)
	Germination index	<i>L. sativum</i>		Raw, tert (anaerobic MBR)	No	WET	(Saddoud et al. 2010)
	Phytotoxkit	<i>S. saccharatum, L. sativum, S. alba</i>		Sec, tert (UV, Cl)	No	WET	(Bakopoulou et al. 2011)
<b>Genotoxicity</b>	Mitotic index, chromosome aberrations	<i>A. cepa</i>	Drinking water	Bottled, glass, PET, sunlight exposure	No	WET	(Evandri et al. 2000)
	MN	<i>Tradescantia</i> (clone #4430)		Bottled, still and carbonated	No	Lyophilised concentrates	(Biscardi et al. 2003)
	MN, mitotic index	<i>A. cepa</i> and <i>Tradescantia</i> (clone #4430)		Bottled, still and carbonated	No	WET	(Ceretti et al. 2010)
	Comet	<i>A. cepa</i>	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	<i>Vicia faba</i> (bean)	Surface water	Rivers China	No	SPE (XAD-2)	(Zhong et al. 2001)
	Mitotic index, MN	<i>A. cepa</i>		Receiving streams, Brazil	No	WET	(Mitteregger et al. 2007)
				Receiving river, Argentina	Y	WET	(Gana et al. 2008)
	Chromosome ab.	<i>A. cepa</i> seeds		Oil contaminated river, Brazil	No	WET	(Leme et al. 2008)
		<i>A. cepa</i>		River, Brazil	Y	WET	(Barberio et al. 2009)
	Mitotic index, MN and more			Creek, Brazil	No	WET	(Santos et al. 2009)
	Comet	<i>Lemna minor L</i>		Receiving river, Croatia. Prim/sec	No	WET	(Radić et al. 2010a)
	<i>Allium</i> root assay	<i>A. cepa</i>		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)
	<i>Allium</i> root assay		Wastewater	Unspecified treatment	Not specified	WET	(Fatima and Ahmad 2006)
	Chromosome ab., MN	<i>A. cepa</i> seeds		Textile effluent	Y	WET	(Carilta and Marin-Morales 2008)
		<i>A. cepa</i>		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)
	<i>Allium</i> root assay			Dom + pharmaceutical, prim/sec	No	WET	(Radić et al. 2010b)
<b>OS</b>	MDA	<i>Lemna minor L</i>	Receiving waters	River Sava, Croatia. Prim/sec	Y (no EC)	WET	(Radić et al. 2010a)
	POD						(Radić et al. 2010a)

Protozoan toxicity								
<b>Cytotoxicity</b>	Growth inhibition, Protoxkit F™	<i>T. thermophila</i>	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)	
	Mortality, Microbiotest	<i>Spirostomum ambiguum</i>	Landfill leachates	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 <sub>3</sub> , coag, AC)	No	WET	(Kontana et al. 2009)	
				Mixed	Y	WET	(Clement et al. 1996)	
				Surface water	Rivers, Poland	No	WET	(Mankiewicz-Boczek et al. 2008)
Mortality, deformities		Wastewater	Prim + unspecified treatment, also deep well water	Y (TU)	WET	(Rojicková-Padrťová et al. 1998)		
<b>Genotoxicity</b>	Comet/SCGE	<i>T. thermophila</i>	Drinking water	Tap + charcoal filtration	No	SPE (XAD-4)	(Lah et al. 2005)	

<sup>1</sup>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 = fluorescein 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<sub>2</sub>LNGal-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** – glucocorticoid 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).

## REFERENCES

- Aerni, H. R., Kobler, B., Rutishauser, B. V., Wettstein, F. E., Fischer, R., Giger, W., Hungerbühler, A., Marazuela, M. D., Peter, A., Schonenberger, R., Vogeli, A. C., Suter, M. J. F. and Eggen, R. I. L. (2004). Combined biological and chemical assessment of estrogenic activities in wastewater treatment plant effluents. *Analytical and Bioanalytical Chemistry***378**(3): 688-696.
- Aguayo, S., Muñoz, M. J., de la Torre, A., Roset, J., de la Peña, E. and Carballo, M. (2004). Identification of organic compounds and ecotoxicological assessment of sewage treatment plants (STP) effluents. *Science of The Total Environment***328**(1-3): 69-81.
- Alam, M. Z., Ahmad, S., Malik, A. and Ahmad, M. (2010). Mutagenicity and genotoxicity of tannery effluents used for irrigation at Kanpur, India. *Ecotoxicology and Environmental Safety***73**(7): 1620-1628.
- Aleem, A. and Malik, A. (2005). Genotoxicity of the Yamuna River water at Okhla (Delhi), India. *Ecotoxicology and Environmental Safety***61**(3): 404-412.
- Allinson, G., Allinson, M., Salzman, S., Shiraishi, F., Myers, J., Theodoropoulos, T., Hermon, K. and Wightwick, A. (2007). Hormones in recycled water. Final report, DPI.
- Allinson, G., Allinson, M. and Shiraishi, F. (2010a). A pilot survey of the aryl hydrocarbon receptor activity of Victorian WWTP effluents. What's in our water, Canberra, Australia. 10-11 November, 2010.
- Allinson, G., Allinson, M. and Shiraishi, F. (2010b). A pilot survey of the retinoid activity of Victorian WWTP effluents using a retinoic acid activity recombinant yeast bioassay. What's in our water Canberra, Australia. 10-11 November.
- Allinson, G., Allinson, M., Shiraishi, F., Salzman, S. A., Myers, J. H., Hermon, K. M. and Theodoropoulos, T. (2008). Androgenic activity of effluent from forty-five municipal waste water treatment plants in Victoria, Australia. *Environmental Toxicology II*. A. Kungolos and M. Zamorano. Southampton, Wit Press. **110**: 293-304.
- Allinson, M., Shiraishi, F. and Allinson, G. (2009). A comparison of the hormonal activity of Victorian and Japanese rivers using a two-hybrid yeast assay. Intersect 09: Where Australian Chemistry meets Biosciences, Materials and Medicine. Melbourne, Australia. 1-2 October, 2009.
- Allinson, M., Shiraishi, F. and Allinson, G. (2010c). A pilot survey of the hormonal activity of the Yarra River using a suite of recombinant yeast bioassays. What's in our water Canberra, Australia. 10-11 November.
- Allinson, M., Shiraishi, F., Salzman, S. and Allinson, G. (2010d). In vitro and immunological assessment of the estrogenic activity and concentrations of 17 $\beta$ -estradiol, estrone, and ethinyl estradiol in treated effluent from 45 wastewater treatment plants in Victoria, Australia. *Archives of Environmental Contamination and Toxicology***58**(3): 576-586.
- Ames, B. N., McCann, J. and Yamasaki, E. (1975). Methods for detecting carcinogens and mutagens with the salmonella/mammalian-microsome mutagenicity test. *Mutation Research/Environmental Mutagenesis and Related Subjects***31**(6): 347-363.
- Aneck-Hahn, N. H., Bornman, M. S. and de Jager, C. (2008). Preliminary assessment of oestrogenic activity in water sources in Rietvlei Nature Reserve, Gauteng, South Africa. *African Journal of Aquatic Science***33**(3): 249-254.
- Aneck-Hahn, N. H., Bornman, M. S. and de Jager, C. (2009). Oestrogenic activity in drinking waters from a rural area in the Waterberg District, Limpopo Province, South Africa. *Water Sa***35**(3): 245-251.
- Ankley, G. T., Bennett, R. S., Erickson, R. J., Hoff, D. J., Hornung, M. W., Johnson, R. D., Mount, D. R., Nichols, J. W., Russom, C. L., Schmieder, P. K., Serrano, J. A., Tietge, J. E. and Villeneuve, D. L. (2010). Adverse outcome pathways: a conceptual framework to support ecotoxicology research and risk assessment. *Environmental Toxicology and Chemistry***29**(3): 730-741.
- Ansari, M. I. and Malik, A. (2009). Genotoxicity of wastewaters used for irrigation of food crops. *Environmental Toxicology***24**(2): 103-115.
- Antonelli, M., Mezzanotte, V. and Panouillères, M. (2009). Assessment of peracetic acid disinfected effluents by Microbiotests. *Environmental Science and Technology***43**(17): 6579-6584.
- Arana, I., Santorum, P., Muela, A. and Barcina, I. (1999). Effect of disinfection upon dissolved organic carbon (DOC) in wastewater: bacterial bioassays. *Letters in Applied Microbiology***31**(2): 157-162.
- Araujo, C. V. M., Nascimento, R. B., Oliveira, C. A., Strotmann, U. J. and da Silva, E. M. (2005). The use of Microtox® to assess toxicity removal of industrial effluents from the industrial district of Camacari (BA, Brazil). *Chemosphere***58**(9): 1277-1281.

- Arienzo, M., Christen, E. W. and Quayle, W. C. (2009). Phytotoxicity testing of winery wastewater for constructed wetland treatment. Journal of Hazardous Materials**169**(1-3): 94-99.
- Assloulj, J. E. L., Amahdar, L., Glouib, K., Kholtei, S., Paaza, N., Verschaeve, L. and Hilali, A. (2009). In vitro genotoxicity of wastewaters from the town of Settat, Morocco. Arhiv Za Higijenu Rada I Toksikologiju**60**(3): 289-296.
- Avishai, N., Rabinowitz, C., Moiseeva, E. and Rinkevich, B. (2002). Genotoxicity of the Kishon River, Israel: the application of an in vitro cellular assay. Mutation Research/Genetic Toxicology and Environmental Mutagenesis**518**(1): 21-37.
- Bakare, A. A., Okunola, A. A., Adetunji, O. A. and Jenmi, H. B. (2009). Genotoxicity assessment of a pharmaceutical effluent using four bioassays. Genetics and Molecular Biology**32**(2): 373-381.
- Bakopoulou, S., Emmanouil, C. and Kungolos, A. (2011). Assessment of wastewater effluent quality in Thessaly region, Greece, for determining its irrigation reuse potential. Ecotoxicology and Environmental Safety**74**(2): 188-194.
- Balaam, J. L., Chan-Man, Y., Roberts, P. H. and Thomas, K. V. (2009). Identification of nonregulated pollutants in north sea-produced water discharges. Environmental Toxicology and Chemistry**28**(6): 1159-1167.
- Balaguer, P., Francois, F., Comunale, F., Fenet, H., Boussioux, A. M., Pons, M., Nicolas, J. C. and Casellas, C. (1999). Reporter cell lines to study the estrogenic effects of xenoestrogens. Science of The Total Environment**233**(1-3): 47-56.
- Balsiger, H. A., de la Torre, R., Lee, W.-Y. and Cox, M. B. (2010). A four-hour yeast bioassay for the direct measure of estrogenic activity in wastewater without sample extraction, concentration, or sterilization. Science of The Total Environment**408**(6): 1422-1429.
- Bandelj, E., Van den Heuvel, M. R., Leusch, F. D. L., Shannon, N., Taylor, S. and McCarthy, L. H. (2006). Determination of the androgenic potency of whole effluents using mosquitofish and trout bioassays. Aquatic Toxicology**80**(3): 237-248.
- Banerjee, P., Talapatra, S. N., Mandal, N., Sundaram, G., Mukhopadhyay, A., Chattopadhyay, D. and Banerjee, S. K. (2008). Genotoxicity study with special reference to DNA damage by comet assay in fission yeast, *Schizosaccharomyces pombe* exposed to drinking water. Food and Chemical Toxicology**46**(1): 402-407.
- Barberio, A., Barros, L., Voltolini, J. C. and Mello, M. L. S. (2009). Evaluation of the cytotoxic and genotoxic potential of water from the River Paraiba do Sul, in Brazil, with the *Allium cepa* L. test. Brazilian Journal of Biology**69**(3): 837-842.
- Barreto-Rodrigues, M., Silva, F. T. and Paiva, T. C. B. (2009). Characterization of wastewater from the Brazilian TNT industry. Journal of Hazardous Materials**164**(1): 385-388.
- Basu, N., Ta, C. A., Waye, A., Mao, J. Q., Hewitt, M., Arnason, J. T. and Trudeau, V. L. (2009). Pulp and paper mill effluents contain neuroactive substances that potentially disrupt neuroendocrine control of fish reproduction. Environmental Science and Technology**43**(5): 1635-1641.
- Beck, I. C., Bruhn, R. and Gandrass, J. (2006a). Analysis of estrogenic activity in coastal surface waters of the Baltic Sea using the yeast estrogen screen. Chemosphere**63**(11): 1870-1878.
- Beck, I. C., Bruhn, R. and Gandrass, J. (2006b). Bioassay-directed fractionation for analyzing estrogens in surface waters of the German Baltic Sea. Acta Hydrochimica Et Hydrobiologica**34**(6): 560-567.
- Beckman Instruments Inc. (1980).
- Behera, B. K. and Misra, B. N. (1982). Analysis of the effect of industrial effluent on growth and development of rice seedlings. Environmental Research**28**(1): 10-20.
- Behnisch, P. A., Hosoe, K. and Sakai, S.-i. (2001). Bioanalytical screening methods for dioxins and dioxin-like compounds -- a review of bioassay/biomarker technology. Environment International**27**(5): 413-439.
- Bengtson Nash, S. M., Goddard, J. and Muller, J. F. (2006). Phytotoxicity of surface waters of the Thames and Brisbane River Estuaries: A combined chemical analysis and bioassay approach for the comparison of two systems. Biosensors and Bioelectronics**21**(11): 2086-2093.
- Bengtson Nash, S. M., McMahon, K., Eaglesham, G. and Muller, J. F. (2005a). Application of a novel phytotoxicity assay for the detection of herbicides in Hervey Bay and the Great Sandy Straits. Marine Pollution Bulletin**51**(1-4): 351-360.
- Bengtson Nash, S. M., Quayle, P. A., Schreiber, U. and Muller, J. F. (2005b). The selection of a model microalgal species as biomaterial for a novel aquatic phytotoxicity assay. Aquatic Toxicology**72**(4): 315-326.
- Bengtson Nash, S. M., Schreiber, U., Ralph, P. J. and Muller, J. F. (2005c). The combined SPE : ToxY-PAM phytotoxicity assay; application and appraisal of a novel biomonitoring tool for the aquatic environment. Biosensors and Bioelectronics**20**(7): 1443-1451.

- Bertanza, G., Pedrazzani, R., Papa, M., Mazzoleni, G., Steimberg, N., Caimi, L., Montani, C. and Dilorenzo, D. (2010). Removal of BPA and NPnEOs from Secondary Effluents of Municipal WWTPs by Means of Ozonation. Ozone-Science and Engineering**32**(3): 204-208.
- Bianchi, J., Espindola, E. L. G. and Marin-Morales, M. A. (in press). Genotoxicity and mutagenicity of water samples from the Monjolinho River (Brazil) after receiving untreated effluents. Ecotoxicology and Environmental Safety.
- Bicchi, C., Schilirò, T., Pignata, C., Fea, E., Cordero, C., Canale, F. and Gilli, G. (2009). Analysis of environmental endocrine disrupting chemicals using the E-screen method and stir bar sorptive extraction in wastewater treatment plant effluents. Science of The Total Environment**407**(6): 1842-1851.
- Biscardi, D., Monarca, S., De Fusco, R., Senatore, F., Poli, P., Buschini, A., Rossi, C. and Zani, C. (2003). Evaluation of the migration of mutagens/carcinogens from PET bottles into mineral water by Tradescantia/micronuclei test, Comet assay on leukocytes and GC/MS. Science of The Total Environment**302**(1-3): 101-108.
- Björkblom, C., Salste, L., Katsiadaki, I., Wiklund, T. and Kronberg, L. (2008). Detection of estrogenic activity in municipal wastewater effluent using primary cell cultures from three-spined stickleback and chemical analysis. Chemosphere**73**(7): 1064-1070.
- Bjorseth, A., Carlberg, G. E. and Moller, M. (1979). Determination of halogenated organic-compounds and mutagenicity testing of spent bleach liquors. Science of The Total Environment**11**(2): 197-211.
- Blaauboer, B. J. (2002). The applicability of in vitro-derived data in hazard identification and characterisation of chemicals. Environmental Toxicology and Pharmacology**11**(3-4): 213-225.
- Blinova, I. (2000). The perspective of microbiotests application to surface water monitoring and effluent control in Estonia. Environmental Toxicology**15**(5): 385-389.
- Bortolotto, T., Bertoldo, J. B., da Silveira, F. Z., Defaveri, T. M., Silvano, J. and Pich, C. T. (2009). Evaluation of the toxic and genotoxic potential of landfill leachates using bioassays. Environmental Toxicology and Pharmacology**28**(2): 288-293.
- Brinkmann, C. and Eisentraeger, A. (2008). Completely automated short-term genotoxicity testing for the assessment of chemicals and characterisation of contaminated soils and waste waters. Environmental Science and Pollution Research**15**(3): 211-217.
- Brown, K. W. and Donnelly, K. C. (1984a). Mutagenic activity of runoff and leachate water from hazardous-waste land treatment. Environmental Pollution Series a-Ecological and Biological**35**(3): 229-246.
- Brown, K. W. and Donnelly, K. C. (1984b). Mutagenic activity of the liquid waste from the production of acetonitrile. Bulletin of Environmental Contamination and Toxicology**32**(6): 742-748.
- Buckley, J. A. (2010). Quantifying the antiestrogen activity of wastewater treatment plant effluent using the yeast estrogen screen. Environmental Toxicology and Chemistry**29**(1): 73-78.
- Burke, M. D. and Mayer, R. T. (1974). Ethoxyresorufin - direct fluorimetric assay of a microsomal o-dealkylation which is preferentially inducible by 3-methylcholanthrene. Drug Metabolism and Disposition**2**(6): 583-588.
- Buschini, A., Carboni, P., Frigerio, S., Furlini, M., Marabini, L., Monarca, S., Poli, P., Radice, S. and Rossi, C. (2004). Genotoxicity and cytotoxicity assessment in lake drinking water produced in a treatment plant. Mutagenesis**19**(5): 341-347.
- Buschini, A., Giordani, F., Pellacani, C., Rossi, C. and Poli, P. (2008). Cytotoxic and genotoxic potential of drinking water: A comparison between two different concentration methods. Water Research**42**(8-9): 1999-2006.
- Caffaro-Filho, R. A., Wagner, R., Umbuzeiro, G. A., Grossman, M. J. and Durrant, L. R. (2010). Identification of alpha-beta unsaturated aldehydes as sources of toxicity to activated sludge biomass in polyester manufacturing wastewater. Water Science and Technology**61**(9): 2317-2324.
- Caffetti, J. D., Mantovani, M. S., Pastori, M. C. and Fenocchio, A. S. (2008). First genotoxicity study of Parana river water from Argentina using cells from the clam *Corbicula fluminea* (*Veneroida Corbiculidae*) and Chinese hamster (*Cricetulus griseus Rodentia, Cricetidae*) K1 cells in the comet assay. Genetics and Molecular Biology**31**(2): 561-565.
- Campora, C. E., Hokama, Y., Tamaru, C. S., Anderson, B. and Vincent, D. (2010). Evaluating the risk of ciguatera fish poisoning from reef fish grown at marine aquaculture facilities in Hawai'i. Journal of the World Aquaculture Society**41**(1): 61-70.
- Cao, N., Miao, T., Li, K., Zhang, Y. and Yang, M. (2009a). Formation potentials of typical disinfection byproducts and changes of genotoxicity for chlorinated tertiary effluent pretreated by ozone. Journal of Environmental Sciences**21**(4): 409-413.

- Cao, N., Yang, M., Zhang, Y., Hu, J., Ike, M., Hirotsuji, J., Matsui, H., Inoue, D. and Sei, K. (2009b). Evaluation of wastewater reclamation technologies based on in vitro and in vivo bioassays. Science of The Total Environment**407**(5): 1588-1597.
- Cargouet, M., Perdiz, D., Mouatassim-Souali, A., Tamisier-Karolak, S. and Levi, Y. (2004). Assessment of river contamination by estrogenic compounds in Paris area (France). Science of The Total Environment**324**(1-3): 55-66.
- Carilta, R. and Marin-Morales, M. A. (2008). Induction of chromosome aberrations in the *Allium cepa* test system caused by the exposure of seeds to industrial effluents contaminated with azo dyes. Chemosphere**72**(5): 722-725.
- Carlberg, G. E., Gjos, N., Moller, M., Gustavsen, K. O., Tveten, G. and Renberg, L. (1980). Chemical characterization and mutagenicity testing of chlorinated trihydroxybenzenes identified in spent bleach liquors from a sulfite plant. Science of The Total Environment**15**(1): 3-15.
- Castillo, M., Alonso, M. C., Riu, J., Reinke, M., Kloter, G., Dizer, H., Fischer, B., Hansen, P. D. and Barcelo, D. (2001). Identification of cytotoxic compounds in European wastewaters during a field experiment. Analytica Chimica Acta**426**(2): 265-277.
- Catarino, J., Mendonca, E., Picado, A., Lanca, A., Silva, L. and de Pinho, M. N. (2009). Membrane-based treatment for tanning wastewaters. Canadian Journal of Civil Engineering**36**(2): 356-362.
- Ceretti, E., Zani, C., Zerbini, I., Guzzella, L., Scaglia, M., Berna, V., Donato, F., Monarca, S. and Feretti, D. (2010). Comparative assessment of genotoxicity of mineral water packed in polyethylene terephthalate (PET) and glass bottles. Water Research**44**(5): 1462-1470.
- Cetojevic-Simin, D., Svircev, Z. and Baltic, V. V. (2009). In vitro cytotoxicity of cyanobacteria from water ecosystems of Serbia. Journal of Buon**14**(2): 289-294.
- Chamorro, S., Hernández, V., Monsalvez, E., Becerra, J., Mondaca, M., Piña, B. and Vidal, G. (2010). Detection of estrogenic activity from kraft mill effluents by the yeast estrogen screen. Bulletin of Environmental Contamination and Toxicology**84**(2): 165-169.
- Chang, J. C., Taylor, P. B. and Leach, F. R. (1981). Use of the Microtox assay system for environmental-samples. Bulletin of Environmental Contamination and Toxicology**26**(2): 150-156.
- Chaparro, T. R., Botta, C. M. and Pires, E. C. (2010). Biodegradability and toxicity assessment of bleach plant effluents treated anaerobically. Water Science and Technology**62**(6): 1312-1319.
- Cheh, A. M., Skochdopole, J., Koski, P. and Cole, L. (1980). Non-volatile mutagens in drinking-water - production by chlorination and destruction by sulfite. Science**207**(4426): 90-92.
- Clement, B., Persoone, G., Janssen, C. and Le Du-Delepierre, A. (1996). Estimation of the hazard of landfills through toxicity testing of leachates. 1. Determination of leachate toxicity with a battery of acute tests. Chemosphere**33**(11): 2303-2320.
- Coleman, H. M., Khan, S. J., Watkins, G. and Stuetz, R. M. (2008). Fate and analysis of endocrine disrupting chemicals in some sewage treatment plants in Australia. Water Science and Technology**58**(11): 2187-2194.
- Coleman, H. M., Troester, M., Khan, S. J., McDonald, J. A., Watkins, G. and Stuetz, R. M. (2009). Assessment of trace organic chemical removal by a membrane bioreactor using gas chromatography/mass spectrometry and a yeast screen bioassay. Environmental Toxicology and Chemistry**28**(12): 2537-2545.
- Collins, F., Gray, G. N. and Bucher, J. R. (2008). Transforming environmental health protection. Science**319**: 906-907.
- Conroy, O., Sáez, A. E., Quanrud, D., Ela, W. and Arnold, R. G. (2007). Changes in estrogen/anti-estrogen activities in ponded secondary effluent. Science of The Total Environment**382**(2-3): 311-323.
- Creusot, N., Kinani, S., Balaguer, P., Tapie, N., LeMenach, K., Maillot-Marechal, E., Porcher, J. M., Budzinski, H. and Ait-Aissa, S. (2010). Evaluation of an hPXR reporter gene assay for the detection of aquatic emerging pollutants: screening of chemicals and application to water samples. Analytical and Bioanalytical Chemistry**396**(2): 569-583.
- Czyz, A., Kowalska, W. and Wegrzyn, G. (2003). *Vibrio harveyi* mutagenicity assay as a preliminary test for detection of mutagenic pollution of marine water. Bulletin of Environmental Contamination and Toxicology**70**(6): 1065-1070.
- da Silva Pereira, T., Rocha, J. A. V., Duccatti, A., Silveira, G. A., Pastoriza, T. F., Bringuenti, L. and Vargas, V. M. F. (2007). Evaluation of mutagenic activity in supply water at three sites in the state of Rio Grande do Sul, Brazil. Mutation Research/Genetic Toxicology and Environmental Mutagenesis**629**(2): 71-80.
- Dagnino, S., Gomez, E., Picot, B., Cavailles, V., Casellas, C., Balaguer, P. and Fenet, H. (2010). Estrogenic and AhR activities in dissolved phase and suspended solids from wastewater treatment plants. Science of The Total Environment**408**(12): 2608-2615.

- Delgado, L. F., Faucet-Marquis, V., Pfohl-Leszkowicz, A., Dorandeu, C., Marion, B., Schetrite, S. and Albasi, C. (in press). Cytotoxicity micropollutant removal in a crossflow membrane bioreactor. Bioresource Technology.
- Desbrow, C., Routledge, E. J., Brighty, G. C., Sumpter, J. P. and Waldock, M. (1998). Identification of estrogenic chemicals in STW effluent. 1. Chemical fractionation and in vitro biological screening. Environmental Science and Technology**32**(11): 1549-1558.
- Dhouib, A., Aloui, F., Hamad, N. and Sayadi, S. (2006). Pilot-plant treatment of olive mill wastewaters by *Phanerochaete chrysosporium* coupled to anaerobic digestion and ultrafiltration. Process Biochemistry**41**(1): 159-167.
- Di Marzio, W. D., Saenz, M., Alberdi, J., Tortorelli, M. and Silvana, G. (2005). Risk assessment of domestic and industrial effluents unloaded into a freshwater environment. Ecotoxicology and Environmental Safety**61**(3): 380-391.
- Dizer, H., Wittekindt, E., Fischer, B. and Hansen, P. D. (2002). The cytotoxic and genotoxic potential of surface water and wastewater effluents as determined by bioluminescence, umu-assays and selected biomarkers. Chemosphere**46**(2): 225-233.
- Eggen, R. and Segner, H. (2003). The potential of mechanism-based bioanalytical tools in ecotoxicological exposure and effect assessment. Analytical and Bioanalytical Chemistry**377**(3): 386-396.
- Ellis, R. J., Van Den Heuvel, M. R., Bandelj, E., Smith, M. A., McCarthy, L. H., Stuthridge, T. R. and Dietrich, D. R. (2003). In vivo and in vitro assessment of the androgenic potential of a pulp and paper mill effluent. Environmental Toxicology and Chemistry**22**(7): 1448-1456.
- Ellman, G. L., Courtney, K. D., Andres jr, V. and Featherstone, R. M. (1961). A new and rapid colorimetric determination of acetylcholinesterase activity. Biochemical Pharmacology**7**(2): 88-90, IN81, 91-95.
- Ellouze, M., Saddoud, A., Dhouib, A. and Sayadi, S. (2009). Assessment of the impact of excessive chemical additions to municipal wastewaters and comparison of three technologies in the removal performance of pathogens and toxicity. Microbiological Research**164**(2): 138-148.
- Embry, M. R., Belanger, S. E., Braunbeck, T. A., Galay-Burgos, M., Halder, M., Hinton, D. E., Léonard, M. A., Lillicrap, A., Norberg-King, T. and Whale, G. (2010). The fish embryo toxicity test as an animal alternative method in hazard and risk assessment and scientific research. Aquatic Toxicology**97**(2): 79-87.
- Emmanuel, E., Perrodin, Y., Keck, G., Blanchard, J. M. and Vermande, P. (2005). Ecotoxicological risk assessment of hospital wastewater: a proposed framework for raw effluents discharging into urban sewer network. Journal of Hazardous Materials**117**(1): 1-11.
- Epler, J. L., Larimer, F. W., Rao, T. K., Nix, C. E. and Ho, T. (1978). Energy-related pollutants in the environment - use of short-term tests for mutagenicity in the isolation and identification of bio-hazards. Environmental Health Perspectives**27**(DEC): 11-20.
- Ergene, S., Celik, A., Cavas, T., Koleli, N. and Aymak, C. (2008). The evaluation of toxicity and mutagenicity of various drinking waters in the human blood lymphocytes (HULYs) in vitro. Food and Chemical Toxicology**46**(7): 2472-2475.
- Escher, B. I., Bramaz, N., Eggen, R. I. L. and Richter, M. (2005a). In-vitro assessment of modes of toxic action of pharmaceuticals in aquatic life. Environmental Science and Technology**39**: 3090-3100.
- Escher, B. I., Bramaz, N., Maurer, M., Richter, M., Sutter, D., von Kanel, C. and Zschokke, M. (2005b). Screening test battery for pharmaceuticals in urine and wastewater. Environmental Toxicology and Chemistry**24**(3): 750-758.
- Escher, B. I., Bramaz, N., Mueller, J. F., Quayle, P., Rutishauser, S. and Vermeirssen, E. L. M. (2008a). Toxic equivalent concentrations (TEQs) for baseline toxicity and specific modes of action as a tool to improve interpretation of ecotoxicity testing of environmental samples. Journal of Environmental Monitoring**10**(5): 612-621.
- Escher, B. I., Bramaz, N. and Ort, C. (2009). JEM Spotlight: Monitoring the treatment efficiency of a full scale ozonation on a sewage treatment plant with a mode-of-action based test battery. Journal of Environmental Monitoring**11**(10): 1836-1846.
- Escher, B. I., Bramaz, N., Quayle, P., Rutishauser, S. and Vermeirssen, E. L. M. (2008b). Monitoring of the ecotoxicological hazard potential by polar organic micropollutants in sewage treatment plants and surface waters using a mode-of-action based test battery. Journal of Environmental Monitoring**10**(5): 622-631.
- Escher, B. I. and Hermens, J. L. M. (2002). Modes of action in ecotoxicology: their role in body burdens, species sensitivity, QSARs, and mixture effects. Environmental Science and Technology**36**: 4201-4217.

- Escher, B. I., Lawrence, M. G., Macova, M., Mueller, J. C., Poussade, Y., Robillot, C., Roux, A. and Gernjak, A. (2011). Evaluation of contaminant removal of reverse osmosis and advanced oxidation in full-scale operation by combining passive sampling with chemical analysis and bioanalytical tools. Environmental Science and Technology, in press 10.1021/es201153k.
- Escher, B. I., Quayle, P., Muller, R., Schreiber, U. and Mueller, J. F. (2006). Passive sampling of herbicides combined with effect analysis in algae using a novel high-throughput phytotoxicity assay (Maxi-Imaging-PAM). Journal of Environmental Monitoring **8**(4): 456-464.
- Evandri, M. G., Tucci, P. and Bolle, P. (2000). Toxicological evaluation of commercial mineral water bottled in polyethylene terephthalate: a cytogenetic approach with *Allium cepa*. Food Additives and Contaminants **17**(12): 1037-1045.
- Fai, P. B. and Grant, A. (2010). An assessment of the potential of the microbial assay for risk assessment (MARA) for ecotoxicological testing. Ecotoxicology **19**(8): 1626-1633.
- Farmen, E., Harman, C., Hylland, K. and Tollefsen, K. E. (2010). Produced water extracts from North Sea oil production platforms result in cellular oxidative stress in a rainbow trout in vitro bioassay. Marine Pollution Bulletin **60**(7): 1092-1098.
- Farré, M., Klöter, G., Petrovic, M., Alonso, M. C., de Alda, M. J. L. and Barceló, D. (2002). Identification of toxic compounds in wastewater treatment plants during a field experiment. Analytica Chimica Acta **456**(1): 19-30.
- Fatima, R. A. and Ahmad, M. (2006). Genotoxicity of industrial wastewaters obtained from two different pollution sources in northern India: A comparison of three bioassays. Mutation Research/Genetic Toxicology and Environmental Mutagenesis **609**(1): 81-91.
- Filidei, S., Masciandaro, G. and Ceccanti, B. (2003). Anaerobic digestion of olive oil mill effluents: Evaluation of wastewater organic load and phytotoxicity reduction. Water Air and Soil Pollution **145**(1): 79-94.
- Furuichi, T., Kannan, K., Suzuki, K., Tanaka, S., Giesy, J. P. and Masunaga, S. (2006). Occurrence of estrogenic compounds in and removal by a swine farm waste treatment plant. Environmental Science and Technology **40**(24): 7896-7902.
- Gagné, F. and Blaise, C. (1998). Estrogenic properties of municipal and industrial wastewaters evaluated with a rapid and sensitive chemoluminescent in situ hybridization assay (CISH) in rainbow trout hepatocytes. Aquatic Toxicology **44**(1-2): 83-91.
- Gana, J. M., Ordonez, R., Zampini, C., Hidalgo, M., Meoni, S. and Isla, M. I. (2008). Industrial effluents and surface waters genotoxicity and mutagenicity evaluation of a river of Tucuman, Argentina. Journal of Hazardous Materials **155**(3): 403-406.
- García-Reyero, N., Grau, E., Castillo, M., De Alda, M. J. L., Barcelo, D. and Pina, B. (2001). Monitoring of endocrine disruptors in surface waters by the yeast recombinant assay. Environmental Toxicology and Chemistry **20**(6): 1152-1158.
- Gartiser, S., Hafner, C., Hercher, C., Kronenberger-Schafer, K. and Paschke, A. (2010). Whole effluent assessment of industrial wastewater for determination of bat compliance. Part I: paper manufacturing industry. Environmental Science and Pollution Research **17**(4): 856-865.
- Gartiser, S., Hafner, C., Oeking, S. and Paschke, A. (2009). Results of a "Whole Effluent Assessment" study from different industrial sectors in Germany according to OSPAR's WEA strategy. Journal of Environmental Monitoring **11**(2): 359-369.
- Gibb, S. (2008). A review of the National Research Council report: Toxicity testing in the 21st century: A vision and a strategy Reproductive Toxicology **25**(1): 136-138.
- Gong, Y. H., Chin, H. S., Lim, L. S. E., Loy, C. J., Obbard, J. P. and Yong, E. L. (2003). Clustering of sex hormone disruptors in Singapore's marine environment. Environmental Health Perspectives **111**(12): 1448-1453.
- Gouider, M., Feki, M. and Sayadi, S. (2010). Bioassay and use in irrigation of untreated and treated wastewaters from phosphate fertilizer industry. Ecotoxicology and Environmental Safety **73**(5): 932-938.
- Gruener, N. (1978). Mutagenicity of ozonated, recycled water. Bulletin of Environmental Contamination and Toxicology **20**(4): 522-526.
- Grung, M., Lichtenthaler, R., Ahel, M., Tollefsen, K.-E., Langford, K. and Thomas, K. V. (2007). Effects-directed analysis of organic toxicants in wastewater effluent from Zagreb, Croatia. Chemosphere **67**(1): 108-120.
- Guerra, R. (2001). Ecotoxicological and chemical evaluation of phenolic compounds in industrial effluents. Chemosphere **44**(8): 1737-1747.
- Günes, E. H., Günes, Y. and Tallin, I. (2008). Toxicity evaluation of industrial and land base sources in a river basin. Desalination **226**(1-3): 348-356.

- Gupta, P., Mathur, N., Bhatnagar, P., Nagar, P. and Srivastava, S. (2009). Genotoxicity evaluation of hospital wastewaters. Ecotoxicology and Environmental Safety**72**(7): 1925-1932.
- Gustavson, K. E., Sonsthagen, S. A., Crunkilton, R. A. and Harkin, J. M. (2000). Groundwater toxicity assessment using bioassay, chemical, and toxicity identification evaluation analyses. Environmental Toxicology**15**(5): 421-430.
- Gustavsson, L. and Engwall, M. (2006). Genotoxic activity of nitroarene-contaminated industrial sludge following large-scale treatment in aerated and non-aerated sacs. Science of The Total Environment**367**(2-3): 694-703.
- Gustavsson, L., Hollert, H., Jonsson, S., van Bavel, B. and Engwall, M. (2007). Reed beds receiving industrial sludge containing nitroaromatic compounds - effects of outgoing water and bed material extracts in the umu-C genotoxicity assay, DR-CALUX assay and on early life stage development in Zebrafish (*Danio rerio*). Environmental Science and Pollution Research**14**(3): 202-211.
- Guzzella, L., Di Caterino, F., Monarca, S., Zani, C., Feretti, D., Zerbini, I., Nardi, G., Buschini, A., Poli, P. and Rossi, C. (2006). Detection of mutagens in water-distribution systems after disinfection. Mutation Research/Genetic Toxicology and Environmental Mutagenesis**608**(1): 72-81.
- Guzzella, L., Monarca, S., Zani, C., Feretti, D., Zerbini, I., Buschini, A., Poli, P., Rossi, C. and Richardson, S. D. (2004). In vitro potential genotoxic effects of surface drinking water treated with chlorine and alternative disinfectants. Mutation Research/Genetic Toxicology and Environmental Mutagenesis**564**(2): 179-193.
- GWRC (2006). *In vitro* assays to detect estrogenicity in environmental waters, Global Water Research Coalition (GWRC).
- Hamers, T., Molin, K. R. J., Koeman, J. H. and Murk, A. J. (2000). A small-volume bioassay for quantification of the esterase inhibiting potency of mixtures of organophosphate and carbamate insecticides in rainwater: Development and optimization. Toxicological Sciences**58**(1): 60-67.
- Hao, O. J., Shin, C. J., Lin, C. F., Jeng, F. T. and Chen, Z. C. (1996). Use of microtox tests for screening industrial wastewater toxicity. Water Science and Technology**34**(10): 43-50.
- Harder, A., Escher, B. I., Landini, P., Tobler, N. B. and Schwarzenbach, R. P. (2003). Evaluation of bioanalytical tools for toxicity assessment and mode of toxic action classification of reactive chemicals. Environ. Sci. Technol.**37**: 4962-4970.
- Hartung, T. (2010). lessons learned from alternative methods and their validation for a new toxicology in the 21st century. Journal of Toxicology and Environmental Health, Part B**13**(2): 277-290.
- Heresztyn, T. and Nicholson, B. C. (2001). Determination of cyanobacterial hepatotoxins directly in water using a protein phosphatase inhibition assay. Water Research**35**(13): 3049-3056.
- Ho, L., Hoefel, D., Palazot, S., Sawade, E., Newcombe, G., Saint, C. P. and Brookes, J. D. (2010). Investigations into the biodegradation of microcystin-LR in wastewaters. Journal of Hazardous Materials**180**(1-3): 628-633.
- Ho, L. N., Gaudieux, A. L., Fanok, S., Newcombe, G. and Humpage, A. R. (2007). Bacterial degradation of microcystin toxins in drinking water eliminates their toxicity. Toxicon**50**(3): 438-441.
- Hollert, H., Durr, M., Holtey-Weber, R., Islinger, M., Brack, W., Farber, H., Erdinger, L. and Braunbeck, T. (2005). Endocrine disruption of water and sediment extracts in a non-radioactive dot blot/RNase protection-assay using isolated hepatocytes of rainbow trout - Deficiencies between bioanalytical effectiveness and chemically determined concentrations and how to explain them. Environmental Science and Pollution Research**12**(6): 347-360.
- Holmes, M., Kumar, A., Shareef, A., Doan, H., Stuetz, R. and Kookana, R. (2010). Fate of indicator endocrine disrupting chemicals in sewage during treatment and polishing for non-potable reuse. Water Science and Technology**62**(6): 1416-1423.
- Huggett, D. B., Foran, C. M., Brooks, B. W., Weston, J., Peterson, B., Marsh, K. E., La Point, T. W. and Schlenk, D. (2003). Comparison of in vitro and in vivo bioassays for estrogenicity in effluent from North American municipal wastewater facilities. Toxicological Sciences**72**(1): 77-83.
- Humpage, A. R., Ledreux, A., Fanok, S., Bernard, C., Briand, J. F., Eaglesham, G., Papageorgiou, J., Nicholson, B. and Steffensen, D. (2007). Application of the neuroblastoma assay for paralytic shellfish poisons to neurotoxic freshwater cyanobacteria: Interlaboratory calibration and comparison with other methods of analysis. Environmental Toxicology and Chemistry**26**(7): 1512-1519.
- Humpage, A. R., Magalhaes, V. F. and Froscio, S. M. (2010). Comparison of analytical tools and biological assays for detection of paralytic shellfish poisoning toxins. Analytical and Bioanalytical Chemistry**397**(5): 1655-1671.
- Hurst, M. R., Chan-Man, Y. L., Balaam, J., Thain, J. E. and Thomas, K. V. (2005). The stable aryl hydrocarbon receptor agonist potency of United Kingdom Continental Shelf (UKCS) offshore produced water effluents. Marine Pollution Bulletin**50**(12): 1694-1698.

- Huuskonen, S., Koponen, K., Ritola, O., Hahn, M. and Lindstrom-Seppa, P. (1998). Induction of CYP1A and porphyrin accumulation in fish hepatoma cells (PLHC-1) exposed to sediment or water from a PCB-contaminated lake (Lake Kernaala, Finland). Marine Environmental Research**46**(1-5): 379-384.
- Inoue, D., Matsui, H., Sei, K., Hu, J., Yang, M., Aragane, J., Hirotsuji, J. and Ike, M. (2009a). Evaluation of effectiveness of chemical and physical sewage treatment technologies for removal of retinoic acid receptor agonistic activity detected in sewage effluent. Water Science and Technology**59**(12): 2447-2453.
- Inoue, D., Nakama, K., Matsui, H., Sei, K. and Ike, M. (2009b). Detection of agonistic activities against five human nuclear receptors in river environments of Japan using a yeast two-hybrid assay. Bulletin of Environmental Contamination and Toxicology**82**(4): 399-404.
- Inoue, D., Nakama, K., Sawada, K., Watanabe, T., Takagi, M., Sei, K., Yang, M., Hirotsuji, J., Hu, J., Nishikawa, J.-i., Nakanishi, T. and Ike, M. (2010). Contamination with retinoic acid receptor agonists in two rivers in the Kinki region of Japan. Water Research**44**(8): 2409-2418.
- Isidori, M., Lavorgna, M., Nardelli, A. and Parrella, A. (2003). Toxicity identification evaluation of leachates from municipal solid waste landfills: a multispecies approach. Chemosphere**52**(1): 85-94.
- Isidori, M., Lavorgna, M., Palumbo, M., Piccioli, V. and Parrella, A. (2007). Influence of alkylphenols and trace elements in toxic, genotoxic, and endocrine disruption activity of wastewater treatment plants. Environmental Toxicology and Chemistry**26**(8): 1686-1694.
- Jadhav, J. P., Kalyani, D. C., Telke, A. A., Phugare, S. S. and Govindwar, S. P. (2010). Evaluation of the efficacy of a bacterial consortium for the removal of color, reduction of heavy metals, and toxicity from textile dye effluent. Bioresource Technology**101**(1): 165-173.
- Jobling, S., Nolan, M., Tyler, C. R., Brighty, G. and Sumpter, J. P. (1998). Widespread sexual disruption in wild fish. Environmental Science and Technology**32**(17): 2498-2506.
- Jolibois, B., Guerbet, M. and Vassal, S. (2003). Detection of hospital wastewater genotoxicity with the SOS chromotest and Ames fluctuation test. Chemosphere**51**(6): 539-543.
- Joung, K. E., Chung, Y. H. and Sheen, Y. Y. (2007). DRE-CALUX bioassay in comparison with HRGC/MS for measurement of toxic equivalence in environmental samples. Science of The Total Environment**372**(2-3): 657-667.
- Jugan, M. L., Oziol, L., Bimbot, M., Huteau, V., Tamisier-Karolak, S., Blondeau, J. P. and Lévi, Y. (2009). In vitro assessment of thyroid and estrogenic endocrine disruptors in wastewater treatment plants, rivers and drinking water supplies in the greater Paris area (France). Science of The Total Environment**407**(11): 3579-3587.
- Kaiser, C., Uhlig, S., Gerlach, T., Körner, M., Simon, K., Kunath, K., Florschütz, K., Baronian, K. and Kunze, G. (2010). Evaluation and validation of a novel *Arxula adenivorans* estrogen screen (nAES) assay and its application in analysis of wastewater, seawater, brackish water and urine. Science of The Total Environment**408**(23): 6017-6026.
- Källqvist, T., Milacic, R., Smital, T., Thomas, K. V., Vranes, S. and Tollefsen, K.-E. (2008). Chronic toxicity of the Sava River (SE Europe) sediments and river water to the algae *Pseudokirchneriella subcapitata*. Water Research**42**(8-9): 2146-2156.
- Kamata, R., Shiraishi, F., Nishikawa, J.-i., Yonemoto, J. and Shiraishi, H. (2008). Screening and detection of the in vitro agonistic activity of xenobiotics on the retinoic acid receptor. Toxicology in Vitro**22**(4): 1050-1061.
- Kargalioglu, Y., McMillan, B. J., Minear, R. A. and Plewa, M. J. (2002). Analysis of the cytotoxicity and mutagenicity of drinking water disinfection by-products in *Salmonella typhimurium*. Teratogenesis, Carcinogenesis, and Mutagenesis**22**(2): 113-128.
- Katsoyiannis, A. and Samara, C. (2007). Ecotoxicological evaluation of the wastewater treatment process of the sewage treatment plant of Thessaloniki, Greece. Journal of Hazardous Materials**141**(3): 614-621.
- Keenan, P. O., Knight, A. W., Billinton, N., Cahill, P. A., Dalrymple, I. M., Hawkyard, C. J., Stratton-Campbell, D. and Walmsley, R. M. (2007). Clear and present danger? The use of a yeast biosensor to monitor changes in the toxicity of industrial effluents subjected to oxidative colour removal treatments. Journal of Environmental Monitoring**9**(12): 1394-1401.
- Keiter, S., Rastall, A., Kosmehl, T., Wurm, K., Erdinger, L., Braunbeck, T. and Hollert, H. (2006). Ecotoxicological assessment of sediment, suspended matter and water samples in the upper Danube River - A pilot study in search for the causes for the decline of fish catches. Environmental Science and Pollution Research**13**(5): 308-319.
- Kerbrat, A.-S., Darius, H. T., Pauillac, S., Chinain, M. and Laurent, D. (2010). Detection of ciguatoxin-like and paralysing toxins in *Trichodesmium spp.* from New Caledonia lagoon. Marine Pollution Bulletin**61**(7-12): 360-366.

- Khoufi, S., Aloui, F. and Sayadi, S. (2006). Treatment of olive oil mill wastewater by combined process electro-Fenton reaction and anaerobic digestion. Water Research**40**(10): 2007-2016.
- Kim, H.-s., Yamada, H. and Tsuno, H. (2007). The removal of estrogenic activity and control of brominated by-products during ozonation of secondary effluents. Water Research**41**(7): 1441-1446.
- Klee, N., Gustavsson, L., Kosmehl, T., Engwall, M., Erdinger, L., Braunbeck, T. and Hollert, H. (2004). Changes in toxicity and genotoxicity of industrial sewage sludge samples containing nitro- and amino-aromatic compounds following treatment in bioreactors with different oxygen regimes. Environmental Science and Pollution Research**11**(5): 313-320.
- Komilis, D. P., Karatzas, E. and Halvadakis, C. P. (2005). The effect of olive mill wastewater on seed germination after various pretreatment techniques. Journal of Environmental Management**74**(4): 339-348.
- Kontana, A., Papadimitriou, C. A., Samaras, P., Zdragas, A. and Yiangou, M. (2008). Bioassays and biomarkers for ecotoxicological assessment of reclaimed municipal wastewater. Water Science and Technology**57**(6): 947-953.
- Kontana, A., Papadimitriou, C. A., Samaras, P., Zdragas, A. and Yiangou, M. (2009). Effectiveness of ozonation and chlorination on municipal wastewater treatment evaluated by a battery of bioassays and biomarkers. Water Science and Technology**60**(6): 1497-1505.
- Körner, W., Bolz, U., Sussmuth, W., Hiller, G., Schuller, W., Hanf, V. and Hagenmaier, H. (2000). Input/output balance of estrogenic active compounds in a major municipal sewage plant in Germany. Chemosphere**40**(9-11): 1131-1142.
- Körner, W., Hanf, V., Schuller, W., Kempter, C., Metzger, J. and Hagenmaier, H. (1999). Development of a sensitive E-screen assay for quantitative analysis of estrogenic activity in municipal sewage plant effluents. The Science of The Total Environment**225**(1-2): 33-48.
- Körner, W., Spengler, P., Bolz, U., Schuller, W., Hanf, V. and Metzger, J. W. (2001). Substances with estrogenic activity in effluents of sewage treatment plants in southwestern Germany. 2. Biological analysis. Environmental Toxicology and Chemistry**20**(10): 2142-2151.
- Krishnamurthi, K., Devi, S. S., Hengstler, J. G., Hermes, M., Kumar, K., Dutta, D., Vannan, S. M., Subin, T. S., Yadav, R. R. and Chakrabarti, T. (2008). Genotoxicity of sludges, wastewater and effluents from three different industries. Archives of Toxicology**82**(12): 965-971.
- Kumar, A., Arienzo, M., Quayle, W., Christen, E. G., Grocke, S., Fattore, A., Doan, H., Gonzago, D., Zandonna, R., Bartrop, K., Smith, L., Correll, R. and Kookana, R. (2009). Developing a systematic approach to winery wastewater management. Final report to Grape and Wine Research and Development Corporation. Project number: CSL05/02, CSIRO.
- Kurelec, B., Matijasevic, Z., Rijavec, M., Alacevic, M., Britvic, S., Muller, W. E. G. and Zahn, R. K. (1979). Induction of benzo (a) pyrene mono-oxygenase in fish and the salmonella test as a tool for detecting mutagenic-carcinogenic xenobiotics in the aquatic environment. Bulletin of Environmental Contamination and Toxicology**21**(6): 799-807.
- Kwon, J. H., Lee, H. K., Kwon, J. W., Kim, K., Park, E., Kang, M. H. and Kim, Y. H. (2008). Mutagenic activity of river water from a river near textile industrial complex in Korea. Environmental Monitoring and Assessment**142**(1-3): 289-296.
- Lah, B., Zinko, B., Narat, M. and Marinsek-Logar, R. (2005). Monitoring of genotoxicity in drinking water using in vitro comet assay and Ames test. Food Technology and Biotechnology**43**(2): 139-146.
- Laingam, S., Froschio, S. M. and Humpage, A. R. (2008). Flow-cytometric analysis of in vitro micronucleus formation: Comparative studies with WIL2-NS human lymphoblastoid and L5178Y mouse lymphoma cell lines. Mutation Research-Genetic Toxicology and Environmental Mutagenesis**656**(1-2): 19-26.
- Langevin, R., Rasmussen, J. B., Slotterduk, H. and Blaise, C. (1992). Genotoxicity in water and sediment extracts from the St Lawrence river system, using the SOS chromotest. Water Research**26**(4): 419-429.
- Latif, M. and Licek, E. (2004). Toxicity assessment of wastewaters, river waters, and sediments in Austria using cost-effective microbiotests. Environmental Toxicology**19**(4): 302-309.
- Lavado, R., Loyo-Rosales, J. E., Floyd, E., Kolodziej, E. P., Snyder, S. A., Sedlak, D. L. and Schlenk, D. (2009). Site-specific profiles of estrogenic activity in agricultural areas of California's inland waters. Environmental Science and Technology**43**(24): 9110-9116.
- Lee, J., Lee, B. C., Ra, J. S., Cho, J., Kim, I. S., Chang, N. I., Kim, H. K. and Kim, S. D. (2008). Comparison of the removal efficiency of endocrine disrupting compounds in pilot scale sewage treatment processes. Chemosphere**71**(8): 1582-1592.

- Leme, D. M., de Angelis, D. D. and Marin-Morales, M. A. (2008). Action mechanisms of petroleum hydrocarbons present in waters impacted by an oil spill on the genetic material of *Allium cepa* root cells. *Aquatic Toxicology***88**(4): 214-219.
- Lemos, A. O., Oliveira, N. C. D. and Lemos, C. T. (in press). In vitro micronuclei tests to evaluate the genotoxicity of surface water under the influence of tanneries. *Toxicology in Vitro*.
- Lemos, A. T., Rosa, D. P., Rocha, J. A. V. and Vargas, V. M. F. (2009). Mutagenicity assessment in a river basin influenced by agricultural, urban and industrial sources. *Ecotoxicology and Environmental Safety***72**(8): 2058-2065.
- Leusch, F. D. L., Chapman, H. F., Korner, W., Gooneratne, S. R. and Tremblay, L. A. (2005). Efficacy of an advanced sewage treatment plant in southeast Queensland, Australia, to remove estrogenic chemicals. *Environmental Science and Technology***39**(15): 5781-5786.
- Leusch, F. D. L., Chapman, H. F., van den Heuvel, M. R., Tan, B. L. L., Gooneratne, S. R. and Tremblay, L. A. (2006a). Bioassay-derived androgenic and estrogenic activity in municipal sewage in Australia and New Zealand. *Ecotoxicology and Environmental Safety***65**(3): 403-411.
- Leusch, F. D. L., De Jager, C., Levi, Y., Lim, R., Puijker, L., Sacher, F., Tremblay, L. A., Wilson, V. S. and Chapman, H. F. (2010). Comparison of five in vitro bioassays to measure estrogenic activity in environmental waters. *Environmental Science and Technology***44**(10): 3853-3860.
- Leusch, F. D. L., van den Heuvel, M. R., Chapman, H. F., Gooneratne, S. R., Eriksson, A. M. E. and Tremblay, L. A. (2006b). Development of methods for extraction and in vitro quantification of estrogenic and androgenic activity of wastewater samples. *Comparative Biochemistry and Physiology C-Toxicology and Pharmacology***143**(1): 117-126.
- Li, J., Ma, M., Cui, Q. and Wang, Z. J. (2008a). Assessing the potential risk of oil-field produced waters using a battery of bioassays/biomarkers. *Bulletin of Environmental Contamination and Toxicology***80**(6): 492-496.
- Li, J., Wang, Z., Ma, M. and Peng, X. (2010a). Analysis of environmental endocrine disrupting activities using recombinant yeast assay in wastewater treatment plant effluents. *Bulletin of Environmental Contamination and Toxicology***84**(5): 529-535.
- Li, N., Wang, D. H., Zhou, Y. Q., Ma, M., Li, J. A. and Wang, Z. J. (2010b). Dibutyl phthalate contributes to the thyroid receptor antagonistic activity in drinking water processes. *Environmental Science and Technology***44**(17): 6863-6868.
- Li, X. M., Luo, F. N., Liu, G. X. and Zhu, P. T. (2008b). Bioassay of estrogenic activity of effluent and influent in a farm wastewater treatment plant using an in vitro recombinant assay with yeast cells. *Biomedical and Environmental Sciences***21**(5): 381-388.
- Liu, Z.-h., Ito, M., Kanjo, Y. and Yamamoto, A. (2009). Profile and removal of endocrine disrupting chemicals by using an ER/AR competitive ligand binding assay and chemical analyses. *Journal of Environmental Sciences***21**(7): 900-906.
- Liviak, D., Wagner, E. D., Mitch, W. A., Altonji, M. J. and Plewa, M. J. (2010). Genotoxicity of water concentrates from recreational pools after various disinfection methods. *Environmental Science and Technology***44**(9): 3527-3532.
- Lundstrom, E., Adolfsson-Erici, M., Alsberg, T., Bjorlenius, B., Eklund, B., Laven, M. and Breitholtz, M. (2010). Characterization of additional sewage treatment technologies: Ecotoxicological effects and levels of selected pharmaceuticals, hormones and endocrine disruptors. *Ecotoxicology and Environmental Safety***73**(7): 1612-1619.
- Ma, M., Li, J. and Wang, Z. J. (2005). Assessing the detoxication efficiencies of wastewater treatment processes using a battery of bioassays/biomarkers. *Archives of Environmental Contamination and Toxicology***49**(4): 480-487.
- Ma, M., Rao, K. F. and Wang, Z. J. (2007). Occurrence of estrogenic effects in sewage and industrial wastewaters in Beijing, China. *Environmental Pollution***147**(2): 331-336.
- Macova, M., Escher, B. I., Reungoat, J., Carswell, S., Chue, K. L., Keller, J. and Mueller, J. F. (2010). Monitoring the biological activity of micropollutants during advanced wastewater treatment with ozonation and activated carbon filtration. *Water Research***44**(2): 477-492.
- Macova, M., Escher, B., Mueller, J. and Toze, S. (2010). Bioanalytical Tools to Evaluate Micropollutants across the Seven Barriers of the Western Corridor Scheme Brisbane, Australia, Urban Water Security Research Alliance **Technical Report No 30**:  
<http://www.urbanwateralliance.org.au/publications/UWSRA-tr30.pdf>
- Macova, M., Toze, S., Hodgers, L., Mueller, J. F., Bartkow, M. E. and Escher, B. I. (2011). Bioanalytical tools for the evaluation of organic micropollutants during sewage treatment, water recycling and drinking water generation. *Water Research***45**: in press.

- Maffei, F., Buschini, A., Rossi, C., Poli, P., Forti, G. C. and Hrelia, P. (2005). Use of the comet test and micronucleus assay on human white blood cells for in vitro assessment of genotoxicity induced by different drinking water disinfection protocols. Environmental and Molecular Mutagenesis**46**(2): 116-125.
- Maffei, F., Carbone, F., Forti, G. C., Buschini, A., Poli, P., Rossi, C., Marabini, L., Radice, S., Chiesara, E. and Hrelia, P. (2009). Drinking water quality: An in vitro approach for the assessment of cytotoxic and genotoxic load in water sampled along distribution system. Environment International**35**(7): 1053-1061.
- Mahjoub, O., Leclercq, M., Bachelot, M., Casellas, C., Escande, A., Balaguer, P., Bahri, A., Gomez, E. and Fenet, H. (2009). Estrogen, aryl hydrocarbon and pregnane X receptors activities in reclaimed water and irrigated soils in Oued Souhil area (Nabeul, Tunisia). Desalination**246**(1-3): 425-434.
- Mankiewicz-Boczek, J., Nalecz-Jawecki, G., Drobniewska, A., Kaza, M., Sumorok, B., Izydorczyk, K., Zalewski, M. and Sawicki, J. (2008). Application of a microbiotests battery for complete toxicity assessment of rivers. Ecotoxicology and Environmental Safety**71**(3): 830-836.
- Manusadzianas, L., Balkelyte, L., Sadauskas, K., Blinova, I., Pöllumaa, L. and Kahru, A. (2003). Ecotoxicological study of Lithuanian and Estonian wastewaters: selection of the biotests, and correspondence between toxicity and chemical-based indices. Aquatic Toxicology**63**(1): 27-41.
- Marabini, L., Frigerio, S., Chiesara, E., Maffei, F., Cantelli Forti, G., Hrelia, P., Buschini, A., Martino, A., Poli, P., Rossi, C. and Radice, S. (2007). In vitro cytotoxicity and genotoxicity of chlorinated drinking waters sampled along the distribution system of two municipal networks. Mutation Research/Genetic Toxicology and Environmental Mutagenesis**634**(1-2): 1-13.
- Marabini, L., Frigerio, S., Chiesara, E. and Radice, S. (2006). Toxicity evaluation of surface water treated with different disinfectants in HepG2 cells. Water Research**40**(2): 267-272.
- Martinovic, D., Denny, J. S., Schmieder, P. K., Ankley, G. T. and Sorensen, P. W. (2008). Temporal variation in the estrogenicity of a sewage treatment plant effluent and its biological significance. Environmental Science and Technology**42**(9): 3421-3427.
- Matsui, S., Takigami, H., Matsuda, T., Taniguchi, N., Adachi, J., Kawami, H. and Shimizu, Y. (2000). Estrogen and estrogen mimics contamination in water and the role of sewage treatment. Water Science and Technology**42**(12): 173-179.
- Matsuoka, S., Kikuchi, M., Kimura, S., Kurokawa, Y. and Kawai, S. (2005). Determination of estrogenic substances in the water of Muko River using in vitro assays, and the degradation of natural estrogens by aquatic bacteria. Journal of Health Science**51**(2): 178-184.
- Meier, J. R. and Bishop, D. F. (1985). Evaluation of conventional treatment processes for removal of mutagenic activity from municipal wastewaters. Journal (Water Pollution Control Federation)**57**(10): 999-1005.
- Mekki, A., Dhouib, A., Feki, F. and Sayadi, S. (2008). Assessment of toxicity of the untreated and treated olive mill wastewaters and soil irrigated by using microbiotests. Ecotoxicology and Environmental Safety**69**(3): 488-495.
- Mendonca, E., Picado, A., Paixao, S. M., Silva, L., Cunha, M. A., Leitao, S., Moura, I., Cortez, C. and Brito, F. (2009). Ecotoxicity tests in the environmental analysis of wastewater treatment plants: Case study in Portugal. Journal of Hazardous Materials**163**(2-3): 665-670.
- Merk, O. and Speit, G. (1999). Detection of crosslinks with the comet assay in relationship to genotoxicity and cytotoxicity. Environmental and Molecular Mutagenesis**33**(2): 167-172.
- Miege, C., Karolak, S., Gabet, V., Jugan, M. L., Oziol, L., Chevreuil, M., Levi, Y. and Coquery, M. (2009). Evaluation of estrogenic disrupting potency in aquatic environments and urban wastewaters by combining chemical and biological analysis. Trac-Trends in Analytical Chemistry**28**(2): 186-195.
- Mispagel, C., Allinson, G., Allinson, M., Shiraishi, F., Nishikawa, M. and Moore, M. R. (2009). Observations on the Estrogenic Activity and Concentration of 17 beta-Estradiol in the Discharges of 12 Wastewater Treatment Plants in Southern Australia. Archives of Environmental Contamination and Toxicology**56**(4): 631-637.
- Mispagel, C., Shiraishi, F., Allinson, M. and Allinson, G. (2005). Estrogenic activity of treated municipal effluent from seven sewage treatment plants in Victoria, Australia. Bulletin of Environmental Contamination and Toxicology**74**(5): 853-856.
- Misra, R. N. and Behera, P. K. (1991). The effect of paper-industry effluent on growth, pigments, carbohydrates and proteins of rice seedlings. Environmental Pollution**72**(2): 159-167.
- Mitteregger, H., da Silva, J., Arenzon, A., Portela, C. S., Ferreira, I. and Henriques, J. A. P. (2007). Evaluation of genotoxicity and toxicity of water and sediment samples from a Brazilian stream influenced by tannery industries. Chemosphere**67**(6): 1211-1217.

- Mnif, W., Dagnino, S., Escande, A., Pillon, A., Fenet, H., Gomez, E., Casellas, C., Duchesne, M.-J., Hernandez-Raquet, G., Cavallès, V., Balaguer, P. and Bartegi, A. (2010). Biological analysis of endocrine-disrupting compounds in Tunisian sewage treatment plants. Archives of Environmental Contamination and Toxicology**59**(1): 1-12.
- Muller, M., Rabenoelina, F., Balaguer, P., Patureau, D., Lemenach, K., Budzinski, H., Barcelo, D., De Alda, M. L., Kuster, M., Delgenes, J. P. and Hernandez-Raquet, G. (2008a). Chemical and biological analysis of endocrine-disrupting hormones and estrogenic activity in an advanced sewage treatment plant. Environmental Toxicology and Chemistry**27**(8): 1649-1658.
- Muller, R., Schreiber, U., Escher, B. I., Quayle, P., Nash, S. M. B. and Mueller, J. F. (2008b). Rapid exposure assessment of PSII herbicides in surface water using a novel chlorophyll a fluorescence imaging assay. Science of The Total Environment**401**(1-3): 51-59.
- Muller, R., Tang, J. Y. M., Thierb, R. and Mueller, J. F. (2007). Combining passive sampling and toxicity testing for evaluation of mixtures of polar organic chemicals in sewage treatment plant effluent. Journal of Environmental Monitoring**9**(1): 104-109.
- Murk, A. J., Legler, J., Denison, M. S., Giesy, J. P., vandeGuchte, C. and Brouwer, A. (1996). Chemical-activated luciferase gene expression (CALUX): A novel in vitro bioassay for Ah receptor active compounds in sediments and pore water. Fundamental and Applied Toxicology**33**(1): 149-160.
- Murk, A. J., Legler, J., van Lipzig, M. M. H., Meerman, J. H. N., Belfroid, A. C., Spenkeliink, A., van der Burg, B., Rijs, G. B. J. and Vethaak, D. (2002). Detection of estrogenic potency in wastewater and surface water with three in vitro bioassays. Environmental Toxicology and Chemistry**21**(1): 16-23.
- Nagy, S. R., Sanborn, J. R., Hammock, B. D. and Denison, M. S. (2002). Development of a green fluorescent protein-based cell Bioassay for the rapid and inexpensive detection and characterization of Ah receptor agonists. Toxicological Sciences**65**(2): 200-210.
- Nakajima, T., Hasegawa, H., Takanashi, H. and Ohki, A. (in press). Ecotoxicity of effluents from hydrothermal treatment process for low-rank coal. Fuel.
- Nestmann, E. R., Kowbel, D. J., Kamraa, O. P. and Douglas, G. R. (1984). reduction of mutagenicity of pulp and paper-mill effluent by secondary-treatment in an aerated lagoon. Hazardous Waste and Hazardous Materials**1**(1): 67-72.
- Nestmann, E. R., Lebel, G. L., Williams, D. T. and Kowbel, D. J. (1979). Mutagenicity of organic extracts from canadian drinking-water in the salmonella-mammalian-microsome assay. Environmental Mutagenesis**1**(4): 337-345.
- Nishikawa, J., Saito, K., Goto, J., Dakeyama, F., Matsuo, M. and Nishihara, T. (1999). New screening methods for chemicals with hormonal activities using interaction of nuclear hormone receptor with coactivator. Toxicology and Applied Pharmacology**154**(1): 76-83.
- Oda, Y., Nakamura, S.-i., Oki, I., Kato, T. and Shinagawa, H. (1985). Evaluation of the new system (umu-test) for the detection of environmental mutagens and carcinogens. Mutation Research/Environmental Mutagenesis and Related Subjects**147**(5): 219-229.
- Oh, S. M., Kim, H. R., Park, H. K., Choi, K., Ryu, J., Shin, H. S., Park, J. S., Lee, J. S. and Chung, K. H. (2009). Identification of estrogen-like effects and biologically active compounds in river water using bioassays and chemical analysis. Science of The Total Environment**407**(21): 5787-5794.
- Ohe, T., Suzuki, A., Watanabe, T., Hasei, T., Nukaya, H., Totsuka, Y. and Wakabayashi, K. (2009). Induction of SCEs in CHL cells by dichlorobiphenyl derivative water pollutants, 2-phenylbenzotriazole (PBTA) congeners and river water concentrates. Mutation Research/Genetic Toxicology and Environmental Mutagenesis**678**(1): 38-42.
- Ohtake, F., Takeyama, K., Matsumoto, T., Kitagawa, H., Yamamoto, Y., Nohara, K., Tohyama, C., Krust, A., Mimura, J., Chambon, P., Yanagisawa, J., Fujii-Kuriyama, Y. and Kato, S. (2003). Modulation of oestrogen receptor signalling by association with the activated dioxin receptor. Nature**423**(6939): 545-550.
- Oishi, K. and Moriuchi, A. (2010). Removal of dissolved estrogen in sewage effluents by [beta]-cyclodextrin polymer. Science of The Total Environment**409**(1): 112-115.
- Ostling, O. and Johanson, K. J. (1984). Microelectrophoretic study of radiation-induced dna damages in individual mammalian-cells. Biochemical and Biophysical Research Communications**123**(1): 291-298.
- Ostra, M., Beklova, M., Stoupalova, M. and Ostry, M. (2009). Ecotoxicity evaluation in municipal and food industry wastewaters. Fresenius Environmental Bulletin**18**(9A): 1674-1680.
- Paixão, S., Silva, L., Fernandes, A., O'Rourke, K., Mendonça, E. and Picado, A. (2008). Performance of a miniaturized algal bioassay in phytotoxicity screening. Ecotoxicology**17**(3): 165-171.

- Palma, P., Alvarenga, P., Palma, V., Matos, C., Fernandes, R. M., Soares, A. and Barbosa, I. R. (2010). Evaluation of surface water quality using an ecotoxicological approach: a case study of the Alqueva Reservoir (Portugal). Environmental Science and Pollution Research**17**(3): 703-716.
- Paustenbach, D. J. (2000). The practice of exposure assessment: A state-of-the-art review (reprinted from Principles and methods of toxicology, 4th edition, 2001). Journal of Toxicology and Environmental Health-Part B-Critical Reviews**3**(3): 179-291.
- Pawlowski, S., Ternes, T., Bonerz, M., Kluczka, T., van der Burg, B., Nau, H., Erdinger, L. and Braunbeck, T. (2003). Combined in situ and in vitro assessment of the estrogenic activity of sewage and surface water samples. Toxicological Sciences**75**(1): 57-65.
- Pawlowski, S., Ternes, T. A., Bonerz, M., Rastall, A. C., Erdinger, L. and Braunbeck, T. (2004). Estrogenicity of solid phase-extracted water samples from two municipal sewage treatment plant effluents and river Rhine water using the yeast estrogen screen. Toxicology in Vitro**18**(1): 129-138.
- Peixoto, F., Martins, F., Amaral, C., Gomes-Laranjo, J., Almeida, J. and Palmeira, C. M. (2008). Evaluation of olive oil mill wastewater toxicity on the mitochondrial bioenergetics after treatment with *Candida oleophila*. Ecotoxicology and Environmental Safety**70**(2): 266-275.
- Pellacani, C., Buschini, A., Furlini, M., Poli, P. and Rossi, C. (2006). A battery of in vivo and in vitro tests useful for genotoxic pollutant detection in surface waters. Aquatic Toxicology**77**(1): 1-10.
- Pelon, W., Whitman, B. F. and Beasley, T. W. (1977). Reversion of histidine-dependent mutant strains of *Salmonella-typhimurium* by Mississippi River water samples. Environmental Science and Technology**11**(6): 619-623.
- Pessala, P., Schultz, E., Nakari, T., Joutti, A. and Herve, S. (2004). Evaluation of wastewater effluents by small-scale biotests and a fractionation procedure. Ecotoxicology and Environmental Safety**59**(2): 263-272.
- Petala, M., Samaras, P., Kungolos, A., Zouboulis, A., Papadopoulos, A. and Sakellaropoulos, G. P. (2006a). The effect of coagulation on the toxicity and mutagenicity of reclaimed municipal effluents. Chemosphere**65**(6): 1007-1018.
- Petala, M., Samaras, P., Zouboulis, A., Kungolos, A. and Sakellaropoulos, G. P. (2008). Influence of ozonation on the in vitro mutagenic and toxic potential of secondary effluents. Water Research**42**(20): 4929-4940.
- Petala, M., Samaras, P., Zouboulis, A., Kungolos, A. and Salkellaropoulos, G. (2006b). Ecotoxicological properties of wastewater treated using tertiary methods. Environmental Toxicology**21**(4): 417-424.
- Pillon, A., Boussioux, A.-M., Escande, A., Ait-Aïssa, S., Gomez, E., Fenet, H., Ruff, M., Moras, D., Vignon, F., Duchesne, M.-J., Casellas, C., Nicolas, J.-C. and Balaguer, P. (2005). Binding of estrogenic compounds to recombinant estrogen receptor- $\alpha$ : application to environmental analysis. Environmental Health Perspectives**113**(3).
- Pinto, B., Garritano, S. and Reali, D. (2005). Occurrence of estrogen-like substances in the marine environment of the Northern Mediterranean Sea. Marine Pollution Bulletin**50**(12): 1681-1685.
- Pinto, B., Garritano, S. L., Cristofani, R., Ortaggi, G., Giuliano, A., Amodio-Cocchieri, R., Cirillo, T., De Giusti, M., Boccia, A. and Reali, D. (2008). Monitoring of polychlorinated biphenyl contamination and estrogenic activity in water, commercial feed and farmed seafood. Environmental Monitoring and Assessment**144**(1-3): 445-453.
- Pinto, B. and Reali, D. (2009). Screening of estrogen-like activity of mineral water stored in PET bottles. International Journal of Hygiene and Environmental Health**212**(2): 228-232.
- Plaza, G. A., Jangid, K., Lukasik, K., Nalecz-Jawecki, G., Berry, C. J. and Brigmon, R. L. (2008). Reduction of petroleum hydrocarbons and toxicity in refinery wastewater by bioremediation. Bulletin of Environmental Contamination and Toxicology**81**(4): 329-333.
- Plewa, M. J., Kargalioglu, Y., Vankerk, D., Minear, R. A. and Wagner, E. D. (2002). Mammalian cell cytotoxicity and genotoxicity analysis of drinking water disinfection by-products. Environmental and Molecular Mutagenesis**40**(2): 134-142.
- Plewa, M. J., Mueller, M. G., Richardson, S. D., Fasano, F., Buettner, K. M., Woo, Y.-T., McKague, A. B. and Wagner, E. D. (2008). Occurrence, synthesis, and mammalian cell cytotoxicity and genotoxicity of haloacetamides: An emerging class of nitrogenous drinking water disinfection byproducts. Environmental Science and Technology**42**(3): 955-961.
- Plewa, M. J., Wagner, E. D., Jazwierska, P., Richardson, S. D., Chen, P. H. and McKague, A. B. (2004a). Halonitromethane drinking water disinfection byproducts: Chemical characterization and mammalian cell cytotoxicity and genotoxicity. Environmental Science and Technology**38**(1): 62-68.
- Plewa, M. J., Wagner, E. D., Richardson, S. D., Thruston, A. D., Woo, Y.-T. and McKague, A. B. (2004b). Chemical and biological characterization of newly discovered iodoacid drinking water disinfection byproducts. Environmental Science and Technology**38**(18): 4713-4722.

- Podgorska, B., Pazdro, K., Pempkowlak, J. and Wegrzyn, G. (2007). The use of a novel *Vibrio harveyi* luminescence mutagenicity assay in testing marine water for the presence of mutagenic pollution. Marine Pollution Bulletin**54**(6): 808-814.
- Pool, E. J. and Magwebeba, T. U. (2009). The screening of river water for immunotoxicity using an in vitro whole blood culture assay. Water Air and Soil Pollution**200**(1-4): 25-31.
- Purdom, C. E., Hardiman, P. A., Bye, V. V. J., Eno, N. C., Tyler, C. R. and Sumpter, J. P. (1994). Estrogenic effects of effluents from sewage treatment works. Chemistry and Ecology**8**(4): 275 - 285.
- Quillardet, P., Huisman, O., Dari, R. and Hofnung, M. (1982). SOS chromotest, a direct assay of induction of an SOS function in *Escherichia-coli* K-12 to measure genotoxicity. Proceedings of the National Academy of Sciences of the United States of America-Biological Sciences**79**(19): 5971-5975.
- Ra, J. S., Kim, S. D., Chang, N. I. and An, K. G. (2007). Ecological health assessments based on whole effluent toxicity tests and the index of biological integrity in temperate streams influenced by wastewater treatment plant effluents. Environmental Toxicology and Chemistry**26**(9): 2010-2018.
- Radić, S., Stipanicev, D., Cvjetko, P., Mikelić, I., Rajčić, M., Širac, S., Pevalek-Kozlina, B. and Pavlica, M. (2010a). Ecotoxicological assessment of industrial effluent using duckweed (*Lemna minor* L.) as a test organism. Ecotoxicology**19**(1): 216-222.
- Radić, S., Stipanicev, D., Vujcic, V., Rajcic, M. M., Sirac, S. and Pevalek-Kozlina, B. (2010b). The evaluation of surface and wastewater genotoxicity using the *Allium cepa* test. Science of The Total Environment**408**(5): 1228-1233.
- Rand, G. (1995). Fundamentals of aquatic toxicology: Effects, environmental fate and risk assessment, Taylor and Francis, Washington DC.
- Rappaport, S. M., Richard, M. G., Hollstein, M. C. and Talcott, R. E. (1979). Mutagenic activity in organic wastewater concentrates. Environmental Science and Technology**13**(8): 957-961.
- Ravindran, P. N. and Ravindran, S. (1978). Cytological irregularities induced by water polluted with factory effluents - preliminary-report. Cytologia**43**(3-4): 565-568.
- Rawson, C. A., Tremblay, L. A., Warne, M. S. J., Ying, G. G., Kookana, R., Laginestra, E., Chapman, J. C. and Lim, R. P. (2009). Bioactivity of POPs and their effects in mosquitofish in Sydney Olympic Park, Australia. Science of The Total Environment**407**(12): 3721-3730.
- Reemtsma, T., Fiehn, O. and Jekel, M. (1999). A modified method for the analysis of organics in industrial wastewater as directed by their toxicity to *Vibrio fischeri*. Fresenius Journal of Analytical Chemistry**363**(8): 771-776.
- Reginatto, V., Amante, E. R., Gerhardy, K., Kunst, S. and Duran, N. (2009). Biodegradation and ecotoxicological assessment of pectin production wastewater. Journal of Environmental Sciences-China**21**(11): 1613-1619.
- Reifferscheid, G., Heil, J., Oda, Y. and Zahn, R. K. (1991). A microplate version of the SOS/umu-test for rapid detection of genotoxins and genotoxic potentials of environmental samples. Mutation Research/Environmental Mutagenesis and Related Subjects**253**(3): 215-222.
- Reifferscheid, G., Ziemann, C., Fieblinger, D., Dill, F., Gminski, R., Grummt, H. J., Hafner, C., Hollert, H., Kunz, S., Rodrigo, G., Stopper, H. and Selke, D. (2008). Measurement of genotoxicity in wastewater samples with the in vitro micronucleus test - Results of a round-robin study in the context of standardisation according to ISO. Mutation Research-Genetic Toxicology and Environmental Mutagenesis**649**(1-2): 15-27.
- Reineke, N., Bester, K., Huhnerfuss, H., Jastorff, B. and Weigel, S. (2002). Bioassay-directed chemical analysis of River Elbe surface water including large volume extractions and high performance fractionation. Chemosphere**47**(7): 717-723.
- Reungoat, J., Escher, B. I., Macova, M. and Keller, J. (2011). Biofiltration of wastewater treatment plant effluent: effective removal of pharmaceuticals and personal care products and reduction of toxicity. Water Research**45**: 2751-2762
- Reungoat, J., Macova, M., Escher, B. I., Carswell, S., Mueller, J. F. and Keller, J. (2010). Removal of micropollutants and reduction of biological activity in a full scale reclamation plant using ozonation and activated carbon filtration. Water Research**44**(2): 625-637.
- Richardson, S. D., DeMarini, D. M., Kogevinas, M., Fernandez, P., Marco, E., Lourencetti, C., Ballesté, C., Heederik, D., Meliefste, K., McKague, A. B., Marcos, R., Font-Ribera, L., Grimalt, J. O. and Villanueva, C. M. (2010). What's in the pool? a comprehensive identification of disinfection by-products and assessment of mutagenicity of chlorinated and brominated swimming pool water. Environmental Health Perspectives**118**(11).

- Richardson, S. D., Plewa, M. J., Wagner, E. D., Schoeny, R. and DeMarini, D. M. (2007). Occurrence, genotoxicity, and carcinogenicity of regulated and emerging disinfection by-products in drinking water: A review and roadmap for research. Mutation Research/Reviews in Mutation Research**636**(1-3): 178-242.
- Richter, M. and Escher, B. I. (2005). Mixture toxicity of reactive chemicals by using two bacterial growth assays as indicators of protein and DNA damage. Environmental Science and Technology**39**: 8753-8761.
- Rigonato, J., Mantovani, M. S. and Jordao, B. Q. (2010). Detection of genotoxicity of water from an urbanized stream, in *Corbicula fluminea* (mollusca) (in vivo) and CHO-K1 cells (in vitro) using comet assay. Archives of Environmental Contamination and Toxicology**59**(1): 31-38.
- Roda, A., Mirasoli, M., Michelini, E., Magliulo, M., Simoni, P., Guardigli, M., Curini, R., Sergi, M. and Marino, A. (2006). Analytical approach for monitoring endocrine-disrupting compounds in urban waste water treatment plants. Analytical and Bioanalytical Chemistry**385**(4): 742-752.
- Rodrigues, F. P., Angeli, J. P. F., Mantovani, M. S., Guedes, C. L. B. and Jordao, B. Q. (2010). Genotoxic evaluation of an industrial effluent from an oil refinery using plant and animal bioassays. Genetics and Molecular Biology**33**(1): 169-175.
- Rojíková-Padrťová, R., Marsálek, B. and Holoubek, I. (1998). Evaluation of alternative and standard toxicity assays for screening of environmental samples: Selection of an optimal test battery. Chemosphere**37**(3): 495-507.
- Romero, J., Ventura, F., Caixach, J. and Rivera, J. (1991). Genotoxic activity of ether insoluble organic-compounds in raw and treated water extracts. Chemosphere**22**(12): 1089-1101.
- Rosa, R., Moreira-Santos, M., Lopes, I., Silva, L., Rebola, J., Mendonca, E., Picado, A. and Ribeiro, R. (2010). Comparison of a test battery for assessing the toxicity of a bleached-kraft pulp mill effluent before and after secondary treatment implementation. Environmental Monitoring and Assessment**161**(1-4): 439-451.
- Routledge, E. J. and Sumpter, J. P. (1996). Estrogenic activity of surfactants and some of their degradation products assessed using a recombinant yeast screen. Environmental Toxicology and Chemistry**15**(3): 241-248.
- Rutishauser, B. V., Pesonen, M., Escher, B. I., Ackermann, G. E., Aerni, H. R., Suter, M. J. F. and Eggen, R. I. L. (2004). Comparative analysis of estrogenic activity in sewage treatment plant effluents involving three in vitro assays and chemical analysis of steroids. Environmental Toxicology and Chemistry**23**(4): 857-864.
- Rydberg, B. and Johanson, K. J. (1978). Estimation of DNA strandbreaks in single mammalian cells. DNA repair mechanisms. P. C. Hanawalt, E. C. Friedberg and C. F. Fox. New York, Academic Press: 465-468.
- Saddoud, A., Abdelkafi, S., Aloui, F. and Sayadi, S. (2010). A comparative study of the industrial discharges effect on the anaerobic treatment of domestic wastewater in both experimental and pilot-plant scales. Environmental Technology**31**(12): 1325-1333.
- Saddoud, A., Abdelkafi, S. and Sayadi, S. (2009). Effects of domestic wastewater toxicity on anaerobic membrane-bioreactor (MBR) performances. Environmental Technology**30**(13): 1361-1369.
- Saddoud, A., Ellouze, M., Dhouib, A. and Sayadi, S. (2007). Anaerobic membrane bioreactor treatment of domestic wastewater in Tunisia. Desalination**207**(1-3): 205-215.
- Salste, L., Leskinen, P., Virta, M. and Kronberg, L. (2007). Determination of estrogens and estrogenic activity in wastewater effluent by chemical analysis and the bioluminescent yeast assay. Science of The Total Environment**378**(3): 343-351.
- Sanchez, P. S., Sato, M. I. Z., Paschoal, C., Alves, M. N., Furlan, E. V. and Martins, M. T. (1988). Toxicity assessment of industrial effluents from S. Paulo State, Brazil, using short-term microbial assays. Toxicity Assessment**3**(1): 55-80.
- Sanfilippo, K., Pinto, B., Colombini, M. P., Bartolucci, U. and Reali, D. (2010). Determination of trace endocrine disruptors in ultrapure water for laboratory use by the yeast estrogen screen (YES) and chemical analysis (GC/MS). Journal of Chromatography B-Analytical Technologies in the Biomedical and Life Sciences**878**(15-16): 1190-1194.
- Santos, T. C. O., Maciel, L. F., Leal, K. S., Bender, A. E. N., Paiva, T. S., Garcias, G. L. and Martino-Roth, M. G. (2009). Mutagenic potential of water from Pelotas Creek in Rio Grande do Sul, Brazil. Genetics and Molecular Research**8**(3): 1057-1066.
- Sarmah, A. K., Northcott, G. L., Leusch, F. D. L. and Tremblay, L. A. (2006). A survey of endocrine disrupting chemicals (EDCs) in municipal sewage and animal waste effluents in the Waikato region of New Zealand. Science of The Total Environment**355**(1-3): 135-144.

- Sato, M., Takigami, H., Hayakawa, K. and Sakai, S. (2010). Water-quality monitoring technique for dioxins during dredging using on-site solid phase extraction with graphitic carbon and analysis with DR-CALUX. Journal of Environmental Science and Health Part a-Toxic/Hazardous Substances and Environmental Engineering**45**(7): 867-874.
- Saxena, J. and Schwartz, D. J. (1979). Mutagens in wastewaters renovated by advanced wastewater-treatment. Bulletin of Environmental Contamination and Toxicology**22**(3): 319-326.
- Schaeffer, D. J. and Kerster, H. W. (1985). Estimating the mass of mutagens in indeterminate mixtures. Ecotoxicology and Environmental Safety**10**(2): 190-196.
- Schilirò, T., Pignata, C., Fea, E. and Gilli, G. (2004). Toxicity and estrogenic activity of a wastewater treatment plant in northern Italy. Archives of Environmental Contamination and Toxicology**47**(4): 456-462.
- Schilirò, T., Pignata, C., Rovere, R., Fea, E. and Gilli, G. (2009). The endocrine disrupting activity of surface waters and of wastewater treatment plant effluents in relation to chlorination. Chemosphere**75**(3): 335-340.
- Schirmer, K., Dayeh, V. R., Bopp, S., Russold, S. and Bols, N. C. (2004). Applying whole water samples to cell bioassays for detecting dioxin-like compounds at contaminated sites. Toxicology**205**(3): 211-221.
- Schirmer, K., Tom, D. J., Bols, N. C. and Sherry, J. P. (2001). Ability of fractionated petroleum refinery effluent to elicit cyto- and photocytotoxic responses and to induce 7-ethoxyresorufin-O-deethylase activity in fish cell lines. Science of The Total Environment**271**(1-3): 61-78.
- Schmitt, M., Gellert, G. and Lichtenberg-Fraté, H. (2005). The toxic potential of an industrial effluent determined with the *Saccharomyces cerevisiae*-based assay. Water Research**39**(14): 3211-3218.
- Schnurstein, A. and Braunbeck, T. (2001). Tail moment versus tail length--application of an in vitro version of the comet assay in biomonitoring for genotoxicity in native surface waters using primary hepatocytes and gill cells from zebrafish (*Danio rerio*). Ecotoxicology and Environmental Safety**49**(2): 187-196.
- Schoff, P. K. and Ankley, G. T. (2002). Inhibition of retinoid activity by components of a paper mill effluent. Environmental Pollution**119**(1): 1-4.
- Schreiber, U., Muller, J. F., Haugg, A. and Gademann, R. (2002). New type of dual-channel PAM chlorophyll fluorometer for highly sensitive water toxicity biotests. Photosynthesis Research**74**(3): 317-330.
- Schreiber, U., Quayle, P., Schmidt, S., Escher, B. I. and Mueller, J. F. (2007). Methodology and evaluation of a highly sensitive algae toxicity test based on multiwell chlorophyll fluorescence imaging. Biosensors and Bioelectronics**22**(11): 2554-2563.
- Schwarzenbach, R. P., Escher, B. I., Fenner, K., Hofstetter, T. B., Johnson, C. A., von Gunten, U. and Wehrli, B. (2006). The challenge of micropollutants in aquatic systems. Science**313**(5790): 1072-1077.
- Schweigert, N., Eggen, R. I. L., Escher, B. I., Burkhardt-Holm, P. and Behra, R. (2002). Ecotoxicological assessment of surface waters: A modular approach integrating in vitro methods. Altex-Alternativen Zu Tierexperimenten**19**: 30-37.
- Shanthamurthy, K. B. and Rangaswamy, V. (1979). Cytological effects of paper-mills effluents on somatic-cells of *Allium-cepa*. Cytologia**44**(4): 921-926.
- Shappell, N. W. (2006). Estrogenic activity in the environment: Municipal wastewater effluent, river, ponds, and wetlands. Journal of Environmental Quality**35**(1): 122-132.
- Shappell, N. W., Billey, L. O., Forbes, D., Matheny, T. A., Poach, M. E., Reddy, G. B. and Hunt, P. G. (2007). Estrogenic activity and steroid hormones in swine wastewater through a lagoon constructed-wetland system. Environmental Science and Technology**41**(2): 444-450.
- Shappell, N. W., Elder, K. H. and West, M. (2010). Estrogenicity and nutrient concentration of surface waters surrounding a large confinement dairy operation using best management practices for land application of animal wastes. Environmental Science and Technology**44**(7): 2365-2371.
- Shaw, M., Negri, A., Fabricius, K. and Mueller, J. F. (2009). Predicting water toxicity: Pairing passive sampling with bioassays on the Great Barrier Reef. Aquatic Toxicology**95**(2): 108-116.
- Shi, W., Wang, X., Hu, G., Hao, Y., Zhang, X., Liu, H., Wei, S., Wang, X. and Yu, H. (2011). Bioanalytical and instrumental analysis of thyroid hormone disrupting compounds in water sources along the Yangtze River. Environmental Pollution**159**(2): 441-448.
- Shi, W., Wang, X. Y., Hu, W., Sun, H., Shen, O. X., Liu, H. L., Wang, X. R., Giesy, J. P., Cheng, S. P. and Yu, H. X. (2009a). Endocrine-disrupting equivalents in industrial effluents discharged into Yangtze River. Ecotoxicology**18**(6): 685-692.

- Shi, Y., Cao, X.-w., Tang, F., Du, H.-r., Wang, Y.-z., Qiu, X.-q., Yu, H.-p. and Lu, B. (2009b). In vitro toxicity of surface water disinfected by different sequential treatments. Water Research**43**(1): 218-228.
- Shiraishi, F., Okumura, T., Nomachi, M., Serizawa, S., Nishikawa, J., Edmonds, J. S., Shiraishi, H. and Morita, M. (2003). Estrogenic and thyroid hormone activity of a series of hydroxy-polychlorinated biphenyls. Chemosphere**52**(1): 33-42.
- Shue, M. F., Chen, F. A. and Chen, T. C. (2010). Total estrogenic activity and nonylphenol concentration in the Donggang River, Taiwan. Environmental Monitoring and Assessment**168**(1-4): 91-101.
- Shue, M. F., Chen, F. A., Kuo, Y. T. and Chen, T. C. (2009). Nonylphenol concentration and estrogenic activity in Kaoping River and its tributaries, Taiwan. Water Science and Technology**59**(10): 2055-2063.
- Simmon, V. F. and Tardiff, R. G. (1976). Mutagenic activity of drinking-water concentrates. Mutation Research**38**(6): 389-390.
- Singh, N. P., McCoy, M. T., Tice, R. R. and Schneider, E. L. (1988). A simple technique for quantitation of low levels of DNA damage in individual cells. Experimental Cell Research**175**(1): 184-191.
- Singhal, A. and Thakur, I. S. (2009). Decolourization and detoxification of pulp and paper mill effluent by *Emericella nidulans* var. *nidulans*. Journal of Hazardous Materials**171**(1-3): 619-625.
- Smith, B. S. (1981). Male characteristics on female mudsnails caused by antifouling bottom paints. Journal of Applied Toxicology**1**(1): 22-25.
- Snyder, S. A., Villeneuve, D. L., Snyder, E. M. and Giesy, J. P. (2001). Identification and quantification of estrogen receptor agonists in wastewater effluents. Environmental Science and Technology**35**(18): 3620-3625.
- Soto, A. M., Sonnenschein, C., Chung, K. L., Fernandez, M. F., Olea, N. and Serrano, F. O. (1995). The E-SCREEN assay as a tool to identify estrogens: an update on estrogenic environmental pollutants. Environmental Health Perspectives**103** (supplement 7): 113-122.
- Sousa, A., Schonenberger, R., Jonkers, N., Suter, M. J. F., Tanabe, S. and Barroso, C. M. (2010). Chemical and biological characterization of estrogenicity in effluents from WWTPs in Ria de Aveiro (NW Portugal). Archives of Environmental Contamination and Toxicology**58**(1): 1-8.
- Stalter, D., Magdeburg, A. and Oehlmann, J. (2010). Comparative toxicity assessment of ozone and activated carbon treated sewage effluents using an in vivo test battery. Water Research**44**(8): 2610-2620.
- Sun, Q. F., Deng, S. B., Huang, J., Shen, G. and Yu, G. (2008). Contributors to estrogenic activity in wastewater from a large wastewater treatment plant in Beijing, China. Environmental Toxicology and Pharmacology**25**(1): 20-26.
- Sundvall, A., Marklund, H. and Rannug, U. (1984). The mutagenicity on salmonella-typhimurium of nitrobenzoic acids and other wastewater components generated in the production of nitrobenzoic acids and nitrotoluenes. Mutation Research**137**(2-3): 71-78.
- Swain, A. P., Cooper, J. E. and Stedman, R. L. (1969). Large-scale fractionation of cigarette smoke condensate for chemical and biologic investigations. Cancer Research**29**(3): 579-and.
- Swart, J. C. and Pool, E. J. (2009). Development of a bio-assay for estrogens using estrogen receptor alpha gene expression by MCF7 cells as biomarker. Journal of Immunoassay and Immunochemistry**30**(2): 150-165.
- Swart, J. C., Pool, E. J. and van Wyk, J. H. (2011). The implementation of a battery of in vivo and in vitro bioassays to assess river water for estrogenic endocrine disrupting chemicals. Ecotoxicology and Environmental Safety**74**(1): 138-143.
- Takahashi, Y., Tojo, T., Nagahora, S. and Yamazaki, K. (2007). Direct determination of estrogenic and antiestrogenic activities using an enhanced plant two-hybrid system. Journal of Agricultural and Food Chemistry**55**(8): 2923-2929.
- Takanashi, H., Kishida, M., Nakajima, T., Ohki, A., Akiba, M. and Aizawa, T. (2009). Surveying the mutagenicity of tap water to elicit the effects of purification processes on Japanese tap water. Chemosphere**77**(3): 434-439.
- Takanashi, H., Urano, K., Hirata, M., Hano, T. and Ohgaki, S. (2001). Method for measuring mutagen formation potential (MFP) on chlorination as a new water quality index. Water Research**35**(7): 1627-1634.
- Tan, B. L. L., Hawker, D. W., Müller, J. F., Leusch, F. D. L., Tremblay, L. A. and Chapman, H. F. (2007). Comprehensive study of endocrine disrupting compounds using grab and passive sampling at selected wastewater treatment plants in South East Queensland, Australia. Environment International**33**(5): 654-669.

- Tarkpea, M., Andren, C., Eklund, B., Gravenfors, E. and Kukulska, Z. (1998). A biological and chemical characterization strategy for small and medium-sized industries connected to municipal sewage treatment plants. Environmental Toxicology and Chemistry**17**(2): 234-250.
- Terasaki, M., Shiraishi, F., Fukazawa, H. and Makino, M. (2009). Development and validation of chemical and biological analyses to determine the antiestrogenic potency of resin acids in paper mill effluents. Environmental Science and Technology**43**(24): 9300-9305.
- Thomas, K. V., Balaam, J., Hurst, M. R. and Thain, J. E. (2004). Identification of in vitro estrogen and androgen receptor agonists in North Sea offshore produced water discharges. Environmental Toxicology and Chemistry**23**(5): 1156-1163.
- Thomas, K. V., Langford, K., Petersen, K., Smith, A. J. and Tollefsen, K. E. (2009). Effect-directed identification of naphthenic acids as important in vitro xeno-estrogens and anti-androgens in North Sea offshore produced water discharges. Environmental Science and Technology**43**(21): 8066-8071.
- Thorpe, K. L., Gross-Sorokin, M., Johnson, I., Brighty, G. and Tyler, C. R. (2006). An assessment of the model of concentration addition for predicting the estrogenic activity of chemical mixtures in wastewater treatment works effluents. Environmental Health Perspectives**114**: 90-97.
- Tilton, F., Benson, W. H. and Schlenk, D. (2002). Evaluation of estrogenic activity from a municipal wastewater treatment plant with predominantly domestic input. Aquatic Toxicology**61**(3-4): 211-224.
- Timourian, H., Felton, J. S., Stuermer, D. H., Healy, S., Berry, P., Tompkins, M., Battaglia, G., Hatch, F. T., Thompson, L. H., Carrano, A. V., Minkler, J. and Salazar, E. (1982). Mutagenic and toxic activity of environmental effluents from underground coal-gasification experiments. Journal of Toxicology and Environmental Health**9**(5-6): 975-994.
- Tollefsen, K. E., Harman, C., Smith, A. and Thomas, K. V. (2007). Estrogen receptor (ER) agonists and androgen receptor (AR) antagonists in effluents from Norwegian North Sea oil production platforms. Marine Pollution Bulletin**54**(3): 277-283.
- Tsuno, H., Arakawa, K., Kato, Y. and Nagare, H. (2008). Advanced sewage treatment with ozone under excess sludge reduction, disinfection and removal of EDCs. Ozone-Science and Engineering**30**(3): 238-245.
- Urbatzka, R., van Cauwenberge, A., Maggioni, S., Vigano, L., Mandich, A., Benfenati, E., Lutz, I. and Kloas, W. (2007). Androgenic and antiandrogenic activities in water and sediment samples from the river Lambro, Italy, detected by yeast androgen screen and chemical analyses. Chemosphere**67**(6): 1080-1087.
- Valente-Campos, S., Dias, C. L., Barbour, E. D. A., de Souza Nascimento, E. and de Aragão Umbuzeiro, G. (2009). The introduction of the Salmonella/microsome mutagenicity assay in a groundwater monitoring program. Mutation Research/Genetic Toxicology and Environmental Mutagenesis**675**(1-2): 17-22.
- Vamvakaki, V. and Chaniotakis, N. A. (2007). Pesticide detection with a liposome-based nano-biosensor. Biosensors and Bioelectronics**22**(12): 2848-2853.
- Van der Linden, S. C., Heringa, M. B., Man, H. Y., Sonneveld, E., Puijker, L. M., Brouwer, A. and Van der Burg, B. (2008). Detection of multiple hormonal activities in wastewater effluents and surface water, using a panel of steroid receptor CALUX bioassays. Environmental Science and Technology**42**(15): 5814-5820.
- Vankreijl, C. F., Kool, H. J., Devries, M., Vankranen, H. J. and Degreef, E. (1980). Mutagenic activity in the rivers Rhine and Meuse in the Netherlands. Science of The Total Environment**15**(2): 137-147.
- Vanparys, C., Depiereux, S., Nadzialek, S., Robbens, J., Blust, R., Kestemont, P. and De Coen, W. (2010). Performance of the flow cytometric E-screen assay in screening estrogenicity of pure compounds and environmental samples. Science of The Total Environment**408**(20): 4451-4460.
- Vermeirssen, E. L., Hollender, J., Bramaz, N., Voet, J. v. d. and Escher, B. I. (2010). Linking toxicity in algal and bacterial assays with chemical analysis in passive samplers deployed in 21 treated sewage effluents. Environmental Toxicology and Chemistry: n/a-n/a.
- Vermeirssen, E. L. M., Bramaz, N., Hollender, J., Singer, H. and Escher, B. I. (2009). Passive sampling combined with ecotoxicological and chemical analysis of pharmaceuticals and biocides - evaluation of three Chemcatcher<sup>TM</sup> configurations. Water Research**43**: 903-914.
- Vermeirssen, E. L. M., Burki, R., Joris, C., Peter, A., Segner, H., Suter, M. J. F. and Burkhardt-Holm, P. (2005). Characterization of the estrogenicity of swiss midland rivers using a recombinant yeast bioassay and plasma vitellogenin concentrations in feral male brown trout. Environmental Toxicology and Chemistry**24**(9): 2226-2233.

- Vermeirssen, E. L. M., Suter, M. J. F. and Burkhardt-Holm, P. (2006). Estrogenicity patterns in the Swiss midland river Lutzelnmurg in relation to treated domestic sewage effluent discharges and hydrology. Environmental Toxicology and Chemistry**25**(9): 2413-2422.
- Wadhia, K. (2008). ISTA13 - International interlaboratory comparative evaluation of microbial assay for risk assessment (MARA). Environmental Toxicology**23**(5): 626-633.
- Wadhia, K., Dando, T. and Thompson, K. C. (2007). Intra-laboratory evaluation of Microbial Assay for Risk Assessment (MARA) for potential application in the implementation of the Water Framework Directive (WFD). Journal of Environmental Monitoring**9**(9): 953-958.
- Wagner, M. and Oehlmann, J. (2009). Endocrine disruptors in bottled mineral water: total estrogenic burden and migration from plastic bottles. Environmental Science and Pollution Research**16**(3): 278-286.
- Wang, L.-S., Hu, H.-Y. and Wang, C. (2007). Effect of ammonia nitrogen and dissolved organic matter fractions on the genotoxicity of wastewater effluent during chlorine disinfection. Environmental Science and Technology**41**(1): 160-165.
- Wang, X., Shi, W., Wu, J., Hao, Y., Hu, G., Liu, H., Han, X. and Yu, H. (2010). Reproductive toxicity of organic extracts from petrochemical plant effluents discharged to the Yangtze River, China. Journal of Environmental Sciences**22**(2): 297-303.
- Wang, X. J., Hayes, J. D. and Wolf, C. R. (2006). Generation of a stable antioxidant response element-driven reporter gene cell line and its use to show redox-dependent activation of Nrf2 by cancer chemotherapeutic agents. Cancer Research**66**(22): 10983-10994.
- Weston, J., Warren, C., Chaudhary, A., Emerson, B., Argote, K., Khan, S. and Willett, K. L. (2010). Use of bioassays and sediment polycyclic aromatic hydrocarbon concentrations to assess toxicity at coastal sites impacted by Hurricane Katrina. Environmental Toxicology and Chemistry**29**(7): 1409-1418.
- Williams, M., Woods, M., Kumar, A., Ying, G. G., Shareef, A. and Karkkainen, M. (2007). Endocrine disrupting chemicals in the Australian riverine environment: A pilot study on estrogenic compounds., CSIRO Land and Water Australia.
- Wolz, J., Engwall, M., Maletz, S., Takner, H. O., van Bavel, B., Kammann, U., Klempt, M., Weber, R., Braunbeck, T. and Hollert, H. (2008). Changes in toxicity and Ah receptor agonist activity of suspended particulate matter during flood events at the rivers Neckar and Rhine - a mass balance approach using in vitro methods and chemical analysis. Environmental Science and Pollution Research**15**(7): 536-553.
- Wood, S. A., Holland, P. T., Stirling, D. J., Briggs, L. R., Sprosen, J., Ruck, J. G. and Wear, R. G. (2006). Survey of cyanotoxins in New Zealand water bodies between 2001 and 2004. New Zealand Journal of Marine and Freshwater Research**40**(4): 585-597.
- Wu, Q.-Y., Hu, H.-Y., Zhao, X. and Li, Y. (2010a). Effects of chlorination on the properties of dissolved organic matter and its genotoxicity in secondary sewage effluent under two different ammonium concentrations. Chemosphere**80**(8): 941-946.
- Wu, Q.-Y., Hu, H.-Y., Zhao, X. and Sun, Y.-X. (2009). Effect of chlorination on the estrogenic/antiestrogenic activities of biologically treated wastewater. Environmental Science and Technology**43**(13): 4940-4945.
- Wu, Q.-Y., Li, Y., Hu, H.-Y., Sun, Y.-X. and Zhao, F.-Y. (2010b). Reduced effect of bromide on the genotoxicity in secondary effluent of a municipal wastewater treatment plant during chlorination. Environmental Science and Technology**44**(13): 4924-4929.
- Xie, S. H., Liu, A. L., Chen, Y. Y., Zhang, L., Zhang, H. J., Jin, B. X., Lu, W. H., Li, X. Y. and Lu, W. Q. (2010). DNA damage and oxidative stress in human liver cell I-02 caused by surface water extracts during drinking water treatment in a waterworks in China. Environmental and Molecular Mutagenesis**51**(3): 229-235.
- Ying, G. G., Rawson, C. A., Kookana, R. S., Peng, P. A., Warne, M. S. J., Tremblay, L. A., Laginestra, E., Chapman, J. C. and Lim, R. P. (2009). Contamination and screening level toxicity of sediments from remediated and unremediated wetlands near Sydney, Australia. Environmental Toxicology and Chemistry**28**(10): 2052-2060.
- Young, F. M., Micklem, J. and Humpage, A. R. (2008). Effects of blue-green algal toxin cylindrospermopsin (CYN) on human granulosa cells in vitro. Reproductive Toxicology**25**(3): 374-380.
- Yu, C. P. and Chu, K. H. (2009). Occurrence of pharmaceuticals and personal care products along the West Prong Little Pigeon River in east Tennessee, USA. Chemosphere**75**(10): 1281-1286.
- Zani, C., Feretti, D., Buschini, A., Poli, P., Rossi, C., Guzzella, L., Caterino, F. D. and Monarca, S. (2005). Toxicity and genotoxicity of surface water before and after various potabilization steps. Mutation Research/Genetic Toxicology and Environmental Mutagenesis**587**(1-2): 26-37.

- Zegura, B., Heath, E., Cernosa, A. and Filipic, M. (2009). Combination of in vitro bioassays for the determination of cytotoxic and genotoxic potential of wastewater, surface water and drinking water samples. Chemosphere**75**(11): 1453-1460.
- Zhang, G. C. and Wang, Z. S. (2000). Mechanism study of the coagulant impact on mutagenic activity in water. Water Research**34**(6): 1781-1790.
- Zhang, H., Yamada, H. and Tsuno, H. (2008). Removal of endocrine-disrupting chemicals during ozonation of municipal sewage with brominated byproducts control. Environmental Science and Technology**42**(9): 3375-3380.
- Zhang, H. Q., Yamada, H., Kim, S. E., Kim, H. S. and Tsuno, H. (2006). Removal of endocrine-disrupting chemicals by ozonation in sewage treatment. Water Science and Technology**54**(10): 123-132.
- Zhang, Z. D., Burch, P. E., Cooney, A. J., Lanz, R. B., Pereira, F. A., Wu, J. Q., Gibbs, R. A., Weinstock, G. and Wheeler, D. A. (2004). Genomic analysis of the nuclear receptor family: New insights into structure, regulation, and evolution from the rat genome. Genome Research**14**(4): 580-590.
- Zhen, H., Wu, X., Hu, J., Xiao, Y., Yang, M., Hirotsuji, J., Nishikawa, J.-i., Nakanishi, T. and Ike, M. (2009). Identification of retinoic acid receptor agonists in sewage treatment plants. Environmental Science and Technology**43**(17): 6611-6616.
- Zhong, Y., Feng, S. L., Luo, Y., Zhang, G. D. and Kong, Z. M. (2001). Evaluating the genotoxicity of surface water of Yangzhong City using the *Vicia faba* micronucleus test and the comet assay. Bulletin of Environmental Contamination and Toxicology**67**(2): 217-224.



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