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Assessment of the application of bioanalytical tools as surrogate measure of chemical contaminants in recycled water

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ABSTRACT

The growing use of recycled water in large urban centres requires comprehensive public health risk assessment and management, an important aspect of which is the assessment and management of residual trace chemical substances. Bioanalytical methods such as *in vitro* bioassays may be ideal screening tools that can detect a wide range of contaminants based on their biological effect. In this study, we applied thirteen *in vitro* assays selected explicitly for their ability to detect molecular and cellular effects relevant to potential chemical exposure via drinking water as a means of screening for chemical contaminants from recycled water at 9 Australian water reclamation plants, in parallel to more targeted direct chemical analysis of 39 priority compounds. The selected assays provided measures of primary non-specific (cytotoxicity to various cell types), specific (inhibition of acetylcholinesterase and endocrine receptor-mediated effects) and reactive toxicity (mutagenicity and genotoxicity), as well as markers of adaptive stress response (modulation of cytokine production) and xenobiotic metabolism (liver enzyme induction). Chemical and bioassay analyses were in agreement and complementary to each other: the results show that source water (treated wastewater) contained high levels of biologically active compounds, with positive results in almost all bioassays. The quality of the product water (reclaimed water) was only marginally better after ultrafiltration or dissolved air floatation/filtration, but greatly improved after reverse osmosis often reducing biological activity to below detection limit. The bioassays were able to detect activity at concentrations below current chemical method detection limits and provided a sum measure of all biologically active compounds for that bioassay, thus providing an additional degree of confidence in water quality.

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1. Introduction

Over the last decade, many populated regions throughout the world have suffered water supply shortages. While the causes of these shortages have been variable, extensive droughts and increasing population demands have been consistent factors. Large urban centres in Australia, the USA and parts of Asia and Europe have found traditional groundwater and surface water sources increasingly limited and difficult to expand. One consequence has been the growth of long-distance inter-basin transfers of water from less populous areas. Many large coastal cities have also begun to develop extensive seawater desalination capacity. However, these alternative water supplies are commonly energy intensive, costly and not available in all areas. An increasingly important alternative has been the use of recycled water for a variety of applications including potable water reuse.

The use of recycled water in large urban centres requires comprehensive public health risk assessment and management, of which residual trace chemical substances are an important aspect (Khan and McDonald, 2010; Rodriguez et al., 2009). A wide variety of substances may be present in reclaimed water at low concentrations depending on the water treatment processes applied. Such complex and poorly-defined mixtures tend to be difficult to characterise and present a number of challenges for risk assessment. Direct chemical analysis is limited by the sheer range of chemicals potentially present and a lack of suitable analytical methods for many. In addition, direct chemical analysis cannot account for potential mixture interaction between individual chemicals, which may lead to either increased (additivity or synergism) or decreased (antagonism) biological activity. Bioanalytical methods such as *in vitro* bioassays may be ideal screening tools that can detect a wide range of contaminants based on their biological activity rather than their chemical structures. This means that less expectation bias is introduced in the analysis (Escher and Leusch, 2012). When used in parallel with chemical analysis, "unknown" biologically active contaminants can be detected and sometimes identified.

Bioanalytical tools have previously been applied to recycled water quality assessment. Until 2005, most of these examples focused on assessment of genotoxicity (NRC, 2012), but since then bioanalytical batteries have started to include additional endpoints such estrogenicity, bacterial and algal toxicity, acetylcholinesterase (AChE) inhibition and aryl hydrocarbon receptor activity (Escher et al., 2009; Leusch et al., 2005; Macova et al., 2011; Poulsen et al., 2011; Reitsema et al., 2010; Reungoat et al., 2010). The selection of these additional endpoints is usually based on chemical consideration (*e.g.*, an algal toxicity assay is a good indicator of herbicides) or as surrogate measures (*e.g.*, the bacterial toxicity assay responds to the presence of a wide variety of compounds; Tang et al., 2013) and not specifically related to human health considerations.

In this study we have assessed the application of a battery of *in vitro* assays selected explicitly for their ability to detect molecular and cellular effects relevant to potential chemical exposure via drinking water as a means of screening for chemical contaminants in recycled water prior to more targeted direct chemical analysis.

2. Materials and methods

2.1. Sites and sample processing

Nine water reclamation plants in 6 Australian states were sampled. These plants were selected to provide a variety of treatment technologies (from pond- to membrane-based systems) in a range of climatic conditions. Samples were taken between 7am and 1pm. Sample types and a brief description of each site is provided in Table 1.

Grab samples (2 × 2 L) were taken of the source (treated wastewater) and product water (reclaimed water) in methanol-rinsed amber glass bottles. Ultrapure water field blanks were also taken as negative controls. In addition, samples of tap water from five Australian capital cities, bottled water and rainwater were taken for comparison. All samples were kept on ice until brought back to the laboratory. Samples were processed within 12 h by passage through 6cc Oasis HLB (Waters Corp) and Supelclean coconut charcoal (Sigma–Aldrich) cartridges in series, stacked on top of each other. All cartridges were individually preconditioned with 5 mL methanol followed by 5 mL ultrapure water. Once dried, the cartridges were eluted with 2 × 5 mL methanol, the extracts blown down to dryness under gentle nitrogen stream, and immediately reconstituted to 1 mL methanol for a final sample enrichment factor of 2000×. The same aliquots were used for chemical and bioassay analysis.

2.2. Bioanalytical tools

Thirteen *in vitro* bioassays were selected for this project based on a review of potential human health effects from exposure to toxic chemicals via drinking water and the current state-of-the-science of bioanalytical methods (Escher and Leusch, 2012). The selected assays provide measures of primary non-specific (cytotoxicity to various cell types), specific (inhibition of AChE and endocrine receptor-mediated effects) and reactive toxicity (mutagenicity and genotoxicity), as well as markers of adaptive stress response (modulation of cytokine production) and xenobiotic metabolism (liver enzyme induction).

Table 2 provides details on the bioassay battery used in this study, as well as method references. Additional details on the bioassay methodology used in this study are available in the Supplementary information.

2.3. Chemical analysis

A list of 39 priority chemicals for screening analysis were selected based on criteria such as the availability of chemical analysis methods, predicted biological activity, actual or perceived toxicity, presence on industrial inventories and likelihood of occurrence in recycled water sources. The priority list (Table 3) includes natural and synthetic hormones, industrial compounds, a personal care product, pharmaceuticals, a veterinary drug, pesticides, and chlorinated and brominated disinfection by-products (DBPs). An additional 23

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