Australian Water Recycling
Centre of Excellence

Water Quality and Public Health:
Risks and prevention, health assessments and global potable use case studies

A report of a study funded by the
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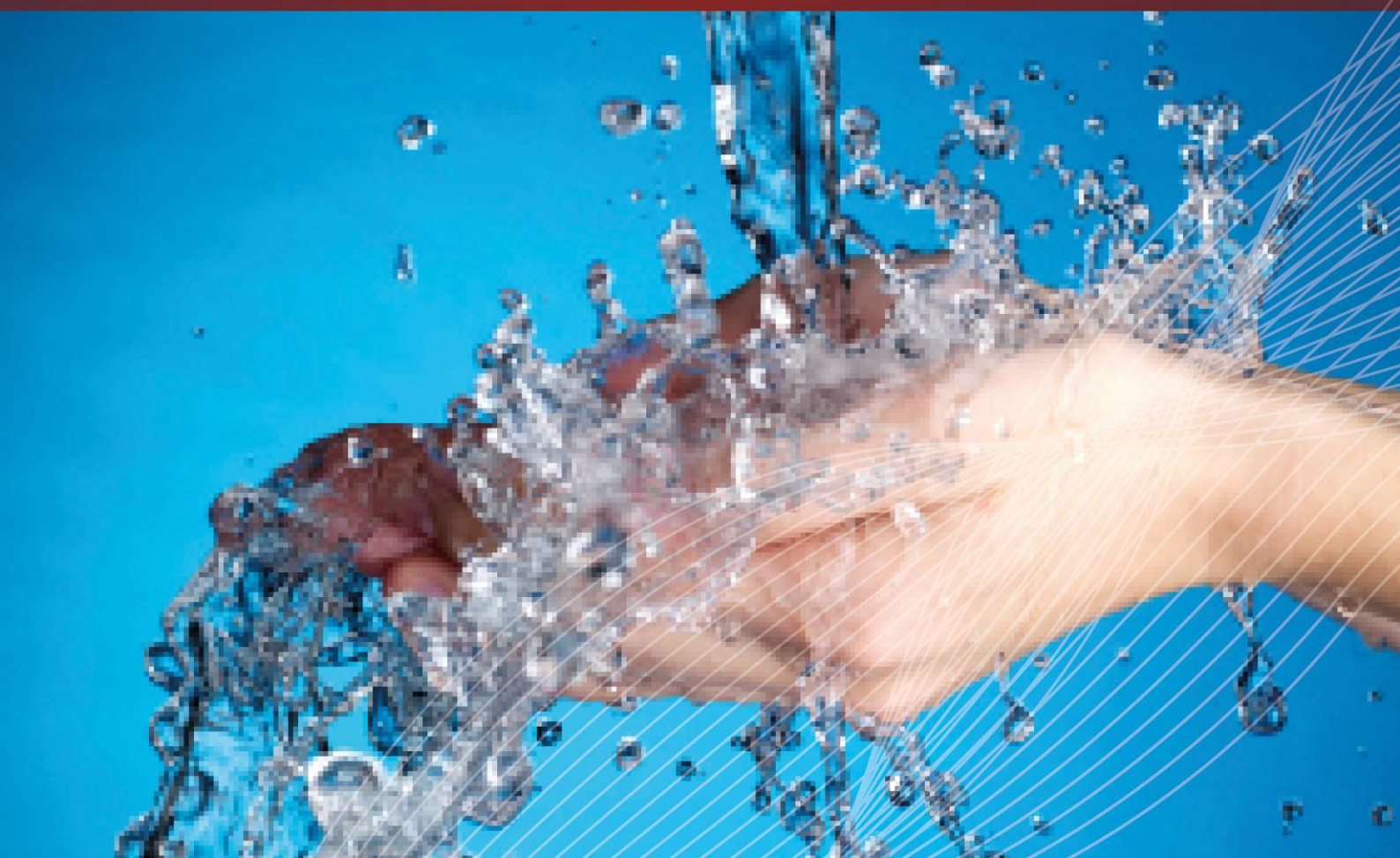

Water Quality and Public Health: Risks and prevention, health assessments and potable use case studies

This report has been prepared as part of the National Demonstration Education and Engagement Program (NDEEP). This Program has developed a suite of high quality, evidence-based information, tools and engagement strategies that can be used by the water industry when considering water recycling for drinking purposes. The products are fully integrated and can be used at different phases of project development commencing at "just thinking about water recycling for drinking water purposes as an option" to "nearly implemented". The information contained in this report was first published on the Public Health pages of a University of New South Wales Wiki website in 2012.

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About the Australian Water Recycling Centre of Excellence

The mission of the Australian Water Recycling Centre of Excellence is to enhance management and use of water recycling through industry partnerships, build capacity and capability within the recycled water industry, and promote water recycling as a socially, environmentally and economically sustainable option for future water security.

The Australian Government has provided $20 million to the Centre through its National Urban Water and Desalination Plan to support applied research and development projects which meet water recycling challenges for Australia's irrigation, urban development, food processing, heavy industry and water utility sectors. This funding has levered an additional $40 million investment from more than 80 private and public organisations, in Australia and overseas.

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- A/Prof Martyn Kirk (ANU – expert in epidemiology of food and waterborne infectious diseases)
- Dr Paul Byleveld (NSW Ministry of Health – Water Unit Manager)
- A/Prof Melissa Haswell (UNSW – expert in environmental and public health)
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1. Public Health

1.1 Introduction

To critically assess the safety of potable reuse and provide a more comprehensive public health assessment, the practise of potable reuse was reviewed by evaluating published literature, online resources, and personal communication received from seven global potable reuse sites. Data collection was extended beyond water quality information to encompass other scheme characteristics and practises pertinent to the production of high quality water (refer to the seven global case studies on the *Water360* website).

The information collated in the database for each case study was presented in wiki pages housed under the Public Health icon of the wiki developed by the University of New South Wales as part of the National Demonstration Education and Engagement Program (NDEEP). The Public Health home page of the wiki provided a brief introduction of the global need for potable reuse, presents the Australian perspective on potable reuse, and the questions the research topic aims to address. The information on the wiki was presented in four main pages:

- Risks & Prevention
- Regulation & Public health
- Health Assessments
- Potable reuse case studies

This report presents the information contained on the Public Health pages of the wiki. It is intended to accompany the report by Wood and Onyango (2014)[1] summarising the work and findings of Sub-stream 1.1 of the NDEEP and the Public Health pages wiki. The report seeks to engage with and provide information to **health and water professionals** with focus on **public health** issues surrounding the acceptability of potable re-use.

Access to safe drinking water is a major global health concern. The WHO estimates that one fifth of the world's population inhabit regions where water is physically scarce, and in every continent, one out of every three people lack access to a potable water supply to meet their daily needs. Consequently, diseases associated with water are a major cause of morbidity and mortality worldwide, and those related to drinking supplies account for approximately **5%** of the global disease burden [1] [2] [3]. In the developed world, a staggering 10 million people are estimated to lack access to potable supplies [4].

In many global regions, a combination of drivers have put a strain on existing potable water sources and many locations experience shortages and restrictions in order to meet demand. While many may agree that water scarcity is indeed a growing global concern, some may question whether recycling wastewater for potable use is necessary and safe.

Some of the drivers cited for potable reuse include:

- Unequal distribution of water sources
- Acute **water shortages**
- Dependence on a single water supply
- Erratic climatic patterns such as frequent & prolonged **drought**, **decline in precipitation**, increased evapo-transpiration
- **Population growth**
- Increased need for **urbanisation** and agricultural applications
- Poor water quality
- Pressure to **reduce discharge of treated effluent into waterways**
- Sewer constraints
- **Water security** (break dependency on local/international supply)
- Seawater intrusion
- Available water reclamation technologies

[1] Wood, J. and Onyango, L. (2014). *Potable Reuse: Practises, Water Quality & Public Health, A Global Perspective*. Australian Water Recycling Centre of Excellence, Brisbane, Australia.

- Other options such as desalination and water transportation can be **economically unsustainable.** [5] [6] [7]

1.2 Water Reuse in Australia

Like many other regions of the world, Australia experiences a number of these factors and has responded to the growing water need through the development of water conservation strategies and use of alternative sources which include wastewater reuse. It is estimated that only about 10% of Australia's municipal wastewater is currently reused [8]. While there is strong support for non-potable reuse applications, Australia has been slow in implementing potable reuse as a viable option to augment drinking supplies, in part due to political pressures, negative media branding, criticism from health and water professionals, and community attitudes and perceptions regarding potential health risks. The latter is understandable given that great gains in public health in developed countries comes from establishing clean water supplies by primarily separating drinking water from sewage contamination [9], while in contrast, diarrhoeal disease remains an important cause of morbidity and mortality in many developing countries due to poor sanitation practises and faecal contamination of potable supplies [10].

Despite technological advancements and the demonstration of treatment and operational efficacy in wastewater reclamation, the acceptance of alternative water sources such as potable reuse hinges on public support, without which some proposed potable reuse projects have failed [11]. Recent research has found that **publics don't feel well informed** about recycled water [12] [13], an example being what are the differences between planned and unplanned potable reuse.

Communities are more inclined to accept alternative waters if their water utilities actively and transparently **engage** and **educate** potential users and build trust within those communities addressing the need to adopt alternative water as well as public health concerns. More information on how community attitudes and values influence recycled water choices is summarised in the Section 2.2 below.

In the past decade, Australia has developed comprehensive guidelines for wastewater reuse - the Australian Guidelines for Water Recycling (AGWR) (Phase 2) (2008) - and since then, two potable reuse projects have been introduced: The Western Corridor Recycled Water Scheme (WCRWS) in **south east Queensland**, and the Groundwater Replenishment Trial (GWRT) in **Perth, Western Australia**. The NDEEP initiative aims to support successful public engagement and address stakeholder concerns through the provision of scientific information on water recycling for potable use within the Australian context.

1.3 Community attitudes and values

1.3.1 Public support for water recycling

A survey of the academic and grey literature reveals significant differences in community acceptance of direct and indirect potable reuse projects around the world. **Highly successful** attempts include:

- NEWater in Singapore,
- Groundwater replenishment in California; and
- Direct Potable Reuse (DPR) in Windhoek, Namibia.

Less successful attempts include the Toowoomba wastewater treatment plant (WWTP) proposal.

The **factors underlying** these differences are not fully clear, but the literature illustrates that probable reasons include:

a. different levels of water scarcity shaping perceived need;
b. differing levels of perceived benefits;
c. differences in community outreach campaigns; and

d. different socio-political environments in which the programs were implemented (including political and media support).

In general, all studies cited show that end-users indicate increasing caution with using recycled water as the vector of contact comes closer to the body. Highest levels of acceptance were found for activities such as gardening and toilet flushing, moderate levels of acceptance for bathing and washing dishes, and lower levels of acceptance for cooking and ingestion.

1.3.2 Community Attitudes and Values

The extant literature and surveys data sets reveal a number of key community concerns about the use of water recycling. The most prominent of these are:

a. the perception of risk and possible implications for health;
b. disgust and emotional response to recycled water;
c. trust in authorities and transparency concerns; and
d. environmentally motivated responses to recycled water.

Within the **Australian context,** data shows significant variation in the level of support for recycled water, in general, in different states, as well as significant differences based on gender, education level, and technical knowledge about recycled water.

There exists a lower level of approval of recycled water amongst those with less formal education and those with less access to telecommunications.

1.3.3 Religion and water recycling

Religious belief and cultural understandings of cleanliness are strong influences on the development of attitudes towards water reuse. Users need to be convinced that it is both not only scientifically safe, but **culturally** acceptable.

While all religions have notions of what is clean and what is unclean, most have important formalised structures of authority that will be critical to negotiate in order to achieve community approval for recycled water.

Two key examples, Islam and Judaism, are particularly important to iterate at this point in the project. Many Muslim countries face acute water shortages, and the societal necessity of water reuse is a key factor as to whether local Islamic law classifies this water as suitable for drinking, and whether it is considered pure for ritual use. Under Islamic law, water is classified into three categories of purity [1] [2]:

a. *Tahur* - the purest degree of water cleanliness. May be used in ablution to clean oneself ritually before prayers, or for everyday use. Once used for ablution, it becomes unsuitable to be reused and becomes *tahir* (relatively clean water that is sufficiently unclean for reuse in ablution).

b. *Tahir* - the second purest degree of water cleanliness. This water is considered generally clean, and may be used for everyday use such as drinking, washing clothes, and bathing. It is regarded as clean enough for bodily use, but not for ritual cleansing. However, it is not considered the highest degree of cleanliness as *tahur* water is, and cannot be used for ritual ablution.

c. *Mutanajjis*: unclean water, which has been defiled through pollution. Any change in colour, odour, or taste will result in *mutanajiis* water.

It is important to note, however, that the consideration of purity is determined by religious authorities taking into account the environmental context, namely the necessity to address water shortages, and the other options available.

Jewish food laws (*kashrut*) dictate what is *kosher* (suitable to eat) and what is not. While observance of differing interpretations of *kashrut* is highly variable [3], this is important for the NDEEP to recognise. Periodic disputes over interpretations of Talmudic law have emerged, for example in New York City in 2004, when the microscopic organisms known as copepods were found in the water supply [4]. Scientists consider copepods acceptable to drink and completely

safe, but reading particular Talmudic verses prohibiting the consumption of insects, several Rabbis objected.

In the case of water reuse in Australia, it will be important for any implementation of reuse to communicate with Jewish religious authorities to ensure they are supportive.

1.4 Questions to be addressed

The following discusses potable reuse from a public health perspective and aims to address the following questions:

1. What data is currently available globally on the practise of potable reuse?
2. Are the regulations and guidelines from different jurisdictions practicing potable reuse adequate to provide information required to assess the impact on public health?
3. What public health information (incidences of infectious disease contagion and other waterborne health impacts) exists for communities that have implemented potable reuse for the period before and after the schemes have been in operation?
4. Does the data that is currently collected from potable reuse provide appropriate information for public health professionals to comment on both the risks associated with potable recycling and the efficacy of the process barriers and preventative measures in mitigating these risks?

We begin by exploring the potential health risks associated with wastewater reuse and discuss their effects (known or perceived) on human health. We examine the treatment and operational control measures that are used in practise and their efficacy in preventing the associated health risks. Regulation forms an important facet in public health protection and as such we examine the regulatory environment under which potable reuse is practised, using relevant evidence drawn from existing global potable reuse schemes. We collate and present evidence from toxicological investigations of water quality, and epidemiological surveillance measures that have been employed to assess the effects of potable reuse on human health. Finally, we present seven case studies of global locations where potable reuse is practiced and their specific institutional architecture in safe water production.

Further information on case studies can be found on the Global Connections Map on the *Water360* website.

2. Risks and Prevention

2.1 Introduction

The traditional approach to ensuring safe drinking water has been to separate potable sources from contaminated waters such as raw or treated sewage, industrial & chemical waste. While not possible in all circumstances (for example unintended re-use along major river systems), the combination of **source water protection** with basic **filtration** and **disinfection** has proved extremely effective in reducing the burden of water-borne disease in developed nations, while in contrast, morbidity and mortality in developing countries due to faecal contamination of drinking water sources is common [1].

Wastewater reuse, whether planned or unplanned, is a common global practice and has been identified to be occurring in at least 47 different countries [2]. Wastewater sources used for reclamation come from various origins including municipal sewage, industrial wastewater, and stormwater runoff, all of which can be used in a variety of potable and non-potable reuse applications (Figure 1). The types and levels of contamination in these waters vary, which in turn will dictate the level of treatment necessary to make each fit-for-intended-use. Although treatment technologies and abundant knowledge exist to achieve this, many jurisdictions express concerns over the health implications of utilising recycled wastewater to drink, despite the already on-going practice of **unintended/unplanned potable reuse** (settings where treated municipal wastewater is released into surrounding waterways and used by downstream communities as their conventional drinking supply).

Figure 1: Recycled water potable and non-potable reuse applications.

Domestic and commercial wastewater (sewage) sources - which form source water for many potable reuse projects - contain elevated levels of a range of **pathogens** that are known causes of a variety of enteric diseases; and a suite of **chemical contaminants** with toxicological potential [3]. Thus, proposals for potable reuse have been met with strong community opposition in some locations with the perceived notion that the practice removes the public health protection that has been established by prior practices.

2.1.1 Microbial contaminants

Pathogens present in wastewater include a spectrum of bacteria, fungi, viruses, parasites, algae, and helminths (worms and nematodes) [4]. Of these pathogens, those of importance in the developed world are the water-borne bacteria, viruses, and parasites which pose real risks to the health of communities as evidenced by reports of **drinking water outbreaks** that have occurred in conventional water systems of many developed nations [5] [6]. Exposure to sufficient doses of these pathogenic hazards can often lead to **acute enteric infections**, the most common of those being **gastroenteritis**, with varying symptoms depending on the pathogen(s)

present (Table 1). An in-depth look at recent drinking water outbreaks is presented in Epidemiological Studies in Section 4.2.

Table 1: Common pathogens in wastewater, minimum infectious dose and predominant symptoms.

Pathogen type	Examples	Minimum infectious dose	Predominant symptoms
Bacteria - the most common pathogens in wastewater	• enterohaemorrhagic *Escherichia coli* • Shigella spp • Campylobacter spp • Salmonella spp	• 10-100 cells for enterohemorrhagic *E. coli* • 100-200 cells for Shigella spp • 500-10,000 cells for *Campylobacter jejuni* • 1,000,000 cells (1-1000 cells for more infectious spp)	• gastroenteritis (severe stomach cramping, nausea, vomiting, bloody watery diarrhoea) • bacilliary dysentry (bloody and mucoid diarrhoea, fever, stomach cramps, malaise) • campylobacteriosis (fever, diarrhoea, stomach cramps, vomiting,may have bloody stool) • salmonellosis (abdominal pain, diarrhoea, chills, fever, malaise); typhoid fever
Virus - highly infectious and cause rapid infection	• rotavirus • norovirus • adenovirus	• 1-100 viral particles (depending on species) • 1-10 viral particles	• gastroenteritis with severe watery diarrhoea, vomiting fever, malaise, headache • epidemic gastroenteritis with non-bloody diarrhoea, explosive vomiting, low grade fever, muscle aches • gastroenteritis (nausea, vomiting, malaise, abdominal pain, fever;) conjuctivitis
Protozoa	• Cryptosporidium spp (oocysts) • Giardia spp (cysts) • *Entamoeba histolytica*	• 1-100 (oo)cysts • 1 or more cysts • 10 cysts	• gastroenteritis(profuse watery diarrhoea, nausea, vomiting, cramping, fever) • giardiasis (smelly diarrhoea with abdominal cramps, flatulence, malaise) • acute enteritis (mild to severe diarrhoea with mucus and blood)

Data extracted from United States Food and Drug Administration 2012 [9], DOH (Victoria) [10].

Removal of the different pathogen classes will depend on the treatment process applied and the intended end-use of the recycled water. Tertiary treatment is commonly employed in instances where close contact with the recycled water may occur [4]. Nonetheless, there is evidence that some viruses and protozoa can still be detected in tertiary treated wastewater [7] [8]. Consequently, for the purpose of potable reuse and public health protection, advanced treatment processes are employed (discussed in Section 2.2).

2.1.2 Chemical contaminants

The chemical composition of municipal wastewater is influenced by discharge from household, commercial, and sometimes industrial sources [11] [12]. Chemicals of concern in wastewater include:

- *personal care products and household products* - eg. caffeine, fragrances, cosmetics, detergents [13] [14]
- *pharmaceuticals* - eg. antimicrobials, aspirins, antidepressants [13]

- *hormones and endocrine disrupting compounds* - eg. progesterone, estradiol, testosterone [15]
- *disinfection by-products* - eg. trihalomethanes, n-nitrosodimethylamine (NDMA), residual chlorine [16]
- *herbicides and pesticides* - eg. atrazine, diuron, simazine [17]
- *heavy metals, nutrients, suspended solids*
- *other industrial inorganic and organic chemicals*

There is growing concern over the long-term human health effects that the range and combinations of natural and anthropogenic chemicals may have on end users of potable reuse. Some reports suggest that these compounds are not entirely removed and can survive the range of treatment processes and end up in receiving waterways [18] [13]. Other reports suggest that the residual chemical water quality from conventionally treated effluent is attenuated so that it does not pose health risks to consumers [4]. Nonetheless, reports suggesting toxicological effects from wastewater effluent such as changes in fertility and reproduction, carcinogenicity, and mutagenicity based on their associated effects on aquatic organisms [13] [19] have not been reassuring to consumers, which makes acceptability of this resource for purposes such as aquifer recharge and augmenting drinking sources an ongoing public concern.

2.2 Wastewater treatment for potable reuse

It is estimated that only 10% of global wastewater produced is actually treated before it is discharged into waterways. Consequently, many global water bodies including those that serve as drinking sources are polluted with contaminants of potential health concern. Within Australia, this is less of a concern as the coastal concentration of the population avoids the issues in continental Europe, where major cities sequentially draw water from large river systems. Nonetheless, the implementation of advanced wastewater treatment and subsequent reuse provides a means to not only manage environmental pollution but also provides water-strained communities with a safe, climate-independent water resource to meet various needs [20]. A portfolio of treatment options exist that can be used to reclaim wastewater and achieve quality suitable for potable purposes. The common approach involves conventional treatment (primary & secondary treatment) proceeded by advanced treatment. Some common advanced treatment technologies used in potable reuse applications include:

- MF/UF
- RO/NF
- AOX/UV light exposure (with hydrogen peroxide) and ozonation.

Despite the advancements in treatment technologies, no single barrier can be deemed 100% effective against all agents of health concern. For instance, while disinfection (eg. through chlorination) is highly effective against most pathogens it is poorly effective against *Cryptosporidium* oocysts. Filter-based technologies are more effective against larger particles (such as protozoa) but less effective against viruses and trace organic compounds such as NDMA. There is no one-size-fits-all approach to choice of treatments for potable reuse. The aim is to incorporate treatment barriers that are reliable and effective in inactivating and removing the range of pathogens and chemicals that can trigger ill health; and consistently meet the prescribed public health standards for drinking water quality. Any number of combinations of advanced treatment barriers can therefore be employed. Both potable reuse projects in Australia, for example, incorporate the three aforementioned technologies in their systems to meet the prescribed public health goals prior to augmentation. Other reuse projects employ different configurations, which are all designed to consistently meet representative jurisdictional drinking water standards.

2.3 How do we know the system is working?

While potable reuse projects are developed with the aim of meeting the water needs of a community, the protection of public health is of uttermost priority in the process of potable water supply. Various measures are put in place to mitigate against health risks.

2.3.1 Multiple Barriers

The ineffectiveness of a single barrier such as treatment alone as a means of safe water production - no matter how robust the technology or pristine the source water - has been demonstrated in many conventional drinking water systems where recent outbreaks have occurred [21]. Lessons learned from these outbreaks reiterate the need for incorporation of measures that extend beyond water treatment in order to prevent similar water contamination events and consequently adverse public health effects. The increased risks in potable reuse due to the nature of the source water calls for incorporation of highly effective risk barriers throughout the system to prevent the range of risks associated with past outbreaks.

The multiple barrier approach incorporates varying combinations of risk prevention measures from source water acquisition all the way to distribution (Figure 2). Each of the barriers functions independently of the other but work collectively to attenuate risk and optimize public health protection. In addition to advanced treatment barriers, other **non-treatment barriers** employed in potable reuse systems include:

- **source water protection** measures such as diversion of industrial wastes from mixing with domestic wastewater;
- **blending** of reclaimed water with conventional sources;
- passage through **environmental buffers** to allow for additional attenuation of risks; and
- storage/**retention** which allows time for additional water quality analysis to be carried out and any mistakes rectified.

Examples of the multiple barrier approach have been incorporated in the Western Corridor Water Recycling Plant (WCWRP) in southeast Queensland, and the pilot Groundwater Recharge Trial (GWRT) in Perth. These barriers are designed to meet the public health targets stipulated in the AGWR document.

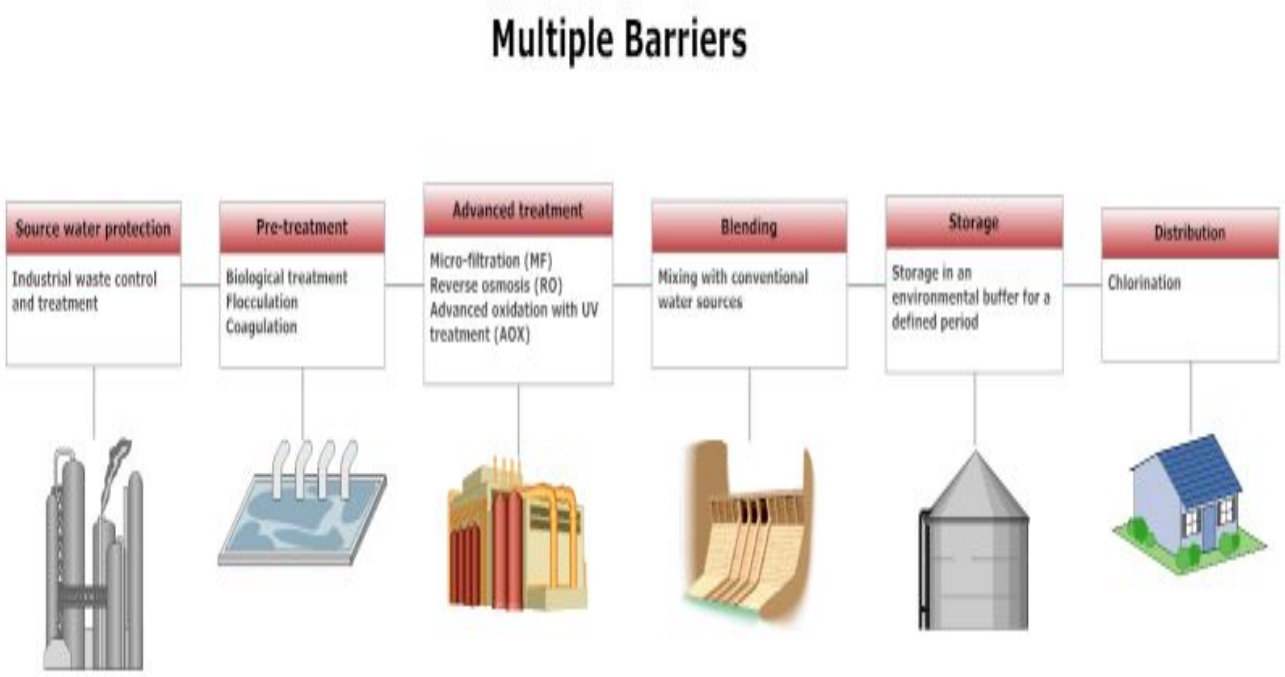

Figure 2: Potential barriers in a potable re-use system.

2.3.2 Incorporation of Critical Control Points

Another way to ensure operational reliability and production of safe water is through incorporation of a hazard analysis and critical control point (HACCP) framework. The process involves identification of hazards/risks that need to be controlled for the protection of public health, then subsequently determining critical control points (CCPs) throughout the reuse system where critical limits for specified parameters must be achieved in order to mitigate risks. These CCPs act as check-points through which water quality is continuously evaluated and the success of treatment trains monitored. Part of the process also includes establishing a series of comprehensive corrective actions that are performed in the event of unsatisfactory performance in order to minimise health risk to consumers [22] [23].

In the pilot GWRT in Perth [2], 13 CCPs were established and the plant was shown to operate within the design of all CCPs for 99.37% of the trial period. There were three instances in which a single CCP failed to operate as designed, but these were found to not influence overall FPW quality, with the remaining 12 CCPs acting to verify this.

2.3.3 Water quality assessments

Rigorous water quality monitoring serves an important role in potable reuse practice and functions to ensure that the desired final product water (FPW) is consistently achieved for consumer satisfaction and health protection. The assessments include monitoring of physical, chemical, and microbiological properties of the water as it goes through the various reclamation steps. This is achieved using **online monitoring** in concert with **composite water sampling**. While modern technologies enable detection of a range of parameters and at levels below what is considered of health importance, it is impractical and not cost-effective to evaluate water samples for the presence of the entire range of contaminants of potential human health impact that are found in wastewater. Instead, the use of a combination of **surrogate parameters and health indicators** provides a more realistic yet rigorous approach at water quality monitoring[24].

The Groundwater Recharge Trial (GWRT) in Perth, Western Australia, takes this approach to enable frequent and effective water quality monitoring. While there are >300 water quality parameters identified, 292 (the RWQP) are regulated for reporting to the DOH, and a subset of 18 key parameters - the RWQI which represent the larger body of the RWQP - is what is more regularly analysed.

2.3.4 Pilot testing

Pilot testing forms an important component of a reuse scheme where the efficacy of the entire system is demonstrated over a period of time to ascertain reliability under different conditions and scenarios, and that the FPW meets the prescribed health target limits. Most potable reuse schemes undergo initial periods of pilot testing before transitioning into full-scale projects. A review of the different pilot testing initiatives that have been carried out in potable reuse systems can be viewed on the Global Connections Map on the *Water360* website.

2.3.5 Health assessments

In some communities where potable reuse is practiced, health studies have been conducted to investigate whether there is any association between consumption of recycled water and increase in **adverse health effects**. This has primarily been done via epidemiological studies which have compared populations exposed to recycled water with control groups on parameters such as infectious diseases (e.g enteric infections), respiratory infections, reproduction & birth defects, carcinogenic effects, etc. There have been few epidemiological studies performed to date due to several reasons including low prominence of global potable reuse, the costly nature and poor sensitivity of the studies. Nevertheless, the findings suggest no observed adverse health effects from planned potable reuse.

Other measures of health effects in potable reuse have been through **toxicological studies** (bio-monitoring). These studies examine the combined toxicological effects of the chemical mixtures in reclaimed water by using various cell lines (bacterial or mammalian) and whole organisms (mice, fish, etc) to evaluate reclaimed wastewater for endocrine, toxicological, and

carcinogenic effects [25]. Although helpful in assessing the chemical water quality, the usefulness of these studies to address human health scenarios has been debated.

Both types of studies have been described in detail in Section 4 on Health Assessments.

Putting risk into perspective

Despite the efficacy of treatment technologies to remove/attenuate substances of health concern in wastewater coupled with the availability of increasingly sensitive water quality monitoring techniques to detect a wide range of contaminants and at very low concentrations, it is impossible to guarantee absolutely no risk in any water system. Instead, what is paramount for water providers is to reduce the level of risk to a standard that is deemed safe and does not pose any adverse health effects to users.

In Australia, national water guidelines have been developed to help provide a unified approach towards health risk management and the provision of safe drinking water. The guidelines advocate a risk preventative approach that utilities can adopt and tailor to fit their specific jurisdictional water needs. Both types of studies have been described in detail in the following Section 3 on Regulation and Public Health.

3. Regulation and Public Health

3.1 Regulation and drinking water in Australia

For a recycled water system to be successful, it needs to consistently demonstrate that the FPW does not pose a danger to human health or the environment for its intended use. This is partly achieved through regulation which forms an important practice in the water reuse industry. Regulations aid recycled water providers understand what requirements they need to meet to ensure public health is maintained. Regulatory bodies ensure compliance by these water agencies and that **best practices** are utilised in delivering water that is **safe for the prescribed end use**. In potable reuse, producing water of the highest quality is crucial to prevent exposing communities to pathogenic agents and contaminant chemicals. In Australia, guidelines and standards on drinking water have been developed at a national level but regulation is under the authority of state governments. The key documents that currently define the national approach in the water industry are the **ADWG** [1] and the **AGWR** - Phase 1 & 2. The latter provides a comprehensive national guideline to managing recycled water and focuses on a **risk management approach** that evaluates for possible public health and environmental risk factors and stipulates ways to prevent them from occurring. The AGWR document was based on two key documents: the ADWG and the WHO Guidelines for Drinking Water Quality (WHO 2004).

Phase 1 addresses water recycling for general, **non-potable purposes** [2] , while Phase 2 of the guidelines focuses specifically on planned augmentation of **drinking water supplies** [3], harvesting and re-use of storm-water, [4] and aquifer recharge [5]. The major difference between potable and non-potable reuse is the level of exposure for end users to the risk factors associated with municipal wastewater reuse. Phase 1 of the guidelines describes maximum risk for non-potable reuse end users as **<1L/person/year** while Phase 2 describe risk of exposure based on consumption of **>700L/person/year** for potable reuse. Thus, the high risk exposure from potable reuse requires these schemes to implement a more conservative preventative risk management strategy that reduces the risk to levels that are acceptable. The guidelines are generic and were intended to be applicable to any size recycled water system and optimised for their particular end use.

Since microbial contaminants pose a greater risk to human health, there is a strong focus on minimising health impacts in the guidelines, with the use of log reduction values (of reference pathogens) to validate treatment and operational processes, and a move to expressing the **tolerable risk** in terms of **DALYs** [6]. This aligns with the WHO approach to WQ [7] and is more generally the WHO unit of choice in discussing the burden of disease in populations. The reference value in the WHO publication is for water to lead to a loss of no more than 1 microDALYs (10^-6 DALYs) per person per year. This equates to about 1 additional case of diarrhoea per 1000 people per year in the population served by the supply. However, there remain some challenges in monitoring these public health targets, with current methods relying on use of QMRA (log reduction requirements) to link water quality measures with microDALYs. Routine infectious disease surveillance detects only a very small proportion of diarrhoeal disease and so is not suited to such monitoring.

The guidelines take a principles-based approach, with 12 key areas outlined that can be developed into a scheme-specific risk management strategy:

- **Protection of public health is of paramount** importance and should never be compromised
- Drinking water augmentation requires **community acceptance and support**
- **Institutional capacity** is required
- Recycled water systems need to include and continuously maintain **robust and reliable multiple barriers**
- Skills and training:
  - Designers, operators and managers of schemes must have **appropriate skills and training**

    - System operators must be able to **respond quickly and effectively** to adverse monitoring signals
    - System operators must maintain a personal **sense of responsibility** and be dedicated to providing consumers with safe water
- **Industrial waste management programs** need to be established and maintained
- All schemes must be subject to **regulatory surveillance**
- The greatest risks to consumers of drinking water are pathogenic microorganisms; protection of water sources and treatment are of paramount importance and must never be compromised
- Any **sudden or extreme change in water quality**, flow or environmental conditions (eg extreme rainfall or flooding) needs to **arouse suspicion that drinking water might become contaminated.**

3.1.1 Role of the Regulator

To ensure that schemes adhere to requirements for the production of safe water for public consumption, they are regulated (monitored) by jurisdictional regulatory bodies. Because the AGWR are not prescriptive and there is limited information on the process of scheme validation, defining what is needed and how to go about validation is left to each jurisdiction and their corresponding regulators. Different jurisdictions have different regulatory requirements that achieve some of the same principles as those established in the AGWR that are tailored for their needs. These requirements make up **scheme permits** with defined operational processes that validate treatments and ensure that safe potable water is produced and consumer health always protected.

In Queensland and Western Australia where potable reuse projects have been commissioned, these locations have adopted the national guidelines in the development of regulation in their locations. In QLD, regulation is administered by the Office of the Water Supply Regulator [8] based on the *Water Supply (Safety and Reliability) Act 2009*. In WA, regulation is a collaborative effort between their Department of Water, Department of Environmental Regulation and Department of Health [9]. WA uses the *Guidelines for the use of Recycled Water in Western Australia*.

3.2 International Guidelines

There is no one-size-fits-all when it comes to regulation of potable reuse. Each location practicing potable reuse has developed their own policies in collaboration with their public health authority and water regulator. These stipulate health based targets and how they should be met. Because of the health implications associated with potable reuse, these guidelines are overly cautious. Some common guidelines that have been used to develop country-specific potable reuse guidelines include:

- The WHO *Guidelines for the Safe Use of Wastewater, Excreta and Greywater* [10]
- USEPA *Guidelines for Water Reuse*

Some common practices included in international frameworks are:

- hazard identification and defining health based targets
- implementation of protective measures (eg. multiple barriers)
- system monitoring and validation
- breach management protocols and reporting
- development of comprehensive water safety plans and notifications
- regulatory surveillance by qualified external parties.

Some examples of international potable reuse projects are presented in Table 2.

Table 2: Examples of international potable reuse projects.

Scheme Name	Permit guidelines	Regulatory surveillance	Validation of operational measures	Operational Monitoring (SCADA)	Identified CCP	Corrective (shutdown) protocols	Water quality approval body	Breach management protocol	Breach reporting
Groundwater replenishment system (GWRS), Orange County, CA, USA	*Based on USEPA and State of California standards	*Random inspections (~3/YR) by the CRWQCB regulatory staff	*Uses a HACCP framework subject to treatment performance measurement	*Online SCADA system *Operational performance of each process unit monitored through CCP	*MF system *RO system *AOX system *FPW	*Automated shut down if CCP parameter or laboratory WQ not met *Processes resume only after parameters are met	*California Department of Public Health *CRWQCB	*Plant either shuts down or runs to waste (ocean outfall) until water quality specifications are met (online CCP or traditional laboratory testing)	*Reported to CRWQCB in summary reports
Groundwater replenishment trial (GWRT), Perth, Western Australia	*Based on AGWR, ADWG and recommend ations from the DOH	*Performed by DOH, DOW, and DER	*All processes were validated on commissioning . Ongoing validation of the UF and RO are done by the technical advisors and process technical officer of the plant.	*Online View X system	*2 at Aeration tanks *1 at Secondary sedimentation tanks *2 at raw water *5 at UF *4 at RO *2 at UV *Final pH	*All CCP instantly divert water on their violation limit. Water is prevented from entering the next treatment step until specified limits reached	*DOH	*Incident Management Protocol in place - breaches are reported to the DOH	*Breaches are reported to the DOH
New Goreangab water reclamation plant (NGWRP), Windhoek, Namibia	*Based on Namibian, WHO, USEPA guidelines and South African Water Quality	*Performed yearly by BV	*Since 2005, BV certification every 3 years based on ISO 9001 QMS standards and HACCP	*Online SCADA & composite water quality monitoring *Automated sampling taken after every process step (24hrs). Manual sampling for microbiological	*DAF unit and MOO	*Penalties incurred when target values not met *Water delivery halted when absolute values for any criteria is	*CoW *Department of Infrastructure, Water and Technical Services	*Plant goes into recycle mode and FPW pumped to Goreangab dam and reprocessed again *Recycle	*Penalties levied by contract if water quality breaches (based on permit guidelines) occur

Scheme Name	Permit guidelines	Regulatory surveillance	Validation of operational measures	Operational Monitoring (SCADA)	Identified CCP	Corrective (shutdown) protocols	Water quality approval body	Breach management protocol	Breach reporting
	Criteria			samples *FPW continuously sampled and analyzed for the range of analytes		surpassed *Plant goes into recycle mode until values restored below threshold		pipelines also connected to CCP units to redirect water back if unsatisfactory	
NEWater, Singapore	*Based on WHO and USEPA guidelines	*Conducted twice a year by the National Environment Agency	*None	*Online Sampling and Monitoring Program investigates total organic carbon, conductivity, pH, turbidity and ammonia levels	*RO permeate	*Automatic shutdown of plant in case of abnormal operating conditions	*Environmental Public Health	*Supply of water ceases within 8min of breach detection	*Incidents reported to the NEA
Torreele / St. Andre water reclamation plant (TSAWRP), Koksijde, Belgium	*Environmental permit issued for the reuse plant (Torreele) and the infiltration scheme (St-André); specific control of certain parameters and frequency were part of the permit	*A yearly report of the monitoring results provided to the Flemish Environmental Agency	*All processes were validated on commissioning (online process monitoring and additional sampling and analysis)	*Online SCADA system provides real time data	*Membrane performances *The distribution system	*Alarms generated when critical parameters exceed threshold values. Automatic shutdown of plant in case of abnormal operating conditions	*Flemish Environmental Agency	*High turbidity stops the UF train automatically *High conductivity stops the RO skid immediately	*No requirement to report operational breaches *Analytical analyses that do not comply are reported to the Flemish Environmental Agency and the Flemish Health Authorities
Upper Occoquan Service Authority	*Virginia Permit Regulation (9VAC25-	*Regular environmental audits from Virginia	*Online process monitoring and additional	*Online real time monitoring (SCADA system), in-situ and automatic. Some	*Nitrification/denitrification *chemical clarification and	*Each CCP has established corrective	*State Water Control Board * Occoquan Policy	*Spill prevention, control and countermeasure	*All spills or permit violations are reported to the VA DEQ

Scheme Name	Permit guidelines	Regulatory surveillance	Validation of operational measures	Operational Monitoring (SCADA)	Identified CCP	Corrective (shutdown) protocols	Water quality approval body	Breach management protocol	Breach reporting
(UOSA), Fairfax, VA, USA	31-10 et seq.) and Virginia Pollutant Discharge Elimination System (VPDES) permit regulation for sewage discharges	Department of Environmental Quality (VA DEQ)	sampling and analysis	samples are taken by automatic composite samplers (primary effluent and final effluent). Other samples are taken manually and monitored	phosphorus removal *pH control *chlorination/dechlorination *final filtration & turbidity control	actions under various operational upsets *An incident report is written after each incident and communicated with staff	* VA DEQ *VPDES permit regulation for sewage discharges	s plan *Stormwater pollution prevention plan *Spill reporting procedures	*Variations from normal unit operations parameters are corrected prior to final effluent discharge
Western Corridor Recycled Water Scheme (WCRWS), Queensland, Australia	*Based on the Australian Guidelines for Water Recycling (AGWR) - Water Supply Act 2009 and regulatory guidelines developed under this Act *Water quality parameters specified in schedule 3B of the Public Health Regulation (2005)	*External audit every 2 years by qualified external auditor (guidelines issued by Queensland Water Supply Regulator) *Yearly internal audit	*Each CCP was validated through desktop "pre-commissioning" submission of evidence as well as "commissioning" validation accompanied by 3 months of intensive monitoring of source water and Final Product Water (FPW).	*Online SCADA system provides real-time data at each CCP and on purified water *Composite sampling and testing of treated wastewater (source) and FPW included	*Biological nitrogen removal activated sludge treatment *MF/UF unit *RO unit *UV-peroxide unit (effectiveness measured by power consumption) *Chlorine unit	*3 alarm levels at each CCP: **Alert** - instigate investigation and operator initiated corrective action; **Action** - automatic shutdown when action alarm levels activated; and **Critical** - alarm if shut-down action or other corrective action not completed	*Queensland Water Supply Regulator (QWSR)	*Recycled Water Management Olan (RWMP) specifies comprehensive response protocol for CCP alarms and purified water quality non-conformance. The QWSR also specifies reporting requirements.	*QWSR

4 Health Assessments

4.1 Introduction

This section presents the epidemiological and toxicological studies that have been conducted around potable reuse. These studies complement the range of water quality assessments and data on the issue surrounding perceived health-related outcomes of potable reuse.

Biomonitoring (*in vitro* bioassays)

In vitro bioassays (sometimes called bioanalytical tools) can provide a measure of water quality based on biological effect in the assay as opposed to the chemical structure used in conventional water quality testing. The principal advantage here is that bioanalytical tools provide a sum measure of all bioactive compounds that act via the mode of action that the assay detects. However, while good measures of exposure, *in vitro* assays cannot be used as predictors of whole organism toxicity, nor are they a replacement of standard chemical-based water quality testing. Instead, they should be seen as a means of covering gaps in testing left by conventional chemical-by-chemical testing as part of an Integrated Testing Strategy that improves the assessment of water quality.

Epidemiological studies

At the other end of the spectrum, one can look to population level data or community-based studies for signs of the impact of potable reuse on health. Broadly speaking, these are epidemiological studies that may look for acute or long-term impacts of potable re-use on human health. Such studies are far less sensitive than chemical tests or bioassays but are more closely linked with standard measures of impacts on human health, such as through measures of quality of life. If done prior to introduction of a scheme, these studies can also help quantify the burden of disease in categories that may be of relevance to water quality, which then provides a useful reference point for future investigations.

4.2 Biomonitoring

4.2.1 What are bioassay tools?

In vitro toxicity tests are tests performed at the **molecular or cellular level** in the laboratory, usually on **concentrated water extracts**. Examples of molecular endpoints include binding to specific enzymes or receptors, while cellular events could include **cell death**, **maturation** or **growth**. An advantage of *in vitro* assays is that they can be based on human cells, thereby eliminating the inter-species predicament of whole animal testing. *In vitro* tests detect the triggering molecular or cellular toxic event that occurs at much lower, environmentally relevant concentrations, often below detection limits of chemical analysis and *in vivo* testing [1]. The main limitation of *in vitro* assays is that they lack some metabolism and transport mechanisms that may modulate toxicity in whole organisms, and that many cell-based assays are based on cancer cell lines. Also, in vitro bioassays were developed for screening purposes and there is still much debate about their ability to predict whole-organism effects [2]. But the gap in our understanding of the link between an *in vitro* response and an adverse outcome in whole organisms is getting narrower: the concept of Adverse Outcome Pathway [3] provides a solid framework to link a molecular/cellular event (as measured using *in vitro* bioassays) to a whole organism effect [4]. *In vitro* bioassays are well-suited to monitoring of water quality, as they are significantly faster and cheaper than *in-vivo* toxicity testing, are amenable to high-throughput screening, and allow the generation of relatively rapid toxicology data without the need for ethically and financially expensive whole-animal experimentation [5]. In recent years, there has been a move towards standardising the various *in vitro* techniques available, with the creation of the European Centre for the Validation of Alternative Methods (ECVAM) in 1991 and the US National Toxicology Program Interagency Centre for the Evaluation of Alternative Toxicological Methods (NICEATM) in 1998. These two programs, and similar efforts by Organisation for

Economic Cooperation and Development (OECD), have published an ever-growing catalogue of defined operating protocols for the testing of chemicals. *In vitro* bioassays are increasingly applied to water quality assessment [6] [7] with recent developments having greatly expanded the number and scope of bioanalytical tools available for recycled water quality testing.

4.2.2 Why do we need bioassay tools?

Conventional drinking water often contains a variety of organic and inorganic chemical contaminants commonly found in surface waters such as agricultural pesticides as well as chemicals formed during water treatment such as disinfection by-products. **Drinking water standards are set by health authorities** for specific chemicals that are likely to be found in water sourced from conventional sources such as surface or ground water. Conservative extrapolation to other exposures is usually applied to set conditions for safe drinking water from conventional sources [8] [9]. However, for less traditional water sources such as reclaimed wastewater, there may be different sets of chemicals present that introduce new, unaccounted risks.

Municipal wastewater often contains a wide range of **natural and synthetic chemicals**, including personal care products, household chemicals, industrial compounds, chemicals excreted by people such as natural and synthetic hormones and pharmaceuticals, and chemicals formed during wastewater treatment. Health authorities have produced new guidelines specifically for reclaimed wastewater, which address this larger collection of chemical contaminants [10], with extensive data from some water recycling schemes e.g DOH [11] [12] augmented by chemical exposure assessment techniques [13] used to determine the likelihood and significance of exceedance of chemical guidelines [14] [15]. However, even this extensive chemical monitoring of 300-400 different chemicals is only a subset of the vast number of chemicals that are likely present. For example, **between 30,000 to 70,000 compounds are in daily use** [16], and there are more than 4,000 pharmaceutical compounds alone [17], each likely to produce several different environmental transformation products. Testing of this scale is infeasible and therefore other approaches based around toxicity testing have been developed to assess the remaining risk from chemicals not specifically tested for [18].

Toxicity testing involves collecting **whole water samples** and subjecting these to tests for a range of toxicological endpoints, either using whole animals (in the case of direct toxicity assessment, DTA) or bioanalytical tools (also known as *in vitro* bioassays). Toxicity testing may include testing for **mutagenic activity, carcinogenic activity, hormonal activity** such as estrogenicity, or various forms of acute and chronic toxicity. Whole animal toxicity testing has been the cornerstone of toxicology for a long time, but **ethical and financial drivers** to reduce, refine and replace whole animal tests [5] combined with recent advances in molecular toxicology [19] [20] [21] [4] have led to an intense interest in alternative techniques, such as *in vitro* bioassays. For more information, see [22].

4.2.3 Categories of bioanalytical tools

There are **five broad categories** of bioanalytical methods based on the **biological response** that is monitored: three are **measures of toxicity** (non-specific, specific and reactive toxicity) and the other two (adaptive stress responses and induction of xenobiotic metabolism pathways) are **measures of the response of a cell when exposed to foreign or toxic chemicals** [6]. Table 3 presents examples of the different categories of bioanalytical tools that have been used in recycled water assessment. More detailed explanations of these assays can be found in Chapman and Leusch [22]. We also note that many more assays may be suitable to water quality testing [7], with many bioassays that are developed for drug discovery applications applicable with some modification to environmental samples. In a recent study, Escher et al. [23] tested 103 different bioassays in a variety of water samples, including reclaimed water, and identified a few endpoints that have so far received little attention but appear to be highly relevant for water quality assessment.

Table 3: Examples of bioanalytical methods applied to recycled water assessment.

Category	Test Details	Examples of Bioanalytical Methods Applied to Recycled Water Assessment
Non-specific toxicity	Non-specific toxicity assays measure toxicity to all types of cells due to interference with basic cellular physiology or chemistry, such as damage to cell membranes or interference with intracellular homeostasis. Toxicity measured by non-specific assays is often termed "baseline toxicity". Many non-specific assays use bioluminescent marine bacteria, which are easy to quantify, while others use mammalian or human cells. The bacteria assays appear more sensitive [23], but of course it is more difficult to relate the results to health risks. On the other hand, some of the human cell based assays have been well correlated with conventional acute rodent toxicity tests [24]. The **Microtox** and the **ToxScreen** are two well-known assays that provide a measure of non-specific toxicity in bacterial systems.	• Bacterial growth inhibition • Caco2-NRU • HepaTOX • LDH leakage assay • Microtox • ToxScreen3 • WIL2NS TOX
Specific toxicity	Specific toxicity assays measure interference with specific biological functions, such as photosynthesis, enzyme or receptor function, endocrine signalling, etc. An example of a well-studied specific toxicity endpoint is estrogenic endocrine disruption, which can lead to feminisation of male fish. The **E-SCREEN** and the **ER-CALUX** are two examples of specific toxicity assays for estrogenic activity.	• AChE (acetylcholinesterase) inhibition • AR-CALUX • CALUX (Chemical Activated Luciferase gene eXpression) • ER-CALUX • E-SCREEN • GeneBLAzer • GR-CALUX • I-PAM (Imaging Pulse-Amplitude Modulated fluorometry) • PR-CALUX • TR-CALUX • YAS (Yeast Androgen Screen) • YES (Yeast Estrogen Screen)
Reactive toxicity	Reactive toxicity is caused by the reaction of the chemical with endogenous molecules, such as protein or DNA. Several reactive toxicity pathways lead to cancer, including mutagenicity (change in DNA code) and genotoxicity (physical damage to DNA). The **Ames test** and the **umuC assays** are two assays for reactive toxicity (specifically mutagenicity and genotoxicity, respectively).	• 6-Thioguanine resistance assay • Ames test • Cytokine production assay (CPA) • Mammalian cell transformation assay • umuC (Umu Chromotest) • WIL2NS FCMN (Flow Cytometry MicroNucleus)
Adapative stress response	Adaptive stress response assays measure the defence mechanisms that cells can initiate to protect against chemically induced damage. These include production of proteins and enzymes to repair DNA damage, isolate reactive oxygen species, etc. Assays for adaptive stress response do not measure toxicity per se, but rather the cell's early response to toxic injury. Assays for adaptive stress response have only recently been tested for water quality assessment, and none of the studies reviewed in this document have used it for recycled water assessment. Adaptive stress response assays	• Assays for adaptive stress response have only recently been tested for water quality assessment, and none of the studies reviewed in this document have used it for recycled water assessment. Adaptive stress response assays appear sensitive and relevant to water samples, particularly oxidative stress and inflammation [23], and future work will likely

	appear sensitive and relevant to water samples, particularly oxidative stress and inflammation [23], and future work will likely explore these endpoints in the context of recycled water. The **AREc32 assay** and the **ARE-GeneBLAzer assay** are two assays that can provide a measure oxidative stress.	explore these endpoints in the context of recycled water.
Xenobiotic metabolism	Xenobiotic metabolism pathway assays measure the induction of biological pathways involved in metabolising xenobiotics, such as the aryl hydrocarbon receptor (AhR) or the peroxisome proliferator-activated receptor (PPAR) responses. This type of assay also does not measure toxicity per se, but the cell's attempt to detoxify foreign compounds. The **AhR-CAFLUX** and the **DR-CALUX** are two assays that can measure induction of an AhR-mediated xenobiotic metabolism pathway.	• AhR-CAFLUX • YDS (Yeast Dioxin Screen) • HepCYP1A2

4.2.4 Application of bioanalytical tools to recycled water quality assessment

Since the 1980s, bioanalytical tools have been incorporated in **validation and/or verification monitoring of water recycling schemes** [2]. Prior to 2000, most applications of bioanalytical tools to recycled water were to detect reactive toxicity, specifically mutagenicity and genotoxicity. In particular, the Ames test for mutagenicity [25] was widely applied. The Ames test, also known as the Salmonella mutagenicity test or the Bacterial Reverse Mutation Assay, measures the **mutagenicity of a test sample by its ability to induce mutations in specific Salmonella bacteria strains.** Unfortunately, bacterial cells have an inherently high degree of gene mutation, and the Ames test has a relatively high rate of both false positive and false negatives, which has challenged the value and significance of Ames test results [2]. After much initial enthusiasm in the promise of *in vitro* methods, the limitations of the Ames test had a negative impact on the perceived value of *in vitro* bioassays as a whole in the 1990s [2]. The development and application of new bioassays since then have led to renewed recognition of the value of bioanalytical tools for water quality monitoring, and bioassay batteries used for testing of water quality have since the mid-2000s expanded in both application and complexity [6] [7]. For a more thorough exploration of the application of bioanalytical tools in recycled water assessment, see Chapter 2 of Chapman and Leusch (2014) [22].

Table 4 highlights the recycled water schemes where *in vitro* bioassays have been used to analyse water quality (during validation and/or verification monitoring).

Table 4: Bioanalytical tools applied to recycled water assessment.

Scheme name	Years of operation	Country	Bioassays
Montebello Forebay Groundwater Recharge Project	1962 - present	USA (CA)	2 assays for mutagenicity [1]
Orange County Water Factory 21 (now Groundwater Replenishment System)	1975 - present	USA (CA)	1 assay for mutagenicity [1] [2]
Potomac Estuary Experimental Water Treatment Plant	1980 - 1982	USA (VA)	2 assays for mutagenicity [3] [1]
San Diego Total Resources Recovery Project	1981 - 1999	USA (CA)	4 assays (mutagenicity, genotoxicity) [1]
Tampa Water Resource Recovery Project	1987 - 1989	USA (FL)	2 assays (mutageniticity, genotoxicity) [1]
Tucson Reclaimed Water System	1989 - present	USA (AZ)	4 assays (estrogenicity, androgenicity) [4]
Five unidentified US water reclamation plants	2006 (analysis)	USA	1 assay for mutagenicity [5]
Windhoek Direct Potable Reuse Scheme	1968 - present	Namibia	5 assays (cytotoxicity to bacteria, cytotoxicity to human cells, mutagenicity, neurotoxicity, immunotoxicity) [6] [7] [8]
Dan Region Sewage Reclamation Project	1960 - present	Israel	1 assay for mutagenicity [9]
Perth Groundwater Replenishment Scheme	2009 - present	Australia (WA)	5 assays (cytotoxicity to bacteria, genotoxicity, estrogenicity, androgenicity, phytotoxicity) [10] [11]
Qld Western Corridor Recycled Water Scheme	2009 - present	Australia (Qld)	6 assays (cytotoxicity to bacteria, genotoxicity, AhR induction, estrogenicity, phytotoxicity, neurotoxicity) [12] [13]
Nine unidentified water reclamation plants in Australia	2010 - 2014 (analysis)	Australia	13 assays (cytotoxicity to human cells, mutagenicity, genotoxicity, endocrine activity, neurotoxicity, immunotoxicity, xenobiotic metabolism) [14] [15]
Landsborough Water Reclamation Plant	2003 - 2005 (analysis)	Australia (Qld)	3 assays (cytotoxicity to bacteria, estrogenicity) [16] [17]
South Caboolture Water Reclamation Plant	2010 - 2012 (analysis)	Australia (Qld)	6 assays (cytotoxicity to bacteria, genotoxicity, AhR induction, estrogenicity, phytotoxicity, neurotoxicity) [18] [17] [19] [20]
Gerringong Water Reclamation Plant	2012 (analysis)	Australia (VIC)	2 assays (cytotoxicity to bacteria, estrogenicity) [17]
One unidentified Qld water reclamation plant	2011 - 2012 (analysis)	Australia (Qld)	4 assays (cytotoxicity to bacteria, androgenicity, estrogenicity, genotoxicity) [21]
Two unidentified Australian water reclamation plants	2013 - 2014 (analysis)	Australia	103 assays (endpoints including non-specific, specific and reactive toxicity as well as adaptive stress response and xenobiotic metabolism) [22]

Notes:

1. ↑ [1.0] [1.1] [1.2] [1.3] [1.4] NRC (1998) Issues in potable reuse – The viability of augmenting drinking water supplies with reclaimed water, National Research Council (NRC), National Academy of Sciences, Washington DC, USA.

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3. ↑ NRC (1984) The Potomac Estuary Experimental Water Treatment Plant. A review of the U.S. Army Corps of Engineers, Evaluation of the Operation, Maintenance, and Performance of the Experimental Estuary Water Treatment Plant., National Academy Press, Washington, DC, USA.
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5. ↑ Drewes, J.E., Hemming, J.D.C., Schauer, J.J. and Sonzogni, W.C. (2006) Removal of endocrine disrupting compounds in water reclamation processes, p. 184, Water Environment Reseach Foundation and IWA Publishing, London, UK.
6. ↑ Faul, A.K., Julies, E. and Pool, E.J. (2013) Oestrogen, testosterone, cytotoxin and cholinesterase inhibitor removal during reclamation of sewage to drinking water. Water SA 39, 499-506.
7. ↑ Iiputa, G.I., Nikodemus, K. and Menge, J. (2008) Strategic drinking water quality monitoring for drinking water safety in Windhoek, Sun City, South Africa.
8. ↑ Menge, J.G. and Slabbert, J.L. (1999) Toxicity testing at Goreangab Reclamation Plant - past, present and future, Pretoria.
9. ↑ Gruener, N. (1978) Mutagenicity of ozonated, recycled water. Bulletin of Environmental Contamination and Toxicology 20(4), 522-526.
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11. ↑ Reitsema, T., Nice, H.E., Leusch, F.D.L., Quayle, P., Chapman, H.F., Khan, S.J., Trinh, T., Coleman, H., Rawson, C., Gagnon, M.M. and Blair, P. (2010) Development of an 'ecotoxicity toolbox' to characterise water quality for recycling, Department of Water, Government of Western Australia,, Perth, WA, Australia.
12. ↑ Macova, M., Escher, B., Mueller, J. and Toze, S. (2010a) Bioanalytical tools to evaluate micropollutants across the seven barriers of the indirect potable reuse scheme, UWSRA.
13. ↑ 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, 4238-4247.
14. ↑ Leusch, F.D.L., Khan, S.J., Laingam, S., Prochazka, E., Froscio, S., Trinh, T., Chapman, H.F. and Humpage, A. (2014b) Assessment of the application of bioanalytical tools as surrogate measure of chemical contaminants in recycled water. Water Research 49(0), 300-315.
15. ↑ NWC (2011) A national approach to health risk assessment, risk communication and management of chemical hazards from recycled water. Chapman HF, Leusch FDL, Prochazka E, Cumming J, Ross V, Humpage AR, Froscio S, Laingam S, Khan SJ, Trinh T, McDonald JA. Waterlines report No 48, National Water Commission (NWC), Canberra, Australia.
16. ↑ Leusch, F.D.L., Chapman, H.F., Körner, 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 & Technology 39(15), 5781-5786.
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4.2.5 What have bioanalytical tests shown?

In addition to being used for validation and/or verification monitoring applications, bioanalytical tools have also been applied to recycled water processes to **test a specific treatment train**.

As previously stated, most bioanalytical testing prior to 2000 focused on reactive toxicity, and specifically mutagenicity and genotoxicity. Several studies have tried identifying mutagenic and genotoxic compounds in water (mostly drinking water, reviewed in [26], [27], [28]). Those studies confirmed that chlorination by-products were likely the cause of the reactive toxicity in water. Several highly mutagenic compounds were identified, such as MX [2], but even those compounds could not account for the total reactive toxicity in water samples, and the identity of the causative compound(s) is still unclear to this day. The results however clearly emphasized that exposure to chlorination disinfection by-products in water should be minimized, although not at the cost of adequate disinfection and removal of pathogens.

More recent Australian studies at full scale plants have shown that:

1. **Conclusions on RO systems from Australian studies:** A suite of studies clearly showed that RO, which is an effective technique to remove organic contaminants, is likewise highly efficient at removing the biological response in *in vitro* assays. Some low residual activity is sometimes detected in membrane-based systems [29] [30] indicating that RO is an effective but not absolute barrier to biologically active compounds, as had been previously demonstrated for individual chemicals [31]. **RO should be used in combination with source control and complementary treatment options such as advanced oxidation (AO)**.
2. **Conclusion on ozonation/BAC systems from Australian studies:** Where ozonation and BAC were used, all of the tested final effluents produced only minimal biological response, if any, in the deployed *in vitro* bioassays. When biological activity was detected, it was always less than 10 times above the assay quantification limit or activity in the ultra-pure laboratory blank. This suggests that, even in those cases where biological activity was detected in the final effluent, activity is **unlikely to be of significant health concern**. Bioanalytical tools thus provide additional evidence that ozonation and BAC are effective technologies to produce high quality purified recycled water.
3. **Conclusion on UF systems from Australian studies:** UF is an effective technique to remove pathogens but is not effective at removing trace organic contaminants [31] or their associated biological response. This was clearly shown in the NWC study [30] [13], with UF/UV treatment having negligible effect on biological response associated with trace organic contaminants.

4.2.6 Frequently asked questions

Below is a list of frequently asked questions about bioanalytical tools in the context of recycled water quality. More information is available in Chapman and Leusch [22].

What do bioassays tell us about water quality?

In vitro bioassays (sometimes called bioanalytical tools) can provide a measure of water quality, just as chemical analysis can be used to determine water quality. Bioassays can detect **chemical contaminants by their biological effect** in the assay rather than by their chemical structure (which is how pollutants are detected by conventional chemical methods). Bioanalytical tools thus detect a wide range of contaminants and provide risk-scaled sum measure of all bioactive compounds that act via the mode of action that the assay detects. *In vitro* bioassays detect the initial interaction of the contaminant at the molecular or cellular level, and as such do not accurately predict toxicity in whole organisms (where defense and compensation mechanisms can eliminate the toxic effect). In other words, *in vitro* bioassays can be used as measures of exposure (i.e., to quantify chemical pollutants in water samples) but not measures of effect (i.e., to predict whole organism toxicity). *In vitro* bioassays testing does not replace conventional chemical analysis or whole animal toxicity testing, however it can fill some of the gaps left by our current chemical-by-chemical approach (specifically

detect unknown compounds and transformation products and provide a measure of mixture interaction) and, when used in an Integrated Testing Strategy, lead to a more rational and cost-effective assessment of water quality.

Bioanalytical testing can also be used to benchmark water samples (e.g., compare current drinking water sources with alternative water sources, or current drinking water with reclaimed water) and to determine the efficacy of different treatment technologies to remove bioactive compounds, including whether the process produced toxic transformation products.

What in vitro bioassays are available and suitable to recycled water?

In vitro bioassay methods can be classified into **five classes** based on their **mode of action**: non-specific, specific, and reactive toxicity, as well as adaptive stress response and xenobiotic metabolism. A good bioassay battery should always include at least one assay from each of the five classes. A large number of in vitro bioassay methods have been developed for screening purposes in drug development, and many (but not all) can be adapted to water quality testing. Prior to 2000, most studies applied only a few bioassays to test recycled water quality, usually only to test mutagenicity (Ames test). In the last decade many more endpoints have been included, such as endocrine activity, bacterial toxicity, photosynthesis inhibition, genotoxicity, immunotoxicity, etc. A recent study [23] tested 103 different bioassays with various water samples (including wastewater, surface water, stormwater, reclaimed water and drinking water) and concluded that the most relevant endpoints at this stage (although this of course may evolve with future research) were bacterial toxicity, estrogenic and glucocorticoid endocrine activity, oxidative stress, xenobiotic metabolism (specifically arylhydrocarbon and pregnane X receptor-mediated), mutagenicity and genotoxicity.

How do bioassays fit into and complement the suite of monitoring tools that can be used for water recycling?

In vitro bioassays are one suite of tools available for recycled water assessment, alongside conventional chemical analysis methods and whole animal toxicity testing. Each has its set of advantages and limitations. *In vitro* bioassay methods can complement conventional chemical methods in water quality assessment because they can 1) detect non-target chemicals, such as unexpected compounds and transformation products, and 2) provide a risk-scaled total measure of bioactive chemicals in the sample by combining potency (i.e., how toxic a chemical is) with concentration for each compound. One of the limitations of *in vitro* assays, however, is that they do not clearly identify the causative chemical(s), although some bioassays are particularly sensitive to certain classes of chemical compounds and can thus direct subsequent chemical analysis. Conventional chemical analysis thus complements bioassay methods because they can identify and quantify individual chemical compounds.

What information is generated by bioassay testing?

In vitro bioassays provide a sum measure of the bioactive compounds present in a water sample that act via a specific mode of action. For example, bioassays for estrogenic activity such as the ER-CALUX can detect any compound that can induce an estrogenic effect through an estrogen receptor genomic mediated effect, such as natural and synthetic hormones, bisphenol A and alkylphenols; bioassays for photosynthesis inhibition such as the I-PAM can detect any compound that can interfere with photosynthesis II in plants, such as herbicides. Depending on the assay, the total activity can be expressed as a bioanalytical equivalent (BEQ), such as estradiol equivalent (EEQ) or diuron equivalent (DEQ), or expressed in terms of how much the sample had to be concentrated (or diluted) to reach a pre-determined bioassay response (such as a toxic unit, or a relative enrichment factor). Bioanalytical tools thus provide a quantitative assessment of the concentration of bioactive compounds present in a water sample.

What are we trying to protect with bioassay testing?

In vitro bioassay methods are widely used during drug development by the pharmaceutical industry, and there is therefore a wide selection of bioassays available. The decision of which bioassay to use for a particular project is generally either driven by chemical consideration (e.g., for dioxin-like compounds, one might choose the AhR-CAFLUX; for herbicides, one might choose the I-PAM), but can also be protection-goal oriented (i.e., to assess recycled water quality for irrigation, one might pick an assay for bacterial toxicity such as the Microtox and an assay representative of important and sensitive plant function such as photosynthesis in the I-PAM). A battery of carefully selected bioassays can detect bioactive chemicals by their mode of action, where the mode of action is related to a negative health outcome (e.g., genotoxicity can lead to tumour formation, and assays for genotoxicity can provide a measure of the potential for carcinogenicity). It needs to be absolutely clear however that bioassay methods only detect the potential for harm, and do not correlate fully with whole organism effects. This is due to toxicokinetic and toxicodynamic modifiers of toxicity (such as absorption, distribution, metabolism and excretion), as well as to the simple fact that *in vitro* methods only detect the primary molecular or cellular response to chemical exposure. That cellular injury does not always lead to whole organism toxicity ("secondary response") thanks to defence and compensation mechanisms in whole animals that can either repair or compensate for the cellular injury.

Bioanalytical methods also provide a sum measure of the bioactive compounds present in a sample. As regulators are acutely aware of the growing (but still incomplete) list of compounds of interest in complex water matrices such as treated sewage, there is a clear need for methods that are able to detect not just those compounds that we know we need to look for (by chemical methods), but also those bioactive compounds that may be present without our knowledge. Bioanalytical tools can provide a simple method to address this to some extent. Certainly they are not perfect methods and will not detect all compounds in all situations, but the improved assessment is still better than not doing anything.

How do we communicate that the inclusion of bioassays is appropriate for water recycling on a cost/benefit basis?

Most people believe that bioanalytical tools will replace chemical methods. This is not correct. Bioanalytical tools provide a way to overcome some of the limitations of conventional chemical methods, but likewise conventional chemical methods overcome some of the limitations of bioanalytical tools. The great cost advantage of including bioassays in routine recycled water quality assessment, however, is that using a combination of carefully selected bioassays and chemical surrogates and indicators can allow the use of a much streamlined analysis, and avoid the need to monitor hundreds of chemical compounds. The application of an intelligent testing strategy that combines tier 1 screening with bioassays and surrogates/indicators, only followed by a more comprehensive tier 2 chemical analysis if those measures are above a pre-determined trigger level, provides a more rational and cost-effective approach to recycled water quality monitoring.

How is that information used? What actions are taken based on the data from these techniques? Who takes action?

There is currently no clear regulatory guidance on how to use the information from *in vitro* bioassays, and there is thus currently no regulatory implications for bioassay testing. Decades of experience however suggests that bioassays can be used to provide an improved monitoring programme with clear operational implications. An effective approach would be to apply the concept of Integrated Testing Strategy (ITS) used in chemical risk assessment. In ITS, a sample is first tested in a screening battery (Tier 1), and only those samples that exceed pre-determined trigger levels proceed to a more systematic chemical assessment (Tier 2). The results of tier 2 then determine whether further action is required, such as a more comprehensive toxicity assessment (Tier 3). Establishing relevant trigger levels is not a

simple and easy task, but several proposals have recently been published that offer a structured approach to establish so-called Effects Based Trigger levels (EBT) in *in vitro* bioassays.

A simple, rational and cost-effective approach would be as follows:

1. On-going Tier 1 screening: regular water samples are collected and tested using a combination of carefully selected bioassays and chemical surrogates and indicators. The results are compared with EBT and surrogate/indicator trigger levels. All samples below trigger levels do not require further testing. Samples that exceed any of the trigger levels would be re-tested to confirm that the exceedance is consistent. If confirmed, this then triggers further (Tier 2) investigations. As an acknowledgment that those trigger levels are not hard standards, the level of exceedance should drive the extent to which further investigations are conducted. For example, 100-fold exceedance of a carefully derived trigger level would warrant thorough investigations, while a 2-fold exceedance may be able to stop after tier 2 even if no causative compounds were identified. The point of all further investigations is to either identify the causative chemical (which can then be compared with guideline levels in the conventional risk assessment approach) or to identify a simple and effective treatment modification that can reduce the tier 1 response below trigger level.
2. Tier 2 chemical analysis: more thorough chemical analysis is conducted on any sample that exceeded the tier 1 trigger levels. This chemical analysis can be directed by the results of tier 1. For example, if the sample showed high estrogenic activity in an estrogenic assay, natural estrogens (17β-estradiol and estrone), synthetic estrogens (ethinylestradiol), bisphenol A and alkylphenol polyethoxylates (nonylphenol and octylphenol) would be targeted first. From this tier 2 chemical data, it is then possible to calculate a predicted bioanalytical equivalent and determine how much of the biological activity detected in tier 1 can be explained by the detected compounds. If most of the biological response is explained by the detected compounds (say >80%), then the conventional guideline approach is used by comparing the chemical concentrations (determined in Tier 2) with the relevant guidelines or standards to determine the need for further action. If however the tier 2 analysis does not identify the causative chemicals, the investigations would advance to tier 3.
3. Tier 3 advanced investigations: there are several methods to proceed, and the operators could choose to use all or select their preferred method:
    a. Tier 3(a): Conduct full chemical analysis for all relevant compounds in the pertinent guideline document and consult with regulator to determine if further action is necessary.
    b. Tier 3(b): Toxicity Identification and Evaluation (TIE): a conventional technique to identify toxic compounds in complex samples (TIE) can be used to chemically identify the most bioactive compounds. In TIE, the sample is treated using various methods that remove specific classes of compounds (e.g., air purging to remove volatile compounds, chelation to remove metals) or fractionated (e.g., using liquid chromatography to separate organic compounds by size or polarity) and re-tested in the bioassay to identify the class of compound responsible for the toxicity, which can then be further fractionated to identify the compound(s). If the causative compound is identified, its concentration would be related to a chemical guideline value and the need for further action determined using the conventional risk assessment method.
    c. Tier 3(c): Identify an effective removal method: using bench-scale testing, the operator could identify what (if any) treatment method (e.g., activated carbon, UV, sand filtration) or simple modification of current treatment can reduce the tier 1 response to below trigger level. If the changes can be easily implemented into the full scale treatment plant at minimal cost, then no further action is required. In

some instances the additional treatment may not be possible, but determining an effective removal method may help to identify the class of compound that is responsible for the tier 1 response.

How is the information generated by bioassays translated into language that can be used by regulators?

There are currently no bioassay-based guideline values, and bioassay testing therefore currently has no regulatory implications. It is important to realise that properly carried out bioassay analysis (i.e., including proper quality assurance and quality control samples, reference compounds, replication and analysing serial dilution series of positive samples) provides repeatable and quantitative results. Bioanalytical results can be expressed as bioanalytical equivalent (BEQ), such as estradiol equivalent (EEQ) or diuron equivalent (DEQ), or expressed in terms of how much the sample had to be concentrated (or diluted) to reach a pre-determined bioassay response (such as a toxic unit, or a relative enrichment factor). Bioassay results can be compared to Effects Based Trigger levels (EBT) to determine the significance of the bioassay result, and determine if further chemical characterisation of the sample is required (see above). Several approaches to derive EBT values have recently been proposed in the scientific literature. EBT values for assays expressed as BEQ can be relatively simply based on currently available chemical guidelines, while assay-specific EBT values can be determined for all other assays.

4.3 Epidemiological studies

4.3.1 Introduction

While the suite of operational processes, treatment advancements, and best industry practises are fashioned to protect consumers against the range of health risks in wastewater, concerns still remain surrounding the actual health outcomes to consumers given the nature of the source water (sewage) and the high level of contact (drinking). Communities considering potable reuse not only want to know that the entire system is technologically reliable, but additionally that public health is not put at risk in the move to provide adequate drinking supply. Although there have been no reports to suggest drinking water related outbreaks in regions where potable reuse has been implemented, this alone is insufficient to instil confidence in communities considering this alternative water. Estimating actual disease occurrences or lack thereof from potable reuse can be a challenging feat. Epidemiological study designs have been employed as a means of measuring these health effects.

4.3.2 Epidemiological studies

Establishing a firm relationship between potable reuse and health effects can be a difficult task to achieve, mainly because there are many other sources - other than drinking water - that can contribute similar ill health effects in individuals. Nonetheless, in the 40-year period that potable reuse has been practiced, a limited number of epidemiological studies have been conducted in three communities where potable reuse has been implemented (Table 4). The studies were all ecological in design and investigations included tracking health parameters such as enteric infections, respiratory infections, reproduction & birth outcomes, carcinogenic effects, and others.

Despite differences in water delivery options (DPR vs IPR), operational & treatment configurations, and proportions of recycled water blended into conventional sources at each study location, these investigations found no evidence to suggest significant increase in disease incidences owing to potable reuse practice in the parameters tested. The findings concluded that the health risks associated with potable reuse were **less than or similar to** those found in **conventional drinking supplies**. However, it must be noted that ecological studies in general and the ones presented here have several limitations and challenges:

- all studies were based on weak ecological study designs assessing health outcomes rather than employing more rigorous study designs such as randomised trials;

- difficulties determining who had been drinking the recycled water, **how much of it**, and **for how long**;
- chemicals of concern in water are **present in many other sources** (food for instance);
- health effects from chemical exposures tend to have **multiple causes** and take many **years to manifest**;
- **costly to administer**;
- **mobility of people** makes it difficult for investigators to follow populations for extended periods; and
- few global communities implementing planned potable reuse, therefore limited population exposure to drinking the recycled water.

Table 4: Epidemiological studies conducted in communities where potable reuse has been implemented.

Scheme location	Type of reuse	Study period	Study details	Results & Conclusions	Reference
Chanute, KA	Indirect potable reuse (surface recharge)	1956-1957	*Clinician observations and analyses of laboratory water quality results (Clinicians were questioned about illnesses observed during reclaimed water use period)	* Laboratory data showed safety of reclaimed water *No known adverse health effects resulting from recirculated water usage in the time period	Emergency use of reclaimed water from potable supply at Chanute [1]
Windhoek, Namibia	Direct potable reuse	2011	*Microbiological risk assessment (Examined the probability of risk infection from Giardia, Norovirus and Cryptosporidium using the quantitative microbial risk assessment)	*Probabilities of infection from Giardia and Norovirus were low compared to Cryptosporidium risks which were mainly due to process failures related to power supply *Suggestion of a UV-light treatment for *Cryptosporidium*	Microbiological risk assessment of the water reclamation plant in Windhoek, Namibia [2]
Windhoek, Namibia	Direct potable reuse (old Goreangab plant)	1974-1983	*Study examined the relationship between diarrhoeal disease, morbidity and mortality, and potable reuse between potable reuse consumers and non-users	*Reclaimed water was safe for human consumption based on bacteriological and virological water quality results *Differences related to socio-economic factors rather than the nature of water supply used	Health aspects of the use of recycled water in Windhoek, SWA/Namibia 1974-1983 [3]
Los Angeles, CA (Montebello Forebay)	Indirect potable reuse (groundwater recharge)	1969-1980	*Study conducted in 3 phases (1969-1971; 1972-1978; 1979-1980) (Examined household areas with high(>5%) and low(<5%) concentrations of reclaimed water added to their drinking	*No significant differences noted in reproductive issues between women (household survey results) *Minimal disease risk due to consumption of	Epidemiologic monitoring of possible health reactions of wastewater reuse; [4] Epidemiologic impact of water reuse in Los

			supplies compared to two control groups; 19 health outcomes examined; Mortality, morbidity, cancer incidences and birth outcomes which included reproductive repercussions in women)	reclaimed water *Health effect differences could not be attributed to increased incidences of using reclaimed water	Angeles county [5]
Los Angeles, CA (Montebello Forebay)	Indirect potable reuse (groundwater recharge)	1987-1991 (Study conducted to update the 1969-1980 study)	*Compared populations receiving reclaimed water (up to 31%) with control groups on cancer, mortality, morbidity, and infectious disease outcomes	*Rates of cancer, death and infectious disease similar between control and potable reuse consumers (independent of high or low usage) *No evidence that reclaimed water use had adverse health effects	Groundwater recharge with reclaimed water: an epidemiologic assessment in Los Angeles County, 1987-1991 [6]
Los Angeles, CA (Montebello Forebay)	Indirect potable reuse (groundwater recharge)	1982-1993	*Adverse birth (reproductive) outcomes due to reclaimed water use (Cohort study design used; 19 birth defects included)	*No relationship between reclaimed water use and adverse birth outcomes	Groundwater recharge with reclaimed water use: birth outcomes in Los angeles county, 1982-1993 [7]
Aqua II, Mission Valley, San Diego, CA	Direct potable reuse	1985-1987	*Developed a baseline of morbidity and mortality parameters for residents in case direct potable reuse was implemented (Examined reproductive health and vital statistics)	*Health risk from using reclaimed water was less than or equal to conventional water supplies	Recycled water - A source of potable water: city of San Diego health effects study [8]
Aqua III, Pasqual Valley, San Diego, CA	Direct potable reuse	1994-1995 (Update of the Aqua II plant study)	*Evaluated the potential risks associated with reclaimed water in comparison to existing city supply	*Treated wastewater supply did not provide elevated health risks to consumers more than city's water supply	The City of San Diego's Total Resource Recovery Program: Health Effects Study on Potable Water Reuse [9]

Because of the studies' conclusions, reliability of the systems in place to protect public health, and consistently high quality water produced in these systems, there has been no move to conduct additional health surveillance studies. Nonetheless, as more global regions implement potable reuse projects in their communities, it may be possible to conduct more rigorous, cost-effective studies that can more convincingly demonstrate an absence of negative health impacts from potable reuse.

4.3.3 Water-borne outbreaks

Although communities may prefer conventional drinking water sources compared to the idea of potable reuse, conventional water systems aren't free of failure events which can result in

adverse health effects. Past reports have shown that when drinking water systems fail, the greatest threat to public health is exposure to and subsequent infection with **water-borne pathogens** and their associated diseases. Many studies have documented drinking water outbreaks that have occurred as a result of weaknesses in conventional drinking water systems in many developed nations, including Australia [10]. Common examples include the Walkerton (Canada) [11] and Milwaukee (USA) [12] outbreaks which exposed large communities to contaminated drinking water, leaving many sick and even resulting in fatalities. Nonetheless, the presence of pathogens in drinking water does not always translate to disease transmission. For an event to be classified as a drinking water outbreak, **two or more** people would have to become infected with the same pathogen, present with the same disease symptoms after consumption of the **same water source**, and there is strong **epidemiological evidence** that implicates the water source as the **vehicle of infection** [13]. Several factors determine whether or not infection is established:

- the type of pathogen;
- quantity and distribution in the water;
- dose necessary for infection;
- effectiveness of treatments/barriers prior to distribution; and
- health status of end user.

A review of potable reuse systems found that there have been **no reported incidences of drinking water-related outbreaks in communities implementing potable reuse**. This is encouraging and supportive of the reliability of the preventive measures in place to protect public health. However, certain limitations must be noted, mainly that outbreaks are often not recorded, with capacity for identification and investigation varying between and within countries, and that potable reuse makes up only a tiny fraction of current drinking water supplies. An analysis of recent (2003-2013) drinking water outbreaks in conventional settings of 19 developed nations identified **>2000** outbreak reports as a result of water system failures and consequent microbial contamination of sources (data extracted from the Global Infectious Disease Online Network (GIDEON) database). Despite this large number of reports in GIDEON, published data records were limited and available for only **70 outbreaks** where failures were directly linked to the conventional municipal drinking water system (Figure 3).

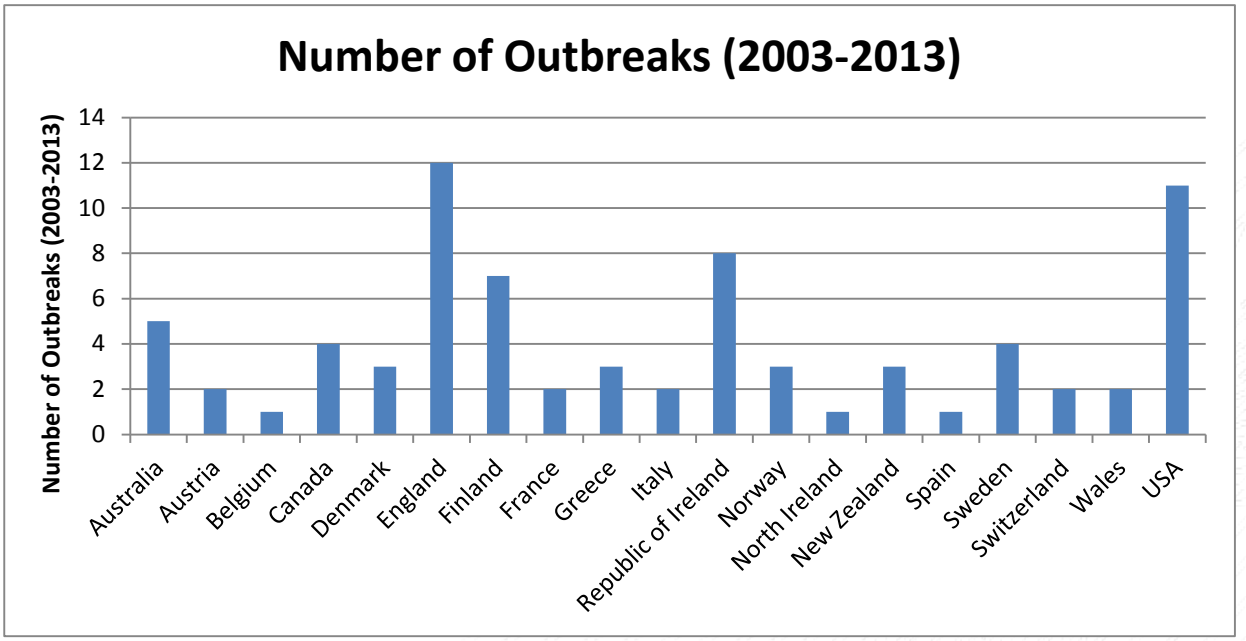

Figure 3: Number of published disease outbreaks in developed countries linked to the conventional municipal drinking water system.

4.3.4 Outbreak detection

In addition to planned epidemiological studies, there exists in many communities, routine collections of health-related outcomes that may have some facility in relation to acute health effects in drinking water systems. In Australia, there is an extensive list of notifiable diseases, including several of concern in water-borne transmission (e.g cryptosporidiosis). **Laboratory confirmation** has been the more widely used technique to identify and confirm pathogenic influences in drinking-water related outbreaks. However, laboratory testing and confirmation is sometimes untimely and notification of the causes of gastroenteritis is often uncommon, therefore only major outbreaks are likely to be detected through this system.

Syndromic Surveillance

In recent years, there has been a focus on a form of surveillance - **syndromic surveillance** - that has the potential to provide more sensitive measures of infectious disease incidence.[14] Syndromic surveillance, as its name suggests, focuses on **data sources** that relate to health syndromes or other activities that may be correlated with outbreaks of infectious disease, rather than requiring specific identification of pathogens. Examples include:

- increased sales of classes of drugs (over the counter (OTC) remedies or prescribed enteric medications);
- increased sales of bottled water;
- hand sanitisers and other sanitary products;
- school and work absenteeism or closures;
- increased complaints or queries to water departments;
- increased calls to national health help lines; and
- increased incidence of web-based queries (as used successfully in Google flu trends).

Syndromic surveillance data sources can provide real-time information about outbreaks as they occur and in that sense tends to be more sensitive and timely than more traditional methods, but with less specificity. Syndromic surveillance has been used in the early detection of diseases such as influenza and pneumonia.[14][15][16] In relation to detection of water-borne illness, there have been mixed results from such systems. Research conducted in San Francisco for example, using OTC drug sales analysis to predict a gastrointestinal infection, found no correlation between the two [17].

4.3.5 Lessons learned

- Drinking water related outbreaks (associated with traditional sources) in developed nations are rare, but still occur.
- Outbreaks are mostly associated with surface water sources open to seasonal flow changes and wastewater effluent contamination.
- Points of the water network susceptible to failures are: catchment, treatment system, and the distribution network.
- Outbreak prevention requires more than good treatment technology. Implementation of best operational & maintenance practices were important factors to be considered too, including:
    - good risk assessment and management plans;
    - using multiple barrier approaches;
    - rigorous water quality monitoring;
    - proper maintenance of infrastructure;
    - employing well trained and skilled workers;
    - developing comprehensive water safety plans and corrective actions; and
    - collaborative initiatives between the multidisciplinary agencies (water professionals, policy makers, and public health units).
- The need for surveillance of emerging water-borne pathogens, including timely detection and notification techniques.

5 Global Potable Reuse Case Studies

5.1 Global water reuse

There are over **3000** water reclamation sites around the world where wastewater (domestic, commercial, industrial and stormwater) is reclaimed, [1] with schemes operating in all continents including Australia.

Reclaimed wastewater is used for **potable** and **non-potable** purposes (Figure 4) which include:

- potable reuse (**augmenting drinking water supplies either directly or by blending**)
- agricultural and landscape **irrigation**
- urban domestic use (toilet flushing, laundry use, car washing)
- recreational use (in rivers, lakes, streams)
- **environmental** uses (aquifer recharge, seawater barrier, improve quality of waterways)
- industrial usage

Figure 4: Potable and non-potable uses of recycled water.

5.1.1 Potable Reuse

Potable re-use can either be **planned** or **unplanned**. Planned reuse is where municipal wastewater sources are **purposefully** utilised to augment existing potable supplies through either aquifer recharge or surface water supplementation. In unplanned potable reuse, wastewater (treated or untreated) is added (intentionally or unintentionally) to a water body (lake, river, stream etc) that is used by a community **downstream** as their source of potable water following traditional treatment. This latter practice also termed **unplanned indirect potable reuse** or de facto reuse is considered to be common place in many communities around the world [2].

Nonetheless, planned potable reuse schemes are increasing in number, with at least **27** schemes identified globally, practicing a diversity of water delivery options including:

- *direct potable reuse* (DPR) eg. in Windhoek, Namibia [3]
- *indirect potable reuse* (IPR) eg. in Orange County, California [2] and piloted in Perth and Brisbane, Australia [4]
- *de facto* / *unacknowledged reuse* / *unplanned indirect potable reuse* eg. in the UK, Netherlands, Australia, USA.

5.1.2 Unsuccessful reuse scheme proposals

Despite the increased demand for water resources and the need to conserve and augment strained potable supplies in many communities, some proposed potable reuse schemes have been unsuccessful. Many around the world (both potable and non-potable) have failed to be implemented primarily due to public opposition and community attitudes towards water recycling [5]. Lack of trust in water utilities, perceived health risks, psychological barriers and environmental concerns were some of the factors identified in these instances. Alternative studies also found that acceptance of reuse declined with increased likelihood of personal contact, with the lowest level of acceptance being for cooking or drinking [6]. Examples of where potable reuse project proposals have not been implemented include:

- Toowoomba IPR, Queensland, Australia - community opposition [7]
- Tampa Water Resource Recovery Project, Florida, USA - community opposed to potable reuse but accepted non-potable reuse
- East Valley Water Recycling Project, Los Angeles, USA - community opposition to groundwater replenishment
- San Diego Total Resource Recovery Project, San Diego, USA - community opposition.

5.1.3 Exploring successful potable reuse schemes

A set of **seven** case studies has been prepared to provide examples of current global potable reuse schemes and these are available for downloading from the *Water360* website. Detailed information has been compiled including **scheme overview, operational infrastructure, water quality data & public health factors, and a portfolio of engagement and educational strategies**. The research reviewed the practise of potable reuse by evaluating published literature, online resources, and personal communication received from the seven global potable reuse sites. These schemes were chosen either reflecting their international importance, or in the Australian context, to illustrate the performance of existing pilot schemes.

These schemes, and a range of other global water reuse initiatives, can be located and explored in more detail on the Global Connections Map and on the *Water360* website.

International Schemes:

- Groundwater replenishment system, Orange County, California, USA;
- Upper Occoquan Service Authority, Fairfax, Virginia, USA;
- NEWater, Singapore;
- New Goreangab Water Reclamation Plant, Windhoek, Namibia; and
- Torreele/St. Andre Water Reclamation Plant, Koksijde, Belgium.

Australian Schemes:

- Western Corridor Recycled Water Scheme, Brisbane, Queensland; and
- Groundwater Replenishment Trial, Perth, Western Australia.

6 References

Section 1 (Public Health)

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