Australian Water Recycling
Centre of Excellence

Demonstration of robust water recycling: Monitoring the levels of trace organic chemicals (TrOCs)

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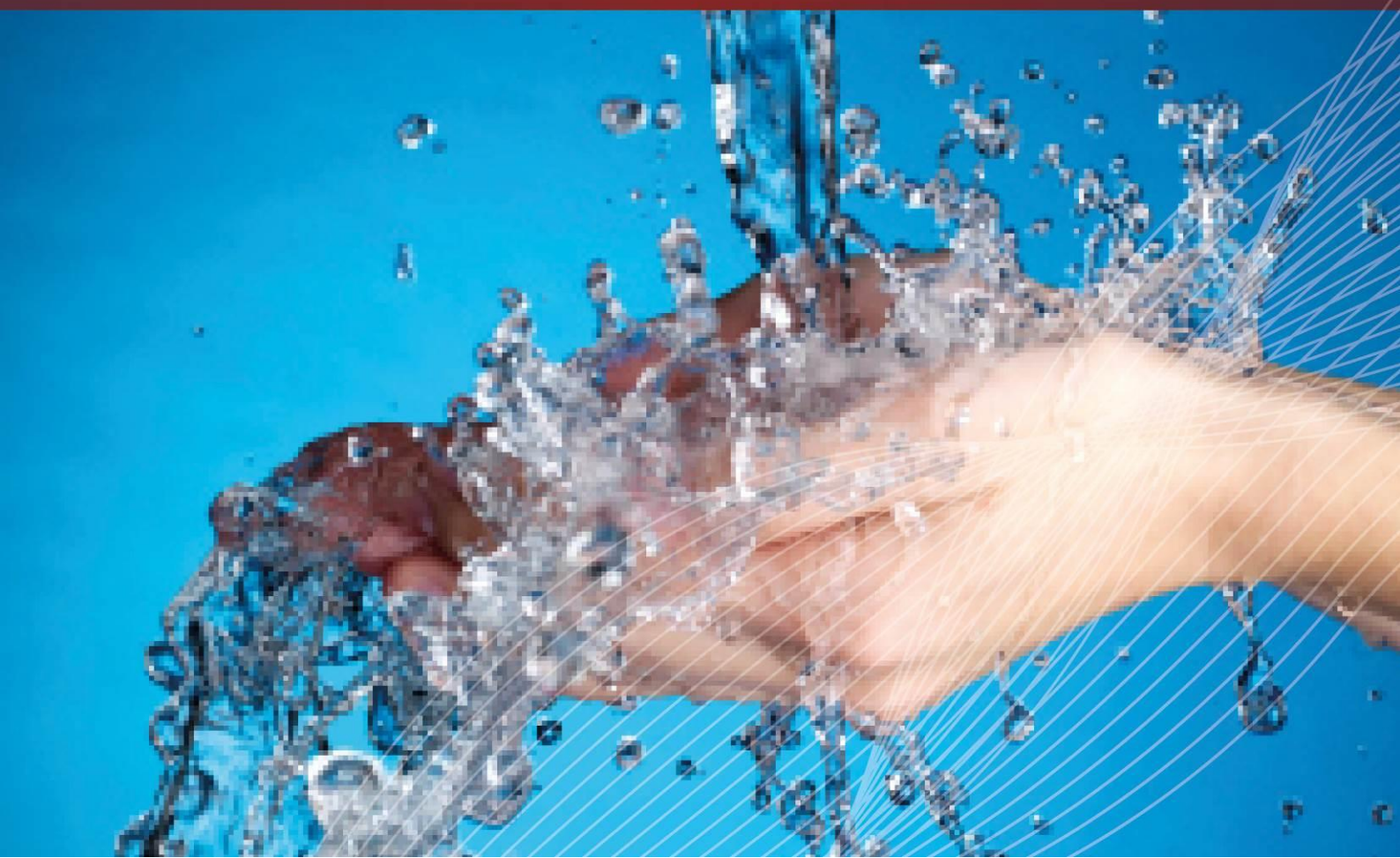
Australian Water Recycling Centre of Excellence

Graeme Allinson, Mayumi Allinson, Kiwao Kadokami, Fujio Shiraishi, Daisuke Nakajima, Jinhua Zhang, Adrian Knight, Stephen Gray, Peter Scales, July 2015



Demonstration of robust water recycling: Monitoring the levels of trace organic chemicals (TrOCs)

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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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Executive Summary

The overall aim of the 'Demonstration of robust water recycling' project is to trial a recycling system capable of producing potable water that requires minimal operator involvement (making it suitable for small and/or remote communities) and a non-toxic by-product wastewater (that can be discharged to the environment with minimal impact). The specific objectives for the TrOCs monitoring team were to:

1. Obtain background water quality information from Self's Point WWTP (to provide background information for demonstration plant operators)
2. Verify that the demonstration plant is operating effectively by screening feed, barriers' and outlet waters for a wide range of TrOCs using multi-residue mass spectrometric techniques**.**
3. Verify that the demonstration plant is operating effectively by screening feed, brine (RO) concentrate and product water for TrOCs of the same mode of biological action ("hormonal activity") using recombinant receptor-reporter gene bioassays**.**
4. Verify that the demonstration plant is operating effectively by screening feed, barriers' and outlet waters for nitrosamines**.**
5. Assess whether the water recycling process produces a brine concentrate fit for disposal in the Antarctic marine environment, and a recyclable product water through comparison of TrOC detection data with regulatory guidelines and ecotoxicology data.

In this study we used two chromatographic-mass spectrometric multi-residue methods to screen TrOCs in feed, environmental discharge (a brine concentrate), and product water. We were able to unambiguously detect almost 80 chemicals in the feed water, but only 20 chemicals in the product water and only 16 chemicals in the environmental discharge (brine concentrate). In that context, we conclude that **most TrOCs were removed from the feed water by the treatment train**.

No residue in the product water exceeded its listed Australian Guidelines for Water Recycling (Augmentation of Drinking Water Supplies) (NRMMC, EPHC, NHMRC 2008) level and all residues were at least four orders of magnitude lower in concentration than the draft drinking water guideline levels (DWGs) calculated by this study. Consequently, we conclude **that drinking the TrOCs observed at the concentrations observed in this study would be unlikely cause adverse effects** on people.

No residue in the brine concentrate waste stream exceeded an ANZECC & ARMCANZ (2000) water quality guideline trigger value for marine waters, or our calculated guideline levels using research quotient and toxicity unit methods of assessment. Consequently, we conclude **that releases of the TrOCs observed at the concentrations observed in this study would be unlikely cause adverse effects** on populations of aquatic organisms in the receiving environment

Sample toxicity was measured using a photobacterium toxicity test. Collected samples of the brine concentrate were only weakly or non-toxic, suggesting that it would have little impact on aquatic organisms and was fit for disposal to the marine environment.

The recombinant receptor-reporter gene bioassay data obtained suggests that there are estrogenic chemicals in the feedwater (human estrogen receptor (hER) activity up to ~ 13 ng/L estradiol equivalents (EEQ); medaka estrogen receptor (medER) activity, up to ~ 23 ng/L EEQ), but that these chemicals are being removed by the treatment train with little or no estrogenic activity observed in product water or brine concentrate.

There are chemicals that stimulate the aryl hydrocarbon (AhR) receptor in the feedwater (AhR up to ~ 380 ng/L β-naphthoflavone EQ), but that these chemicals are mostly being removed by the treatment train, with little AhR activity observed in product water or brine concentrate.

The constitutive androstane receptor (CAR) receptor data is consistent with that reported for the ER and AhR assays, i.e. that there are chemicals that stimulate the CAR receptor in the feedwater (CAR up to ~ 3.4 μg/L p-tert-octylphenol EQ), but that these chemicals are mostly removed by the treatment train.

The TrOCs team conducted six rounds of sampling for N-nitrosamines. A small amount of NDMA (< 10 ng/L on average) entered the plant in the feed water. The concentration of NDMA then increased post-ozone, and remained relatively constant at ~ 45 ng/L until the RO concentrate, but was not observed post-RO in the environmental discharge water (the brine concentrate) or in the product water. In that context, we conclude that the final product water quality met the ADWG for NDMA (<100 ng/L), and that **NDMA releases at the concentrations observed in this study would be unlikely cause adverse effects on populations of aquatic organisms** in the receiving marine environment.

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Glossary of Terms

Abiotic	Non-living factors of the environment, including light, temperature, inorganic soil particles and rocks, water, and atmospheric gases
ADWG	Australian Drinking Water Guidelines
Agrochemical	For the purpose of this report an agrochemical includes any substance or organism used to: • Destroy, stupefy, repel, inhibit the feeding of, or prevent pests on plants or other things; • Destroy a plant or to modify its physiology; • Modify the effect of another agricultural chemical product; or • Attract a pest for the purpose of destroying it. This encompasses all herbicides, insecticides and fungicides. Dairy cleansers for on-farm use, crop markers, insect repellents for use on humans, swimming pool disinfectants, algaecides, rodenticides, antifouling paints, timber preservatives, some pest traps and barriers using chemical attractants, and household and home garden products for pest and weed control are also encompassed by the above definition. Fertilisers are not considered an agrochemical for the purposes of this report unless they modify the physiology of a plant.
Agonist	A chemical which initiates a physiological response when combined with a receptor
AGWR	Australian Guidelines for Water Recycling
AhR	Aryl hydrocarbon receptor
Androgen	Generic term for any natural or synthetic compound, but usually a steroid hormone, that stimulates or controls the development and maintenance of masculine characteristics in vertebrates by binding to androgen receptors. This includes the activity of the accessory male sex organs and development of male secondary sex characteristics. Androgens are also the precursor of all estrogens, the female sex hormones. The primary and most well-known androgen is testosterone
AIQS-DB	Automated Identification and Quantification System - database
ANZECC	Australia and New Zealand Environment and Conservation Council.
Aquatic life	The biological life (e.g. algae, fish, frogs etc.) in or on fresh, marine or estuarine waters (surface or ground waters).
ARMCANZ	Agriculture and Resource Management Council of Australia and New Zealand
Bioassay	A test that exposes living organisms to several levels of a substance that is under investigation, and evaluates the organisms' responses
CAR	Constitutive androstane receptor

Contaminant	A chemical that is present in the environment as a consequence of anthropogenic activity. A material described as a 'contaminant' is one that is either not naturally present in the environment being examined, or is present in unnatural concentrations. However, in being described as a contaminant, no judgement is being made about whether or not the material is having an adverse effect on the environment, or organisms therein – the material is simply present in the environment.
DBP	Disinfection by-product
Drinking water	Water suitable for human consumption without deleterious health risk. Synonymous with 'potable water,' but the preferred term since it is better understood by the community at large
Endocrine disrupting chemicals	Exogenous agents that interfere with the production, release, transport, metabolism, binding, action, or elimination of the natural hormones in the body (of a human and/or wildlife species) responsible for the maintenance of homeostasis and the regulation of developmental processes. Also defined as: • Exogenous substance or mixture that alters function(s) of the endocrine system and consequently causes adverse health effects in an intact organism, or in its progeny, or (sub)-populations.
Environmental hazard	Anything with the potential to cause injury, illness and damage to both living and non-living things within the environment. A danger posed to the environment, whether imminent or otherwise, resulting from any activities, practices, the location, storage or handling of any substance having toxic, corrosive, flammable, explosive, infectious or otherwise dangerous characteristics (adopted from the Environment Protection Act 1970 (Vic), Section 4).
Environmental impact:	Any impact on plants, animals or the environment caused by human activities is an environmental impact. Impacts may be reversible or irreversible, minor or major, affect a whole ecological community or only a few individuals.
Environmental impact assessment	Environmental impact assessment (EIA), also called ecological risk assessment (ERA), is the practice of measuring or estimating the nature and likelihood of effects of an action (e.g. the application of pest control products or practices) on one or more environmental parameters.
Estradiol	Also oestradiol, or 17β-estradiol, this is the major sex hormone in female vertebrates, although it is also produced by males. Estradiol represents the major estrogen in humans. Estradiol has not only a critical impact on reproductive and sexual functioning, but also affects other organs including bone structure
Estriol	Also oestriol, is one of the three main estrogens produced by humans, although this steroid hormone is only produced in significant amounts during pregnancy (as it is made by the placenta).
Estrogen	Generic term for any natural or synthetic compound, but usually a steroid hormone, that stimulates or controls the development and maintenance of masculine characteristics in vertebrates by binding to estrogen receptors.

	This includes the activity of the accessory female sex organs and development of female secondary sex characteristics. The primary and most well-known androgen is estradiol
Estrone	Also oestrone, is an estrogenic steroid hormone derived from androstenedione secreted by the ovary. The least prevalent of the three major steroid estrogens (estradiol being most prevalent), estrone is relevant to health and disease due to its conversion to estrone sulfate, a long-lived derivative that acts as a pool of estrone which can be converted as needed to the more active estradiol. Estrone enters a wastewater treatment system either directly from excretion of humans (in the free form or as glucuronide or sulfate conjugates) or from the oxidation of 17β-estradiol in the treatment plant itself.
GC-MS	Gas chromatography – mass spectrometry
Guideline	Numerical concentration limit or narrative statement to support and maintain designated water use
Guideline trigger value	These are the concentrations (or loads) of the key performance indicators measured, below which there exists a low risk that adverse biological (ecological) effects will occur. They indicate a risk of impact if exceeded and should 'trigger' some action, either further specific investigations or implementation of management/remedial actions.
hER	Human estrogen receptor
In vivo	(Biological) process occurring or made to occur within a living organism or natural setting.
In vitro	(Biological) process made to occur in a laboratory vessel or other controlled experimental environment rather than within a living organism or natural setting
LC-MS	Liquid chromatography - mass spectrometry
LC-MS/MS	Liquid chromatography - tandem mass spectrometry
LC-TOF-MS	Liquid chromatography - time of flight mass spectrometry
LOR	Limit of Reporting; the smallest concentration or amount of a substance that can be reported as present with a specified degree of certainty by an analytical procedure
ND	Not detected
PAH	Polynuclear aromatic hydrocarbon
PB test	Photobacterium test. A baseline bioluminescence inhibition toxicity test using the photobacterium *Vibrio fischeri* adapted for a 96-well plate. Toxicity is correlated to the amount of luminescence emitted by the bacteria. In this study, toxicity is reported as an ICR50 value, which is effectively how much the sample would have had to be diluted to inhibit luminescence in 50% of the photobacteria. In short, the lower the ICR50 reported, the higher the

	toxicity of the sample (and vice versa, i.e. the higher the ICR50, the lower the toxicity).
PCB	Polychlorinated biphenyl
Pesticide	See 'agrochemical.'
Potable water	Water suitable for human consumption without deleterious health risks
PPCPs	Pharmaceutical and personal care products
Recycled water	Water recycled from the effluent of sewage treatment plants (synonymous with reclaimed water)
Reclaimed water	Water which, as a result of treatment of waste, is suitable for a direct beneficial use or a controlled use that would not otherwise occur (synonymous with recycled water)
Teratogenicity	The potential of a chemical to cause structural malformations or defects in offspring; the production of structural malformations or defects in offspring.
TrOC	Trace organic chemical
Toxicant	A chemical that can produce adverse health effects
TU	Toxicity unit
Yeast bioassay	High-throughput cell-based in vitro toxicity testing method developed to target levels of chemical toxicity pathways.
WWTP	Wastewater treatment plant

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The Authors would also like to thank Michael Packer (AAD) and Kathy Northcott (Veolia) for their help with this program, particularly their insights into the operation of the AWTP and other WWTPs.

1 Introduction

The Australian Antarctic Division (AAD) operates Australia's Davis Station in the Antarctic. In 2005, Davis station's wastewater treatment plant failed, and since then untreated, macerated effluent has been discharged to the ocean. Although disposal of the station's effluent by this method meets the minimum requirements specified by international agreements, an environmental impact assessment has identified that there is a clear a need for enhanced sewage treatment

The AAD and has decided to install a new advanced water treatment plant (AWTP) in conjunction with a secondary waste water plant to ensure discharges to the environment meet world's best practice. The AWTP will also have the potential to augment water supply to the station. The process requirements of the AWTP are small (~20 kL/day) and although the inputs to the plant are source defined, without the dilution achieved in large scale water treatment plants, there is potential for spikes in chemical contaminants.

The AWTP was designed and built by Victoria University, the University of Melbourne, the Australian Antarctic Division, Veolia Water and AECOM, and supported by the Australian Water Recycling Centre of Excellence. The plant was operated at a waste water treatment plant (WWTP) in Tasmania (Selfs Point) and underwent testing over twelve months. The feed water (secondary effluent) is treated through a multi-barrier process involving ozonation, ceramic microfiltration, biologically activated carbon filtration, reverse osmosis, ultraviolet disinfection and chlorination. The outputs of the plant are a brine concentrate from the reverse osmosis barrier and water suitable for recycle.

The overall aim of the 'Demonstration of robust water recycling' project is to trial a recycling system capable of producing recyclable water that requires minimal operator involvement (making it suitable for small and/or remote communities) and a non-toxic by-product wastewater (that can be discharged to the environment with minimal impact).

1.1 Scope of works

The scope of the TrOCs monitoring team was limited to the analysis of field-collected water samples on a monthly basis, with additional desk-top examination of the data to assess the environmental and human health risks of trace organic chemicals observed in the brine concentrate and product water, respectively.

1.2 Aims and Objectives

The broad aim of the TrOCs monitoring was to demonstrate that the water recycling process produces a saline effluent fit for disposal to the aquatic environment, and a product water fit for recycling. In that context, specific objectives for the TrOCs monitoring team were to:

1. Obtain background water quality information from the Selfs Point WWTP (to provide background information for demonstration plant operators)
2. Verify that the demonstration plant is operating effectively by screening feed, barriers' and outlet waters for a wide range of TrOCs using multi-residue mass spectrometric techniques.
3. Verify that the demonstration plant is operating effectively by screening feed, RO concentrate and outlet waters for TrOCs of the same mode of biological action ("hormonal activity") using recombinant receptor-reporter gene bioassays.
4. Verify that the demonstration plant is operating effectively by screening feed, barriers' and outlet waters for nitrosamines.

5. Assess whether the water recycling process produces a brine concentrate fit for disposal in the Antarctic marine environment, and a recyclable product water through comparison of TrOC detection data with regulatory guidelines and ecotoxicology data.

1.3 Sub-project outcome

By June 2015, the 'Robust Recycling' team, Australian Antarctic Division, Australian Water Recycling Centre of Excellence and project partners will have information on the extent of residues of TrOCs in the plant Feed Water, environmental discharge (brine concentrate) and final Product (recycled) water, as well as after selected barriers within the plant, with which to assess overall and barrier performance in removing TrOCs, and potential risks to the environment and human heath.

2 Summary of methods

2.1 Sampling and analysis

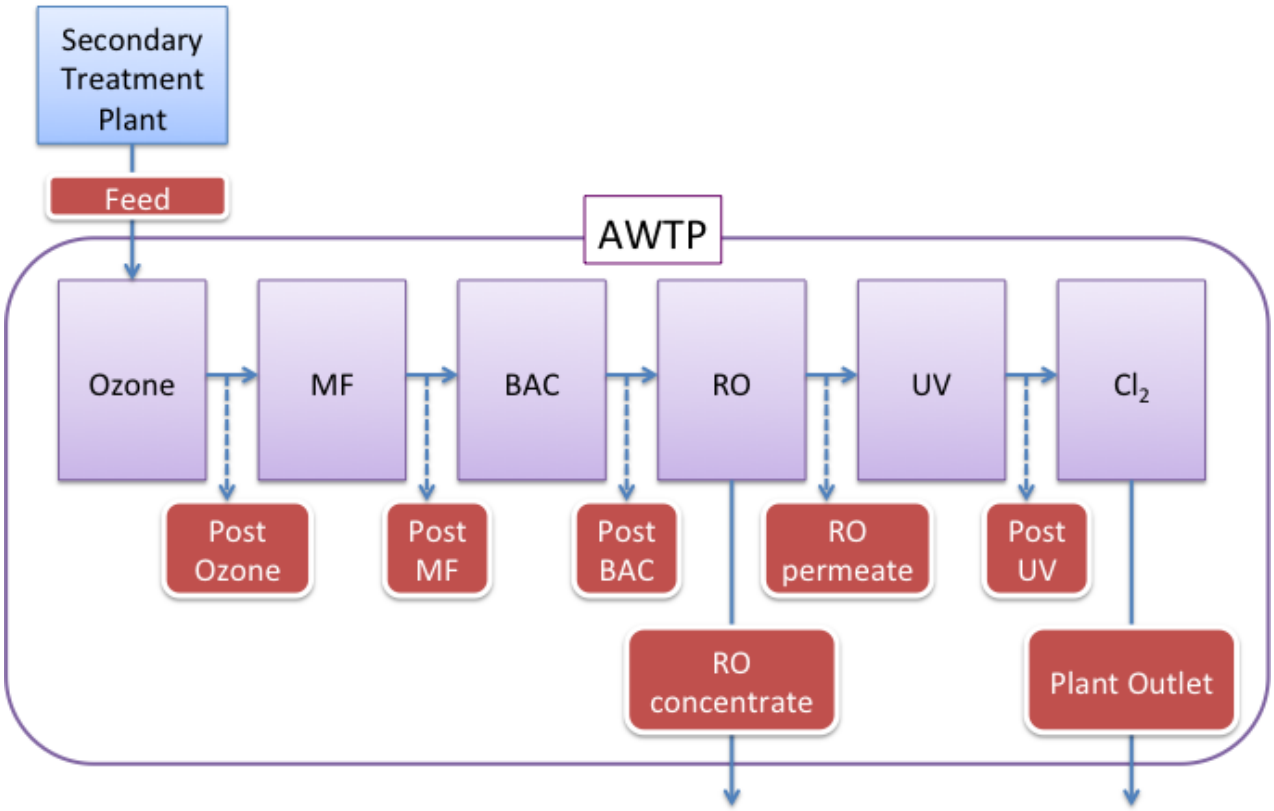


Figure 2.1 Summary of sampling locations at AWTP

Unfiltered water samples were collected from up to 8 locations in the AWTP (Figure 2.1) and sent stored on ice to the project's laboratory in the School of Chemistry at the University of Melbourne. Within 24-48 hours, water samples were split into four exact volumes for the different sample process procedures: 1 L each for GC-MS analysis and yeast bioassay, 0.5 L for nitrosamine analysis, 0.2 L for LC-MS analysis with addition of appropriate buffers and/or internal standards or other reagents. All samples were extracted by solid phase extraction (SPE) methods using appropriate sorbent media for each method: Empore™ SPE disks (SDB-XC for GC and C18FF for bioassay), coconut charcoal cartridges (nitrosamines), and SepPak PS-2 and AC-2 SPE cartridges for LC-MS. The samples were refrigerated at the SPE extract stage until analysis. For full details of sample extraction, see Appendix A

Professor Kadokami (University of Kitakyushu (UKK)) has innovative gas chromatography – and liquid chromatography mass spectrometry (GC-MS and LC-MS/MS) systems that together can screen samples for more than 1200 TrOCs (Kadokami et al. 2005; Kong et al. 2015). These multi-residue methods were used to screen for organic pollutants that would not be otherwise possible to measure. Full analytical method details are provided in Appendix A

NDMA concentrations were determined by UNSW Water Research Centre using the method described in McDonald et al. (2012). This method separates the desired analytes from the sample mixture using solid phase extraction (SPE), with determination using gas chromatography (GC) - tandem mass spectrometry (MS-MS) with electron ionization (EI). The target chemicals were N-nitrosodimethylamine (NDMA), N-

nitrosomethyethylamine (NMEA), N-nitrosodiethylamine NDEA), N-nitrosodipropylamine (NDPA), N-nitrosodi-n-butylamine (NDBuA), N-nitrosodiphenylamine (NDPhA), N-nitrosopyrrolidine (NPyr), N-nitrosopiperidine (NPip), and N-nitrosomorpholine (NMorph).

Managing the effects of large numbers of contaminants ultimately requires information on sample toxicity and receptor activity. In that context, measurement of toxicity on sample extract was conducted using the PB test method of Shiraishi et al. (1999), with data reported as the ICR50, which is a measure of how much the sample has to be concentrated to inhibit luminescence in 50% of the photobacteria. Full toxicity test method details are provided in Appendix B

Measurement of estrogenic and xenobiotic activity was undertaken with a yeast two-hybrid recombinant receptor-reporter gene bioassay system in accordance with the method of Shiraishi et al. (2000) using yeast cells (*Saccharomyces cerevisiae* Y190) into which the human estrogen receptor ERα or the estrogen receptor from Japanese medaka (*Oryzias latipes*) or human constitutive adrostane receptor CAR had been inserted (hERα, medERα and CAR, respectively; Nishikawa et al. 1999). Measurement of AhR activity was undertaken in accordance with the method of Kamata et al. (2009) using yeast cells (YCM3) carrying the response element for the AhR complex, XRE5 (Miller 1999).

Positive controls were used with all assays: hERα and medERα assays, 17β-estradiol (Wako Pure Chemical Industries Ltd, Osaka, Japan); CAR, p-tert-octylphenol (OP; Wako Pure Chemical Industries Ltd, Osaka, Japan); AhR, β-naphthoflavone (βNF; Wako Pure Chemical Industries Ltd, Osaka, Japan). A solvent (vehicle) control (DMSO, Nacalai Tesque Inc., Kyoto, Japan) was used in all cases. The agonist activities of the A/D and MeOH florisil fractions of the sample extracts were measured; data is reported as the sum of the activity observed in all two fractions. The bioassay method's limits of reporting (LOR) for the hERα and medERα systems were 0.1 and 0.3 ng/L 17β-estradiol equivalents (EEQ), respectively; for the CAR assay, 7 ng/L OPEQ; and for the AhR bioassays, 2 ng/L βNFEQ. Full toxicity test method details are provided in Appendix B

2.2 Methods for describing limits of reporting (LOR)

2.2.1 GC-MS and LC-MS AIQS-DB data:

The AIQS-DB method identifies and quantifies chemical substances by using a combination of retention times, mass spectra, and internal standard calibration curves registered in the database. In order to obtain accurate results, a GC–MS has to be adjusted to designated conditions that are almost the same as the instrumental conditions when the database was constructed. The results obtained from performance check standards (Naginata criteria sample mix 3: Hayashi Pure Chemical, Osaka, Japan) were evaluated against three criteria (Kadokami et al. 2004, 2005): spectrum validity, inertness of column and inlet liner, and stability of response. When the results for the performance check standards satisfy the criteria, the difference between the predicted and actual retention times is less than 3 s, and chemical concentrations obtained (excluding some highly polar compounds which are difficult to measure by GC), are comparable to those obtained by conventional internal standard methods (Kadokami et al. 2005; Miyazaki et al. 2011).

The GC-MS and LC-MS AIQS-DB methods were validated using a performance-based approach that included analysis of procedural blanks, duplicate samples, and certified reference materials (NIST 1941a and NMIJ CRM7302-a (Marine Sediment for Heavy Metals; National Metrology Institute of Japan, Tsukuba, Japan)). In addition, recoveries of 41 surrogates (a range of substances covering the broad range of physico-chemical properties of the targeted chemicals) spiked into the samples were examined to ascertain whether each analysis was correct or not.

2.2.2 Bioassay data:

Measurement of receptor activity was undertaken with a yeast-based bioassay system using yeast cells (*Saccharomyces cerevisiae* Y190) in accordance with the method of Shiraishi et al. 2000 (described in English in Allinson et al. 2008). Agonist activity was recorded as the EC×10 (defined as the concentration of test solution producing a chemiluminescent signal 10 times that of the blank (negative) control). Positive controls were run alongside test samples, with the bioassay LOR determined as the EC×10 for the positive controls.

2.2.3 NDMA data:

NDMA concentrations were determined by UNSW Water Research Centre using the method described in McDonald et al. (2012). This method separates the desired analytes from the sample mixture using solid phase extraction (SPE), with determination using gas chromatography (GC) - tandem mass spectrometry (MS-MS) with electron ionization (EI). The use of direct isotope analogues for isotope dilution analysis of all analytes ensures accurate quantification, accounting for analytical variabilities that may occur during sample processing, extraction and instrumental analysis. The LORs reported by the determining laboratory were determined according to Method 1030 C from Eaton et al. (2005).

2.2.4 Data analysis:

Only detects are presented in the report. The aim of our report is to present the number of observations of TrOCS in water samples from our surveys of the AWTP samples in 2014-15. In that context, we are interested in positive detects, and any exceedances of regulatory or ecotoxicological benchmarks. For that reason we have truncated the data, and for the most part report and discuss only positive detects.

Extraction of water samples using SPE cartridges or disks generally involves the conditioning and washing of the cartridges with small volumes deionised water and solvents prior to, and post-loading of the samples on to the cartridge. This provides an avenue for sample contamination in the extracting laboratory. Laboratory blanks are used to provide an understanding of the potential level of laboratory contamination. These are samples of deionised water of the same volume as the extracted samples that are subjected to the sample preparation steps as the real samples. Thereafter, blank subtraction of the data can be performed to remove the potentially confounding effect of laboratory contaminants from the data. However, blank subtraction will naturally result in under-estimation of analyte concentrations in test samples (because the volume of water used for the laboratory blanks is two orders of magnitude higher than that used during sample preparation), so determination of target analytes in the blanks significantly overestimates the level of laboratory contamination cf. the test SPE extraction. Consequently, in those few occasions where chemicals were observed in laboratory blanks, the blank subtraction value was set at 10% of the maximum observed concentration in the blank.

Lack of chemical detected above LOR does not mean there is none of the chemical in a sample. Nor does "ND" (not detected). Obviously, if one uses a numerical value as a surrogate for "ND," the more "ND" there are for any particular chemical, the lower the average, median, and minimum values would be. So, what value to use when no chemical is detected in a sample? That depends on what one is going to do with the data.

Over the years, researchers have used a range of intuitive practices to select a value for an analyte when the response is below formal LORs. Some analyst chemists use 0, other analysts use the instrumental LOD, others use the formal method LOR itself, others split the difference and use half of the LOR, or some

percentage between 0 and the LOR. Finally, some analytical chemists regard almost all values below LOR as indeterminate and report as "ND" or "<LOR" and leave the value missing (UNLESS there is a statistical need to provide a value, i.e. to allow for robust temporal and spatial comparisons).

Where statistical comparisons required all data to have a numerical value, then <LOR values were set at 0.5LOR. When calculating toxicity units (TUs), any 'trace' levels were set at 0.5LOR, and non-detects were set at "0" as per the methods followed.

2.3 Toxicity assessment

2.3.1 Environmental risk assessment

The project addressed its objective to determine *whether the water recycling process produces a brine concentrate fit for disposal in the Antarctic marine environment*, through comparison of TrOC detection data with regulatory guidelines and ecotoxicology data. For all samples with TrOC detections above the limits of reporting (LOR), concentrations were compared with the ANZECC and ARMCANZ (2000) water quality guidelines trigger values for water discharged to receiving marine aquatic environments. For TrOCs without guideline levels, thereafter the potential risk from the observed water concentrations was assessed using risk quotients (RQs) and TUs methods.

The risk quotient (RQ) method is a deterministic method in which a risk ratio is generated, expressed as a measured environmental concentration (MEC) divided by a no-effect concentration (NEC). The RQ is calculated using:

$$RQ = MEC/NEC \qquad (1)$$

An RQ of more than 1 is considered problematic for the receiving aquatic environment.

In this study, we applied the method used by the WHO (2002) in their hyper-conservative analysis of long-term chemical exposure effects of NDMA for aquatic plants and animals. In this case, we used our median and maximum measured concentrations as estimated exposure values (EEV) to generate general case (RQ_{median}) and the worse case (RQ_{max}) scenarios using equation (2). The estimated no-effects value (ENEV), was calculated by dividing a critical toxicity value (CTV; an acute ecotoxicology value obtained from the literature), by an application factor (usually 100).

$$RQ = EEV/ENEV \qquad (2)$$

Again, only an RQ of more than 1 is considered problematic for the receiving aquatic environment. Short term EC50 (lethal/effect) data for fish, aquatic invertebrates, aquatic plants and algae was first sourced from US EPA (2015), then from ECHA (2015) and Kegley et al. (2011), or failing those sources, by searching the internet for information.

The TU concept compares the detected concentration of chemical with the respective toxicity of the substance. We calculated the TU for each chemical in each water sample according to Liess and Von Der Ohe (2005):

$$\text{Log TU} = \log (Cp / Toxp) \qquad (3)$$

where TU is the toxic unit of the chemical (presented as the logarithm); Cp is the concentration of chemical observed in the sample; and Toxp is a measure of the toxicity value of the chemical. The potential effect on aquatic organisms of individual chemicals was assessed for all chemicals detected in water samples by calculating $\log_{10}TU_f$ using the maximum observed concentration and short-term (acute) ecotoxicological data for fish, aquatic invertebrates, aquatic plants and algae sourced first from US EPA (2015), then from ECHA (2015) and Kegley et al. (2011), or failing those sources, by searching the

internet for information. Liess and Von Der Ohe (2005) reported a significant change in community structure between $\log_{10}$TU(D. magna) < -4 and >-3, and so a $\log_{10}$TU of -3 or higher is considered to pose some risk to assessed organisms.

2.3.2 The relevance of the *in vitro* bioassay (bioanalytical methods) data to drinking water guidelines

In the recombinant receptor-reporter gene bioassays used in this study, whole cells were exposed to mixtures of interest and monitored for specific responses. For a chemically-induced impact to occur, a chemical has to interact with a receptor at the molecular level. Organisms, however, have defense and detoxification mechanisms that can prevent molecules reaching the receptors, and it is only when those defense mechanisms are overcome that observable receptor activity occurs. This means that *in vitro* effects are likely to occur and be observable at significantly lower doses than *in vivo* effects (Figure 2.2); it also means that a chemical can be toxic *in vitro* but not *in vivo* (enHealth 2012).

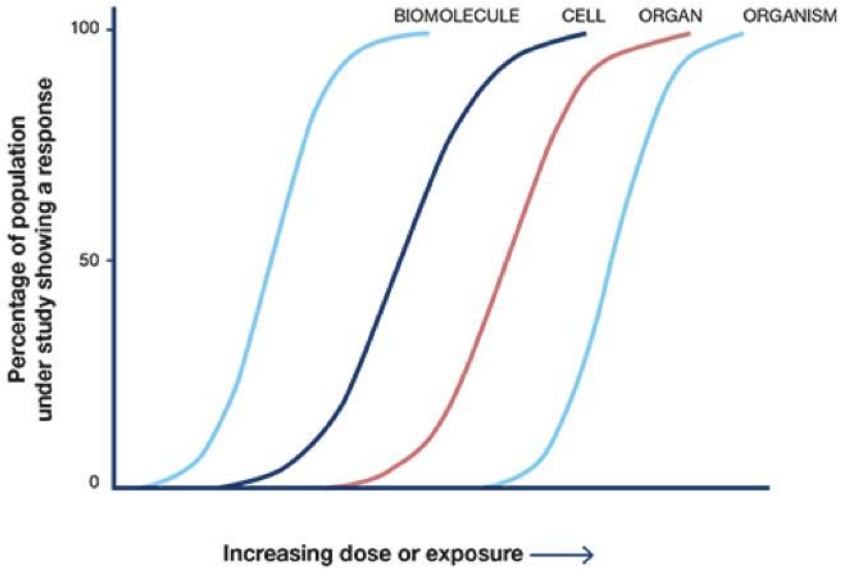


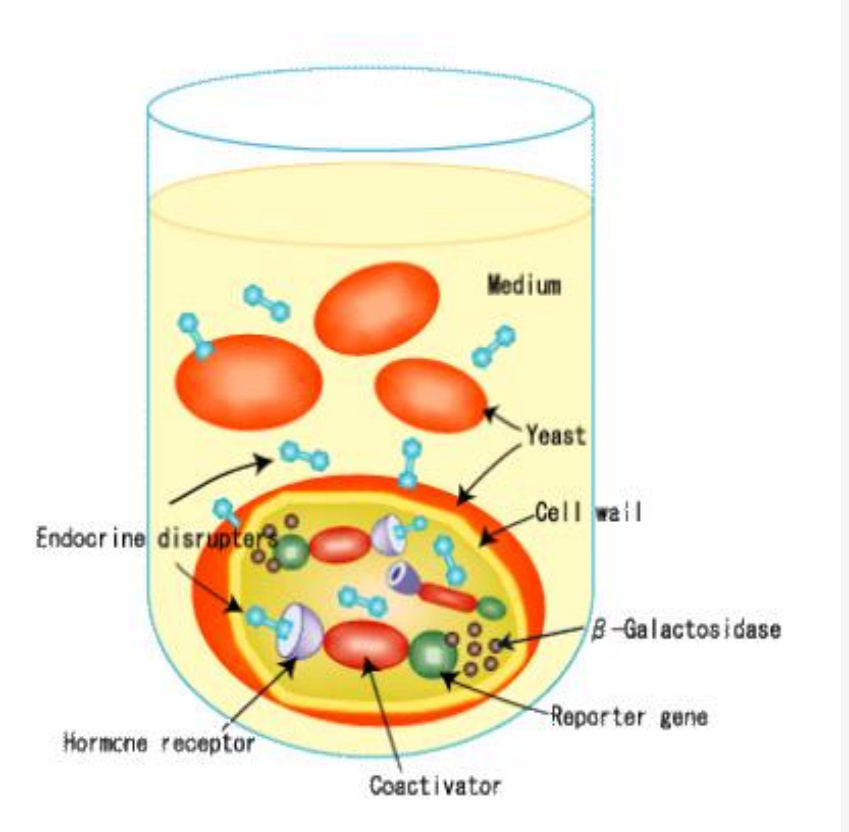
Figure 2.2 A continuum of toxicity. To induce a toxic effect at organism-level generally requires a greater dose or exposure (from enHealth 2012).

The other important limitation to these *in vitro* assays that needs to be made very clear is that there is no incorporation of toxicokinetics. Toxicokinetics include absorption, distribution, metabolism and excretion (ADME), all of which can significantly affect the ability of a substance to reach a receptor (enHealth 2012).

In vitro assays were developed for screening purposes and there is still much debate about their ability to predict whole organism effects and so regulatory agencies have generally been wary of using *in vitro* bioassay data to predict human health effects. Because of their limitations, the enHealth (2012) committee suggest that *in vitro* bioassays should not be used as a measure of effect, but confirmed they are well suited to monitoring water quality (exposure assessment), as they are significantly faster and cheaper than *in vivo* exposures and are amenable to high throughput screening.

Information Box 1: *In Vitro* Assays

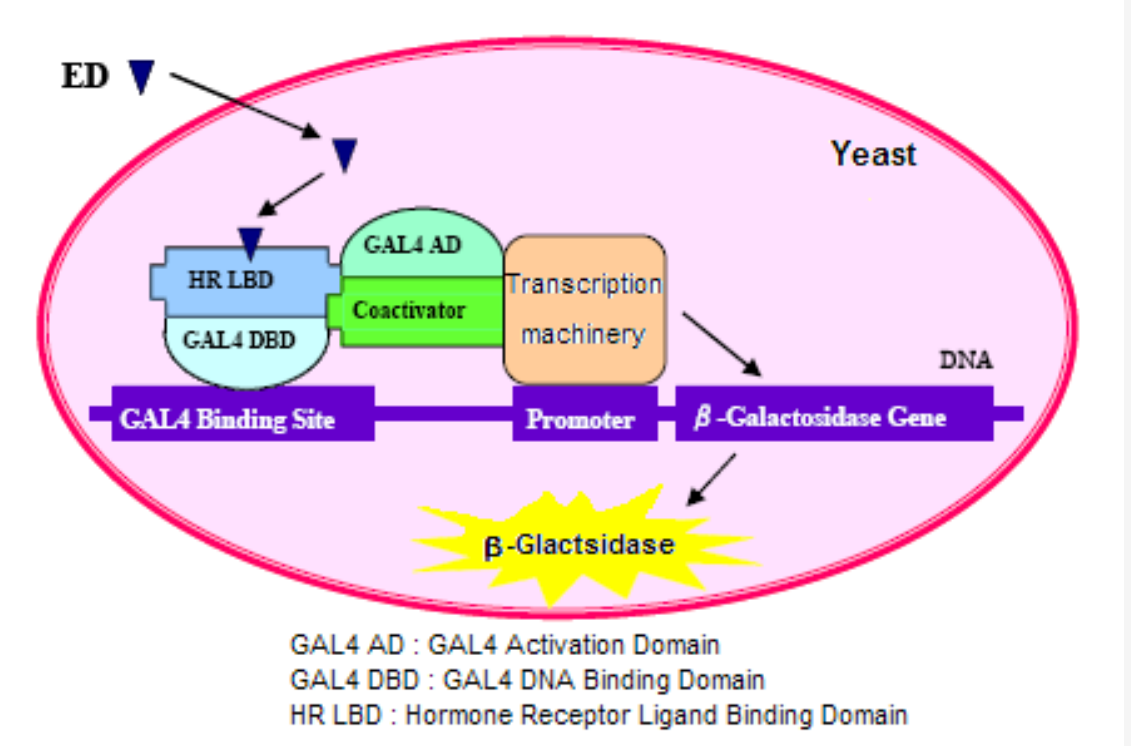
The molecular mechanism of the *in vitro* tests used in this study can be described using the mode of action of estrogen action. In that case, the effects of endogenous and/or xenoestrogens are mediated by the estrogen receptor (ER). Inactive ERs exist in large complexes associated with heat shock proteins. When a compound binds to the ER, the heat shock proteins dissociate, and a conformational change activates the receptor and causes dimerisation. The resulting homodimer complex (HDC) shows high affinity for EREs (estrogen response elements) in the regulatory region of estrogen-inducible genes in the nucleus. After binding to the ERE, the HDC recruits transcription factors to the target gene promoter, leading to gene activation and transcription, and subsequent translation of RNA into the proteins that ultimately stimulate the observed responses.



The **Yeast Two-Hybrid Assay** that is used in this project is based on genetically engineered yeast cells into which specific DNA sequences called estrogen response elements (ERE) have been added and linked to a reporter gene. Essentially, the assay works by quantifying the ability of a chemical to stimulate ER-dependent transcriptional activity. In this assay, reporter gene expression is the result of a cascade of molecular events following receptor activation, considered to provide a more integral indication of the estrogenic activity of a compound than competitive ligand binding or cell proliferation assays. Specifically, the **Yeast Two-Hybrid Assay** is based on the ligand-dependent interaction of two proteins, a hormone receptor and a coactivator, and hormonal activity is detected by β-galactosidase activity.

Two expression plasmids, pGBT9-HRLBD and pGAAD424-TIF-2 are introduced into yeast cells, which carry a β-galactosidase reporter gene. Because the yeast strain harbours a GAL4 binding site upstream of a *lacZ* reporter gene, GAL4DBD-ER binds to the regulatory region of the *lacZ* gene. If GAL4DBD-ER interacts with GAL4AD-coactivator, GAL4AD recruits the basal

transcriptional machinery to the promoter region of the *lacZ* gene, resulting in production of β-galactosidase. Therefore, the β-galactosidase activity corresponds to the strength of interaction between ER and coactivator. The protein-protein interaction between ER and coactivator are strictly dependent on the presence of 17β-estradiol



Adapted from:

1. WHO/IPCS (2002). Global assessment of the state-of-the-science of endocrine disrupters. Edited by: Damstra T, Barlow S, Bergman A, Kavlock R, Van Der Kraak G. World Health Organisation / International Program on Chemical Safety.
2. Kinnberg K (2003). Evaluation of in vitro assays for determination of estrogenic activity in the environment (No. 43). Danish Environmental Protection Agency, Danish Ministry of the Environment.
3. Nishikawa J, Saito K, Goto J, Dakeyama F, Matsuo M, Nishihara T (1999). New screening methods for chemicals with hormonal activities using interaction of nuclear hormone receptor with coactivator. Toxicology and Applied Pharmacology 154:76-83.
4. Nishihara T, Nishikawa J, Kanayama T, Dakeyama F, Saito K, Imagawa M, Takatori S, Kitagawa Y, Shinjiro H, Utsumi H (2000). Estrogenic activities of 517 chemicals by yeast two-hybrid assay. Journal of Health Science 46:282-298.
5. Shiraishi F, Shiraishi H, Nishikawa J, Nishihara T, Morita M (2000). Development of a Simple Operational Estrogenicity Assay System using the Yeast Two-Hybrid System. Journal of Environmental Chemistry 10:57-64.

Testing with in vitro bioassays has some advantages over analytical techniques, such as the relatively low capital equipment needs and staff operating costs, the possibility of high-throughput methods, the possibility of identifying mixture effects, and their preference over in vivo tests due to lower ethical permissions requirements (Sjerps et al. 2012; enHealth 2012). However, factors that are hindering the use of *in vitro* bioassays in regulatory frameworks include:

1. The costly and time-consuming formal validation procedures.
2. The need for a battery of *in vitro* tests to realistically replace one *in vivo* test
3. The need for proven relevance of the use of *in vitro* bioassays for drinking water

In the above context, the recombinant receptor-reporter gene assays were used only to measure the activation of a receptor, and to allow for quantification of 'hormonal' activity without having to know the precise chemical make up of the sample. In that context, **the assay data** provides a measure of the efficiency of TrOCs removal in the AWTP, but **are but not used for comparison with regulatory guidelines for environmental or drinking water quality**.

2.3.3 Human toxicity assessment of product water

Organic chemicals are usually present in Australian drinking water in very low concentrations because, for the most part, our drinking water comes from protected or otherwise very little contaminated catchments. In our case, our 'catchment' is a WWTP, and our drinking water source the WWTP effluent, so a wide range of chemicals could be present in the AWTP product water were it not working properly. Some chemicals will still get through, generally at low sub-µg/L concentrations. So what might the effect of drinking these chemicals day after day be?

The project addressed its objective *to determine whether the water recycling process produces a recyclable product water* through comparison of TrOC detection data with regulatory guidelines and toxicology data. A drinking water guideline value is the concentration that does not result in any significant risk to the health of the consumer over a lifetime of consumption and is consistent with water of good quality (NHMRC & NRMMC 2011; NRMMC, EPHC & NHMRC 2008). As a first step in the risk assessment process, for all product water samples with TrOC detections above the limits of reporting (LOR), concentrations were compared with water quality guidelines, e.g. the ADWG (NHMRC & NRMMC 2011) or AGWR (NRMMC, EPHC & NHMRC 2008) for potable and recycled water. Where a guideline value did not exist, a draft drinking water guideline (Draft DWG) was calculated.

The ADWG and AGWR guideline values are generally based on the highest dose that causes no adverse effects in long-term experiments on laboratory animals (NOEL or NOAEL levels), and it was this process that was followed for those chemicals that did not have a current drinking water guideline. The draft DWG was calculated using the following formula:

$$\text{Guideline value} = \frac{\text{animal dose x human weight x proportion of intake from water}}{\text{volume of water consumed x safety factor}} \quad (4)$$

In using equation (4), it is necessary to make some assumptions.

1. Experiments on laboratory animals provide toxicological data on the effects of exposure to chemical agents. Ideally, these are long-term studies involving ingestion of the compound dissolved in water or present in food, and the guideline value is based on the highest dose that

causes no adverse effects (NOAEL) in long-term experiments on laboratory animals. Where there was no published NOEL or NOAEL (which was the case for about two thirds of the chemicals observed in the product water), then short-tem (acute) toxicity data the $LD50_{rat}$ was used, with additional safety factors.

2. The average human weight is 70 kg
3. The proportion of chemical ingested through water is set at 10%
4. The amount of water consumed is 2L per adult human per day
5. Safety factors can be used to address the uncertainty inherent in extrapolating from animal studies to human populations (NHMRC & NRMMC 2011). Where an NOEL was available, the safety factor applied was 100 (to account for variations between rats and humans, because rats may be less sensitive than humans, and in many cases human sensitivity is unknown). Where there was no long-term information, and acute toxicity study data was used, a safety factor of 1000 was applied

3 Results and Discussion

3.1 Status of works

Water samples have been collected as 'grab' or spot samples by project staff from up to eight locations in the demonstration plant. Samples were directly collected in glass amber bottles, stored on ice, and then at 4°C until processed at University of Melbourne by Mayumi Allinson and then either analysed in-house or despatched to partner laboratories for analysis. Twelve rounds of sampling were undertaken between March 2014 and April 2015, and while not all TrOCs monitoring team testing was undertaken on all samples on every occasion (see Table 3.1), all of the data is available for this report.

Table 3.1 Summary of sampling activity and status of samples in analytical process

Sample round	N	GC-MS-DB	LC-MS-DB	Bioassay	nitrosamines
R1 (26 Mar 2014)	12	Reported		Reported	
R2 (7 May 2014)	10	Reported	Reported	Reported	
R3 (6 Aug 2014)	13	Reported	Reported	Reported	
R4 (20 Aug 2014)	19	Reported	Reported		
R5 (29 Aug 2014)	24	Reported	Reported	Reported	Reported
R6 (29 Sep 2014)	28	Reported	Reported	Reported	Reported
R7 (25 Nov 2014)	25	Reported	Reported	Reported	Reported
R8 (16 Dec 2014)	22	Reported	Reported	Reported	Reported
R9 (20 Jan 2015)	23	Reported	Reported	Reported	Reported
R10 (17 Feb 2015)	23	Reported		Reported	Reported
R11 (17 Mar 2015)	14	Reported		Reported	
R12 (14 Apr 2015)	14	Reported		Reported	

N, number of samples; In train, samples have entered analytical process, i.e. sample preparation, extraction and elution, chemical analysis; data available, final data checking underway prior to write up by team; reported, data incorporated into project reporting

, Feed water only tested; , three barriers tested (Feed water, RO concentrate, Plant outlet); , eight barriers tested (Feed water, Post ozone, Post MF, Post BAC, RO concentrate, Post RO, Post UV, Plant outlet)

The project addressed its first objective (*to obtain background water quality information from the chosen validation WWTP*) by collecting samples in March, May and August 2014 (R1-3, respectively), and subjecting them to multi-residue and bioassay screening. Collection, processing and analysis of the samples provided an ideal opportunity to refine project methods. Unless otherwise stated, the data collected in R1-3 was of sufficient quality that it has been included in analysis of subsequent sampling rounds.

3.2 Multi-residue data

We addressed the project's second objective (*to verify that the demonstration plant is operating effectively by screening feed, barriers' and outlet waters for a wide range of TrOCs*) using multi-residue mass spectrometric techniques in house and through collaboration with Professor Kiwao Kadokami (University of Kitakyushu, Japan)

There are more than 300 target TrOCs in the Australian Drinking Water Guidelines (ADWG; NHMRC & NRMMC 2011) and/or Australian Guidelines for Water Recycling (AGWR; NRMMC, EPHC &

NHMRC 2008) for potable and recycled water. The 344 chemicals in these two sets of guidelines have a wide range of structures, physico-chemical characteristics and toxicities. To measure 344 TrOCs usually means the use of many analytical methods, with the concomitant financial implications associated with conducting multiple quantitative tests. Such analytical programs will be problematic for small and/or remote facilities where both the analytical cost per unit of water produced becomes prohibitive and the deployment of expertise to site is also costly (relative to large facilities). Options to overcome these real and perceived barriers to chemical measurement include measuring a reduced number of chemicals and/or to use new rapid screening methods.

The selection of a representative subset of chemicals to monitor from the many TrOCs known to be present in wastewaters is a difficult task. It is known that the number and type of wastewater-derived chemicals is related to the type of treatment process operating in a WWTP, its 'catchment' or sources of water, and the physico-chemical properties and biodegradability of chemicals. One option is to simply adopt a set of indicators chemicals that has been used and approved by regulatory authorities elsewhere. In that context, reduced lists of wastewater-derived chemicals that might be useful in the assessment of indirect/direct-potable-reuse systems include:

- In the USA, Drewes et al. (2008) list of 64 'indicator,' or 'surrogate' chemicals (Table A2).
- In Western Australia, Water Corporation's set of 12 organic chemicals termed recycled water quality indicators' to represent a much larger group of chemical hazards (Table A3; Water Corporation 2013)

The relevance of Drewes et al. (2008) or Water Corporation (2013) or any other list of surrogate chemicals for our purposes is debateable, in part because of the limited overlap between such lists and the more than 300 chemicals in the ADWG and AGWR documents. For instance, only 29 of the 64 compounds in Drewes et al. (2008) list are on both the ADWG and AGWR lists. The overlap between the Water Corporation (2013) list and the chemicals in the ADWG and AGRW documents is similarly low, with only 2 of the 12 chemicals on both lists. Furthermore, using a list of surrogates may not reduce analytical costs as much as one might expect. For instance, Drewes et al.'s (2008) list still requires significant resources since analysis of the 64 chemicals requires sixteen separate methods, while at least 7 methods are required to determine the twelve chemicals on the Water Corporation (2013) list, with many complex sample preparation and analytical methods (Table A2, A3).

The project team considered the published indicator lists, but in the end determined that it was <u>not</u> the purpose of the monitoring program to provide the AAD with a recommended list of indicator chemicals for the future operation of the AWTP at Davis Station. The determination was made because it *is* known that the number and type of wastewater-derived chemicals is related to the type of treatment process operating in a WWTP and its source water. In that context, the AWTP's feed water during the trials at Selfs Point will be very different to the feed delivered from the secondary treatment plant upstream of the AWTP when it is installed and operated at Davis Station. Therefore, determination of an indicator list must be based on the number and type of chemicals found at Davis Station and the relative risk of their being observed in the feed to the AWTP.

The project team also determined that it was not the purpose of the monitoring program to recommend to the AAD how to measure TrOCs in any operational performance assurance program when the AWTP is at Davis Station. Once the AAD has determined which surrogate (performance) TrOCs it wishes to monitor, and the method it wishes to use (i.e. spot vs. passive vs on-line sampling) then the number and type of analytical screens can be determined.

The considerations of the previous two paragraphs notwithstanding, the ADWG and AGWR guidelines and Drewes et al. (2008) or Water Corporation (2013) lists of surrogates are well-known to regulators in Australia and so it's perhaps not unreasonable to compare the numberof TrOCs screened by the multi-residue methods with these lists. In that context, approximately half of the TrOCs in the ADWG and AGWR guidelines (~100 and 80 chemicals, respectively), approximately half the TrOCs in Water Corporation (2013) list, and approximately one quarter of the TrOCs in Drewes et al. (2008) list are in one of (or both of) the two multi-residue screens used by this project, suggesting that even though we chose not to screen for either of these latter two pre-selected list of indicator chemicals, the multi-residue methods still provided a representative indicator subset of chemicals found in WWTP effluent.

When considering the number and type of TrOCs to monitor, the project team was limited only by the need *to verify that the demonstration plant was operating effectively*. In essence, we needed to ensure our screening covered the widest possible range of physico-chemical characteristics found in TrOCs in WWTP effluent. For this study, key physico-chemical parameters of interest are molecular weight, water solubility (as expressed by a chemical's octanol-water partition coefficient logKow or logP), and affinity for organic carbon (as expressed by a chemical's organic carbon partition coefficient logKoc). Molecular weight is a reasonable proxy for molecular size, which affects membrane and RO removal performance; Kow and Koc affect a molecule's solubility, and in turn their removal by barriers such as BAC.

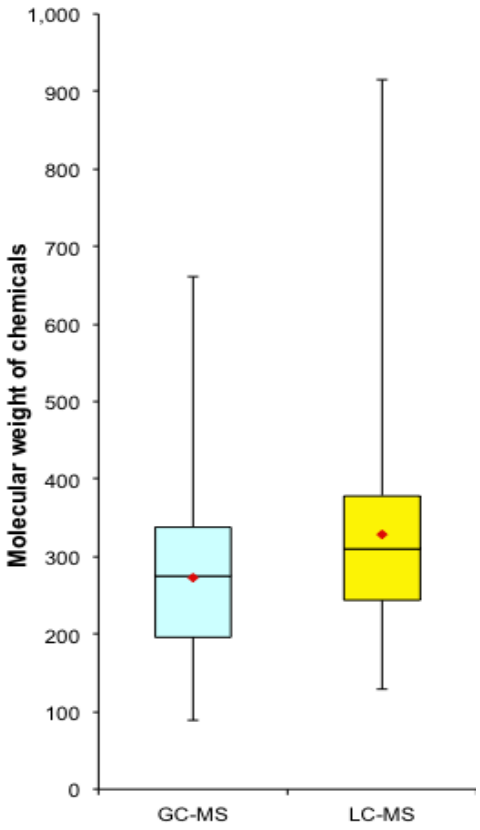


Figure 3.1 Summary of molecular weight distribution of screened chemicals

◆, arithmetic mean; dividing line within data boxes, data median; upper and lower boundaries of boxes, 75th and 25th percentile of data; error bars represent the range.

Approximately two-thirds of the TroCs investigated in this study were screened using Kadokami et al.'s (2005) GC-MS-AIQS-DB method. This method can screen samples for 940 semi-volatile TrOCs, including numerous halogenated and non-halogenated hydrocarbons; polycyclic aromatic hydrocarbons (PAHs); polychlorinated biphenyl compounds (PCBs); a range of pharmaceutical and personal care products (PPCPs); and agricultural compounds (see Table A4). We also used an LC-TOF-MS method that can screen samples for 265 non-volatile and polar chemicals, including 180 agricultural compounds and 70 pharmaceuticals (antibiotics, antidepressants, beta blockers, analgesics, etc.; see Table A5). In that context, the two chromatographic methods screened for chemicals in the range 89 – 916 amu (Figure 3.1), logKow in the range -4.2 – 18.3 and logKoc in the range 0.0 – 7.5 (Figure 3.2). These ranges are better than Water Corporation (2013) or Drewes et al. (2008) lists, further suggesting the multi-residue methods covered a representative set of chemicals found in WWTP effluent.

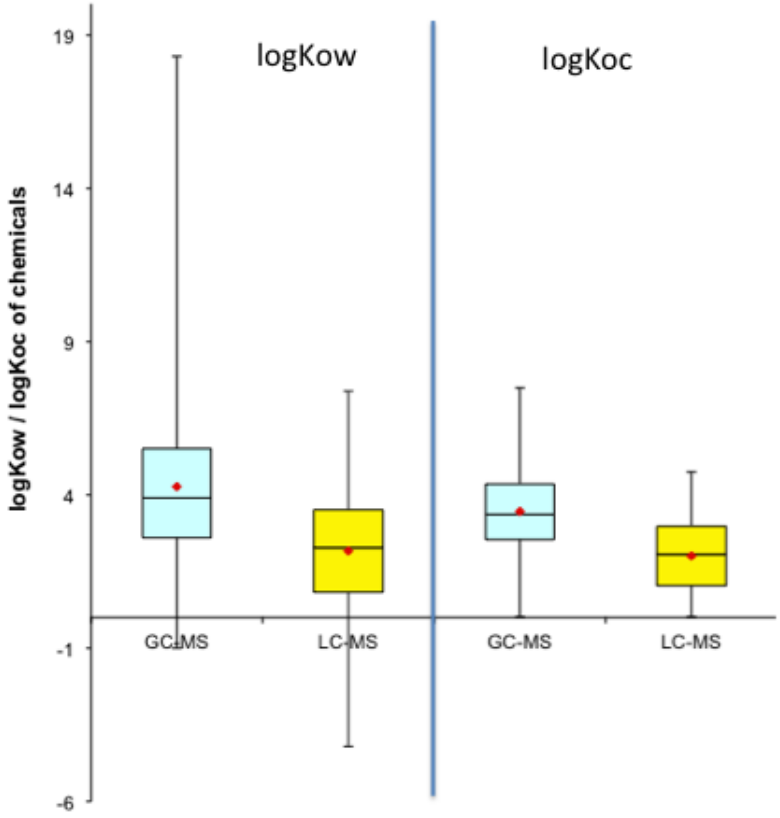


Figure 3.2 Summary of Kow and Koc distribution of screened chemicals

◆, arithmetic mean; dividing line within data boxes, data median; upper and lower boundaries of boxes, 75th and 25th percentile of data; error bars represent the range.

We were able to unambiguously detect almost 80 chemicals in the feed water (Table 3.2) using the multi-residue methods, of which 40% were detected on more than one occasion, including disinfection by-products (1), pesticides (7), non-steroidal pharmaceuticals (1), antibiotics (7), disinfection by-products (1), pesticides (7), antibiotics (8), other pharmaceuticals (18), antioxidants (2), fatty acid methyl esters (5), fire retardants (3), fragrances (3), sterols/stanols (11), and other miscellaneous organic chemicals (20). Of those chemicals that were observed in more than 80% of samples, some were natural compounds, such as coprostanol and stigmasterol, but the majority were antibiotics and PPCPs, including chemicals such as carbamazepine, sulfamethoxazole and triclosan.

Of the 18 chemicals in the Drewes et al. (2008) list that are in one of (or both of) the two multi-residue screens used in this study, 13 (72%) were observed on one or more occasion in the feed water; of the 5 chemicals in the Water Corporation (2015) list that are in one of (or both of) the two multi-residue screens used in this study, 1 (20%) was observed on one or more occasion in the feed water. This again suggests the multi-residue methods used in this study covered a representative set of chemicals found in WWTP effluent.

Only twenty (20) chemicals were observed in the product water (Table 3.3), all at sub-µg/L levels. In that context, the GC-MS-DB and LC-MS-DB data suggests **most chemicals are being removed from the feed water by the treatment train** (see Figure 3.3). Only one chemical was observed in more than 50% of product water samples (2-pheonoxy ethanol), indeed almost half of the chemicals were observed only once. Half the chemicals observed in the feedwater were close to their respective LORs, and all chemicals observed on more than one occasion were of the same order of magnitude as their LOR. The 20 chemicals observed in the product water included other pharmaceuticals (2), antioxidants (1), fatty acid methyl esters (2), fire retardants (2), fragrances (5), sterols/stanols (2) and other miscellaneous organic chemicals (6). There are no ADWG guideline values for any of the chemicals we detected in the product water, and of the nine chemicals that have AGWR guideline values, **no residue exceeded its listed AGWR level** (see Table 3.3). Moreover, all residues were at least four orders of magnitude lower in concentration than the draft DWGs calculated by this study. Taking these assessments together, we can conclude that **at the concentrations observed in this study, the TroCs observed in the product water would be unlikely cause adverse effects** were this water to be recycled into a potable water supply.

One final way to assess whether the AWTP was working efficiently was to analyse the number and type of chemicals observed in brine (or RO) concentrate. Only sixteen (16) chemicals were observed in the brine concentrate (Table 3.4), all bar two of which were at sub-µg/L levels. In that context, the GC-MS-DB and LC-MS-DB data again suggests **most chemicals are being removed from the feed water by the treatment train** (see Figure 3.3). The 16 chemicals observed in the brine (or RO) concentrate included other pharmaceuticals (5), antioxidants (1), fatty acid methyl esters (2), fire retardants (1), fragrances (3), and other miscellaneous organic chemicals (4). In part, this stream represents the brine stream that will be discharged to the environment. As such it is most relevant to compare chemical residues in the discharge water with the ANZECC & ARMCANZ (2000) water quality guidelines for protection of 95% marine organisms. In that context, there are only thirteen (13) chemicals with a marine water quality trigger values (see Table 3.5); nine of these chemicals were in this study's screening regime, but none were observed in the brine concentrate. However, by again using the World Health Organisation's hyper-sensitive approach to assessing the risks of chemicals to aquatic organisms, we can a say that our objective with this testing was achieved because we find that the RQs were well below 1 (Table 3.6). Moreover, the $\log_{10}$TUs for fish and invertebrates were well below -3 (Table 3.7; with the exception of metformin, $\log_{10}$TU -2.9). Taking these assessments together, we can conclude that **that releases at the concentrations observed in this study would be unlikely cause adverse effects** on populations of aquatic organisms in the receiving environment.

Table 3.2 Summary of chemicals detected in AWTP feed water using the GC- and/or LC-MS multi-residue methods

Chemical	n	FOD	Average	Median	Min	Max	DI*	WCI*
			(µg/L)					
2-(Methylthio)-benzothiazole	14	100	0.28	0.26	0.14	0.53		
Candesartan	7	100	0.10	0.11	0.06	0.12		
Carbamazepine	14	100	1.03	0.83	0.42	2.81	□	□
Cholesterol	14	100	1.54	1.70	0.02	3.02		
Coprostanol	14	100	2.43	2.01	0.67	4.21		
Coprostanone	14	100	0.39	0.30	0.12	0.71		
Cotinine	7	100	0.18	0.07	0.04	0.54		
Diethyltoluamide	14	100	0.15	0.11	0.02	0.50	□	
Diltiazem	7	100	0.14	0.15	0.09	0.17		
Diuron	7	100	0.11	0.11	0.03	0.17		
Lidocaine	7	100	0.18	0.18	0.13	0.25		
Roxithromycin	7	100	0.22	0.20	0.13	0.38		
Sotalol	7	100	1.59	0.52	0.31	5.00		
Sulfamethoxazole	7	100	0.95	0.55	0.08	3.09	□	
Sulfapyridine	7	100	0.57	0.53	0.46	0.75		
Trimethoprim	7	100	0.17	0.16	0.09	0.24		
Epicoprostanol	13	93	0.76	0.71	0.28	1.47		
Ergosterol	13	93	1.01	0.72	0.20	2.29		
Thiabendazole	6	86	0.04	0.04	0.03	0.04		
Cholestanol	11	79	0.68	0.57	0.15	1.36		
Tris(2-chloroethyl) phosphate	11	79	0.31	0.31	0.22	0.42	□	
1,4-Dichlorobenzene	10	71	0.08	0.08	0.04	0.13		
4-tert-Octylphenol	10	71	0.05	0.04	0.02	0.09		
Azithromycin	5	71	0.09	0.09	0.05	0.14		

n, number of detections; FOD, frequency of detection; text in blue chemicals detected by GC-MS, in orange by LC-MS; DI, Drewes et al. (2008) indicator; WC, Water Corporation (2015) indicator

Table 3.2 (continued)

Chemical	n	FOD	Average	Median	Min	Max	DI*	WCI*
beta-Sitosterol	10	71	0.84	0.58	0.18	2.35		
Clarithromycin	5	71	0.13	0.14	0.06	0.18		
Erythromycin	5	71	0.23	0.22	0.21	0.28	□	
Griseofulvin	5	71	0.08	0.07	0.03	0.15		
Metformin	5	71	0.69	0.13	0.06	1.98		
Propranolol	5	71	0.07	0.07	0.06	0.07	□	
Tributyl phosphate	10	71	0.11	0.11	0.04	0.23		
Triclosan	10	71	0.10	0.08	0.06	0.21	□	
Tris(1,3-dichloro-2-propyl) phosphate	10	71	0.14	0.13	0.10	0.22	□	
Campesterol	9	64	0.29	0.19	0.08	0.75		
Stigmasterol	9	64	0.88	0.42	0.13	2.86		
Atenolol	4	57	3.95	2.90	0.76	9.25	□	
24-Ethyl coprostanol	8	57	0.41	0.30	0.12	1.25		
Metoprolol	3	43	0.26		0.21	0.32	□	
Simazine	3	43	0.04		0.04	0.05		
Stigmastanol	6	43	0.10	0.11	0.03	0.20		
2-Methoxyphenol	5	36	0.05	0.04	0.02	0.08		
4-Methyl-2,6-di-t-butylphenol (BHT)	5	36	0.13	0.13	0.09	0.18		
2(3H)-Benzothiazolone	5	36	0.21	0.15	0.03	0.61		
3,4-Dichloroaniline	4	29	0.10	0.08	0.07	0.17		
Amitriptyline	2	29	0.05		0.04	0.06		
Bis(2-ethylhexyl)phthalate (DEHP)	4	29	0.53	0.19	0.19	1.56		
Dibenzyl ether	4	29	0.11	0.11	0.07	0.12		
Dipyridamole	2	29	0.06		0.06	0.06		
Disopyramide	2	29	0.02		0.01	0.02		

n, number of detections; FOD, frequency of detection; text in blue chemicals detected by GC-MS, in orange by LC-MS; DI, Drewes et al. (2008) indicator; WC, Water Corporation (2015) indicator

Table 3.2 (continued)

Chemical	n	FOD	Average	Median	Min	Max	DI*	WCI*
e-Caprolactam	4	29	0.13	0.13	0.10	0.17		
2,6-Dichlorophenol	3	21	0.04		0.01	0.07		
Benzyl alcohol	3	21	0.23		0.06	0.36		
Dimethyl phthalate	3	21	0.21		0.06	0.29		
Ethanol, 2-phenoxy-	3	21	0.21		0.16	0.24		
Ibuprofen	3	21	0.21		0.15	0.32	□	
1,2-Dichlorobenzene	2	14	0.13		0.01	0.25		
4-Nonylphenol	2	14	0.29		0.22	0.36	□	
2,4,5-Trichlorophenol	2	14	0.04		0.03	0.05		
2,5-Dichlorophenol	2	14	0.03		0.03	0.03		
Caffeine	2	14	0.08		0.02	0.13	□	
L-Menthol	2	14	0.02		0.02	0.03		
Methyl myristate	2	14	0.05		0.04	0.07		
Dicyclohexylamine	1	14	0.55					
2-tert-Butyl-4-methoxyphenol	1	7	0.28					
2,6-Dinitrotoluene	1	7	0.80					
3-&4-Methylphenol	1	7	0.02					
alpha-Terpineol	1	7	0.12					
Bifenthrin	1	7	0.47					
Elaidic acid methyl ester	1	7	0.06					
Methyl decanoate	1	7	0.03					
Methyl dodecanoate	1	7	0.05					
Methyl palmitate	1	7	0.01					
Methyl palmitoleate	1	7	0.12					
Phenylethyl alcohol	1	7	0.04					

n, number of detections; FOD, frequency of detection; text in blue chemicals detected by GC-MS, in orange by LC-MS; DI, Drewes et al. (2008) indicator; WC, Water Corporation (2015) indicator

Table 3.2 (continued)

Chemical	n	FOD	Average	Median	Min	Max	DI*	WCI*
Piperonyl butoxide	1	7	0.03					
Simetryn	1	7	0.07					
Thymol	1	7	0.03					
trans-Decahydronaphthalene	1	7	0.02					

n, number of detections; FOD, frequency of detection; text in blue chemicals detected by GC-MS, in orange by LC-MS; DI, Drewes et al. (2008) indicator; WC, Water Corporation (2015) indicator

Table 3.3 Summary of chemicals detected in AWTP product water samples

Name	n	FOD	Average	Median	Min	Max	AGWR	Draft DWG
				(µg/L)			(µg/L)	(µg/L)
Ethanol, 2-phenoxy-	6	67	0.28	0.20	0.01	0.89		4400
(9Z)-9-Tetradecenoic acid, methyl ester	3	33	0.04		0.01	0.08		10500
Benzyl alcohol	3	33	0.34		0.07	0.85		18000
1-Nonanol	3	33	0.01		0.01	0.01		12400
4-Methyl-2,6-di-t-butylphenol (BHT)	2	22	0.04		0.03	0.06		3000
4-Nonylphenol	2	22	0.04		0.03	0.05	500	
Dimethylterephthalate	2	22	0.04		0.01	0.06	2	
Octanol	2	22	0.03		0.03	0.04		17500
Tributyl phosphate	2	22	0.03		0.03	0.04	0.5	
Tris(2-chloroethyl) phosphate	2	22	0.08		0.03	0.14	1	
Diethyltoluamide	2	22	0.02		0.01	0.02	2500	
4-tert-Octylphenol	1	11	0.01				59	
Bisphenol A	1	11	0.03				200	
2,4,6-Tribromophenol	1	11	0.27					10500
Dimethyl phthalate	1	11	0.01				29	
Methyl myristate	1	11	0.03					10500
alpha-Terpineol	1	11	0.02					500
Cholesterol	1	11	0.05				7	
Coprostanone	1	11	0.01					n.d.
L-Menthol	1	11	0.01					11500

n, number of detections; FOD, frequency of detection; text in blue chemicals detected by GC-MS, in orange by LC-MS; AGWR, Australian Guidelines for Water recycling (NRMMC, EPHC, NHMRC 2008); Draft DWG, drinking water guideline calculated in this study n.d., no data.

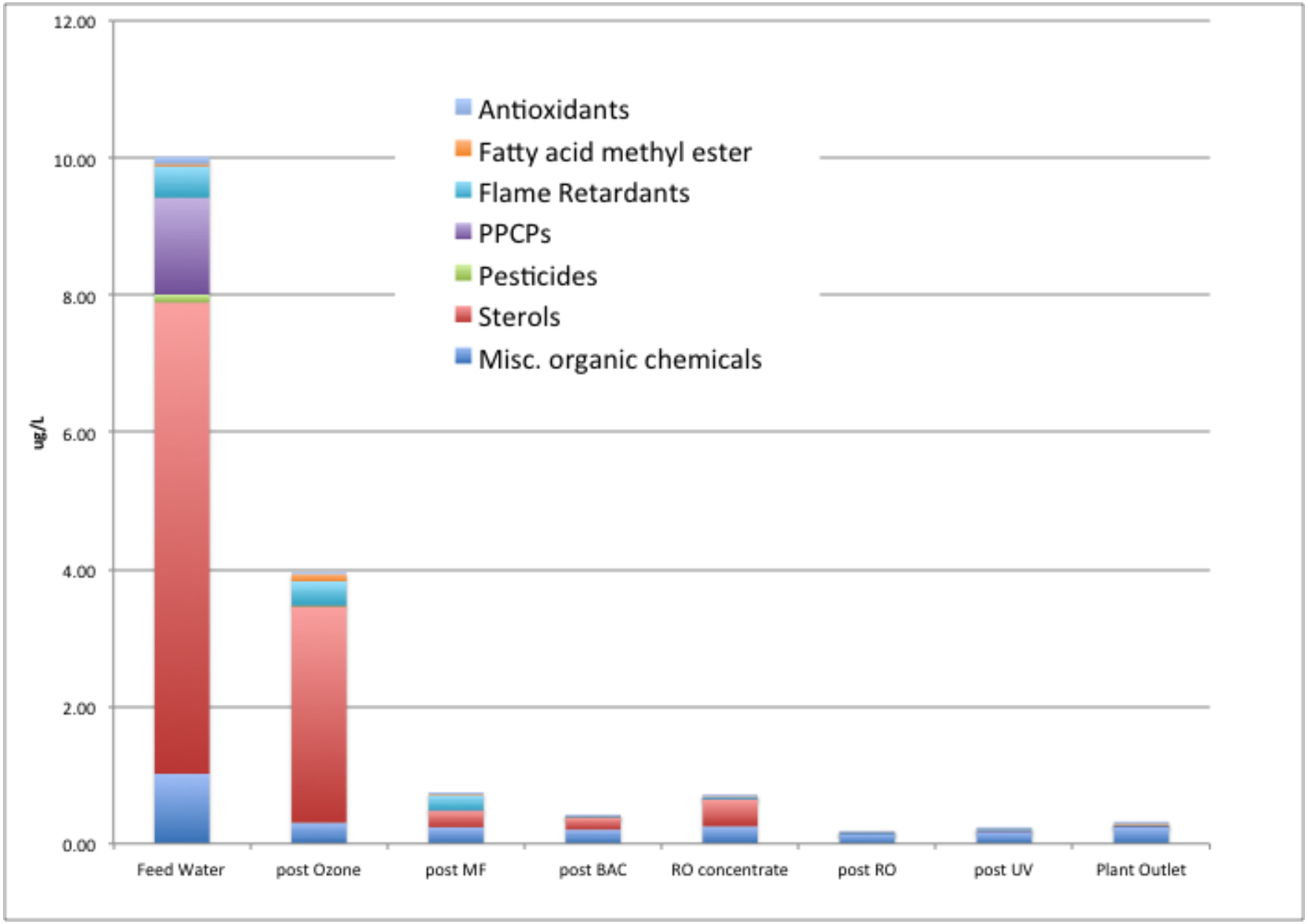


Figure 3.3 Summary of chemical detects through the AWTP treatment train

Table 3.4 Summary of chemicals detected in AWTP brine concentrate (RO conc) samples

Name	n	FOD	Average	Median	Min	Max
			(µg/L)			
Metformin	5	83	0.54	0.18	0.15	1.39
Dimethyl phthalate	5	56	0.038	0.037	0.007	0.063
Benzyl alcohol	4	44	0.616	0.215	0.026	2.008
Bisphenol A	3	33	0.022	0.022	0.015	0.028
1-Nonanol	3	33	0.014	0.015	0.010	0.016
Ethanol, 2-phenoxy-	3	33	0.251	0.222	0.212	0.320
Octanol	2	22	0.018		0.006	0.030
Tributyl phosphate	2	22	0.034		0.017	0.050
Tris(2-chloroethyl) phosphate	2	22	0.024		0.012	0.036
3-&4-Methylphenol	1	11	0.259			
4-Methyl-2,6-di-t-butylphenol (BHT)	1	11	0.018			
4-tert-Octylphenol	1	11	0.011			
Dimethylterephthalate	1	11	0.010			
(9Z)-9-Tetradecenoic acid, methyl ester	1	11	0.033			
L-Menthol	1	11	0.009			
Diethyltoluamide	1	11	0.004			

n, number of detections; FOD, frequency of detection; text in blue chemicals detected by GC-MS, in orange by LC-MS

Table 3.5 Trigger values for TrOCs in marine waters at alternative ANZECC levels of protection (adapted from ANZECC & ARMCANZ 2000).

Chemical	Trigger values for marine water (μg/L)				In screens ?	Detected?	
	Level of protection (% species)						Max conc
	99%	95%	90%	80%	Y/N	Y/N	(μg/L)
Chlorinated alkanes							
1,1,2-trichloroethane	140	1900	5800 [1]	18000 [1]	N	-	-
Anilines							
3,4-dichloroaniline	85	150	190	260	Y	N	-
Aromatic and PAHs							
Benzene	500 [1]	700 [1]	900 [1]	1300 [1]	N	-	-
Naphthalene	50 [1]	70 [1]	90 [1]	120 [1]	Y	N	-
Chlorbenzenes and chloronaphthalenes							
1,2,4-trichlorobenzene [2]	20	80	140	240	Y	N	-
Phenols and xylenols							
Phenol	270	400	520	720	Y	N	-
Pentachlorophenol [4], [2]	11	22	33	55 [3]	Y	N	-
Miscellaneous chemicals							
Poly(acrylonitrile-co-butadiene-costyrene)	200	250	280	340	N	-	-
Corexit 9527	230	1100	2200	4400 [3]	N	-	-
Organochlorine insecticides							
Endosulfan [1]	0.005	0.01	0.02	0.05 [3]	Y	N	-
Endrin [1]	0.004	0.008	0.01	0.02	Y	N	-
Organophosphorus insecticides							
Chlorpyrifos [1]	0.0005	0.009	0.04 [3]	0.3 [3]	Y	N	
Temephos [1]	0.0004	0.05	0.4	3.6 [3]	Y	N	-

Notes: classifications as per ANZECC Water Quality Guidelines (ANZECC & ARMCANZ 2000):

- Most trigger values listed are Moderate reliability figures, derived from acute LC50 data.

1. Figure may not protect key test species from chronic toxicity.
2. Chemicals for which possible bioaccumulation and secondary poisoning effects should be considered.
3. Figure may not protect key test species from acute toxicity (and chronic).
4. Tainting or flavour impairment of fish flesh may possibly occur at concentrations below the trigger value

Table 3.6 Summary of information used to calculate RQ, and calculated RQs for each chemical observed in the brine (RO) concentrate

Chemical	MEC		Short term ecotoxicological effect value			Selected CTV	Risk factor	RQmed *	RQmax
	Median	Max	Fish [b]	Aquatic invertebrates [b]	Algae [b]				
	(µg/L)			(µg/L)					
(9Z)-9-Tetradecenoic acid, methyl ester		0.03	389400	-	-	389400	100		8E-06
1-Nonanol		0.02	18000	25000	-	18000	100		9E-05
3-&4-Methylphenol		0.26	3360	5000	-	3360	100		8E-03
4-Methyl-2,6-di-t-butylphenol (BHT)		0.02	57000	17000	400	400	50		2E-03
4-tert-Octylphenol		0.01	110			110	100		1E-02
Benzyl alcohol	0.22	2.01	15000	-	-	15000	100	1E-03	1E-02
Bisphenol A	0.02	0.03	9400	226	-	226	100	1E-02	1E-02
Diethyltoluamide		0.00	71250	100000	-	71250	100		6E-06
Dimethyl phthalate	0.04	0.06	58000	-	-	58000	100	6E-05	1E-04
Dimethylterephthalate		0.01	9600	30400	32300	9600	100		1E-04
Ethanol, 2-phenoxy-		0.320	344000			344000	100		9E-05
L-Menthol		0.01	13200	25000	20000	13200	100		7E-05
Metformin	0.18	1.39	1000	-	-	1000	100	2E-02	1E-01
Octanol		0.03	75000	93000	-	75000	100		4E-05
Tributyl phosphate		0.050	4200		1100	4200	100		1E-03
Tris(2-chloroethyl) phosphate		0.01	190000	-	-	190000	100		6E-06
NDMA	0.05	0.09	940000	300000	-	300000	100	2E-05	3E-05
NDEA	0.03	0.05	775000	230000	-	230000	100	1E-05	2E-05

* Calculated only for chemical with more than 4 reported residues; MEC, measured environmental concentration

RQ >1 indicates a concentration of significance.

Table 3.7 Summary of information used to calculate TU, and calculated TUs for each trophic level for chemicals observed in brine (RO) concentrate

Chemical	MEC	Short term (acute) ecotoxicological effect value				TUf	TUzp	TUpl	TUalg
	Max	Fish [b]	Aquatic invertebrates [b]	Aquatic plants [b]	Algae [b]				
				(µg/L)					
(9Z)-9-Tetradecenoic acid, methyl ester	0.03	389400	-	-	-	-7.1		-	
1-Nonanol	0.02	18000	25000	-	-	-6.1	-6.2	-	
3-&4-Methylphenol	0.26	3360	5000	-	-	-4.1	-4.3	-	
4-Methyl-2,6-di-t-butylphenol (BHT)	0.02	57000	17000	-	400	-6.5	-6.0	-	-4.4
4-tert-Octylphenol	0.01	110				-4.0			
Benzyl alcohol	2.01	15000	-	-	-	-3.9		-	
Bisphenol A	0.03	9400	226	-	-	-5.5	-3.9	-	
Diethyltoluamide	0.00	71250	100000	-	-	-7.2	-7.4	-	
Dimethyl phthalate	0.06	58000	-	-	-	-6.0		-	
Dimethylterephthalate	0.01	9600	30400	-	32300	-6.0	-6.5	-	-6.5
Ethanol, 2-phenoxy-	0.32	344000				-6.0			
L-Menthol	0.01	13200	25000	-	20000	-6.2	-6.5	-	-6.4
Metformin	1.39	1000	-	-	-	-2.9		-	
Octanol	0.03	75000	93000	-	-	-6.4	-6.5	-	
Tributyl phosphate	0.05	4200			1100	-4.9			-4.3
Tris(2-chloroethyl) phosphate	0.01	190000	-	-	-	-7.2		-	
NDMA	0.09	940000	300000	-	-	-7.0	-6.5	-	
NDEA	0.05	775000	230000	-	-	-7.2	-6.6	-	

TU >-3 indicates a concentration of signifcance

3.3 Bioassay data

The project addressed it's third objective (*to verify that the demonstration plant is operating effectively by screening feed, RO concentrate and outlet waters for TrOCs of the same mode of biological action ("hormonal activity")*) using recombinant receptor-reporter gene bioassays in house and through collaboration with Dr Daisuke Nakajima and Dr Fujio Shiraishi (National Institute for Environmental Studies (NIES), Tsukuba, Japan).

Sample toxicity was measured using a modified photobacterium test. With this photobacterium test system, the lower the ICR50 reported for the disk extracts, the higher the toxicity of the waters sampled by the disk, with samples considered highly toxic to the photobacterium at an ICR50 < 100, and non-toxic at an ICR50 > 400. In this context, in R1, R2 and R3 only the feed water was tested, whereas the feed water, product water and RO concentrate was tested in R5-R9. For the purposes of this summary report, unless otherwise mentioned, only the data from R5 – R12 will be discussed.

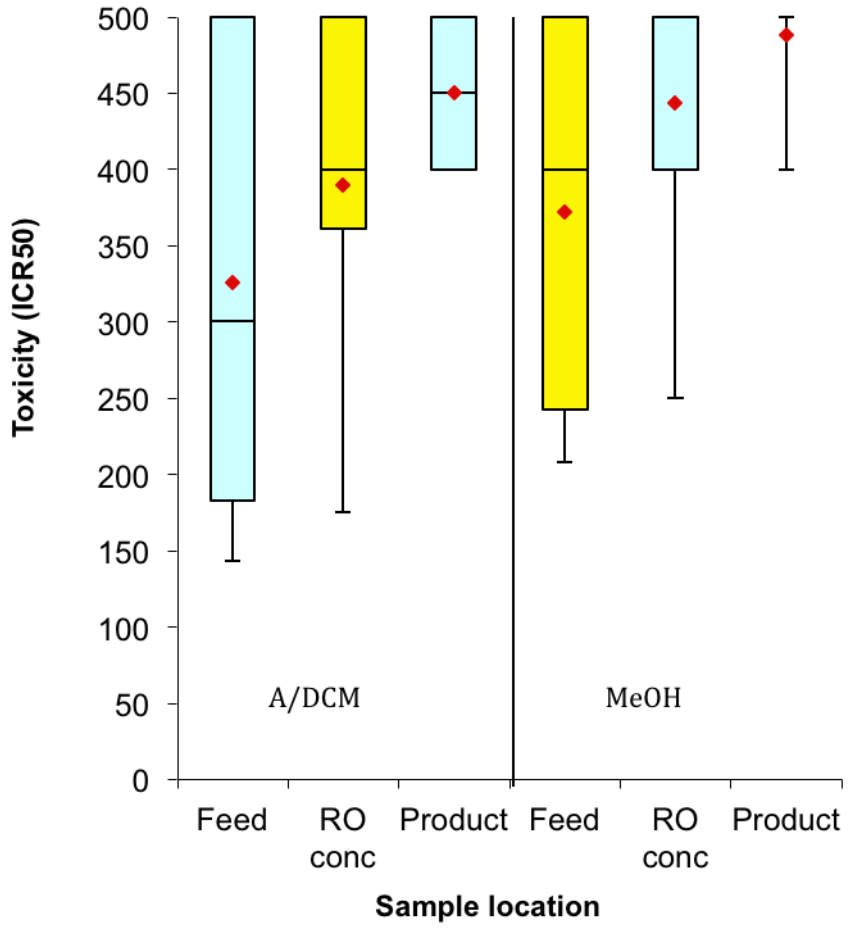


Figure 3.4 Summary of general toxicity (as measured using P.B. test)
Note: the lower the ICR50, the higher the toxicity.

For R5-R9, most of the samples were considered to be only weakly or non- toxic (Figure 3.4), although some of the samples of feed water and RO concentrate were weakly toxic. The product water was non toxic. The lack of toxicity of the brine concentrate samples suggests that were this water to be discharged to the marine environment, it would be unlikely to cause an adverse effect on aquatic organisms.

Many in vitro assays have been developed to screen for chemicals of similar modes of action in natural waters (Escher et al. 2014), including the recombinant receptor-reporter gene assays such as the yeast two-hybrid bioassays used in this study. Recombinant receptor-reporter gene assays, such as the yeast two-hybrid bioassays used in this study, measure the activation of a receptor, and allow for quantification of 'hormonal' activity without having to know the precise chemical make up of the sample. In that context, the bioassays were used as a measure of the performance of the AWTP only, not for comparison with regulatory guidelines.

In R1-R3, very little estrogenic activity was observed in the feed water. Given the data reported by Allinson et al (2010) this was considered unusual. The results of the positive controls run at the same time as the samples suggest the bioassays were performing to expectation, and thus capable of measuring low ng/L levels of hormonal activity. At that time, the samples were tested as a 'whole sample extract' and subsequent fractionation of samples by NIES staff suggested that there were anti-estrogenic chemicals in the samples, and that these anti-estrogenic chemicals were 'masking' the estrogenic activity. Consequently, for R5 – R12 the initial sample extracts were subjected to a florisil fractionation process.

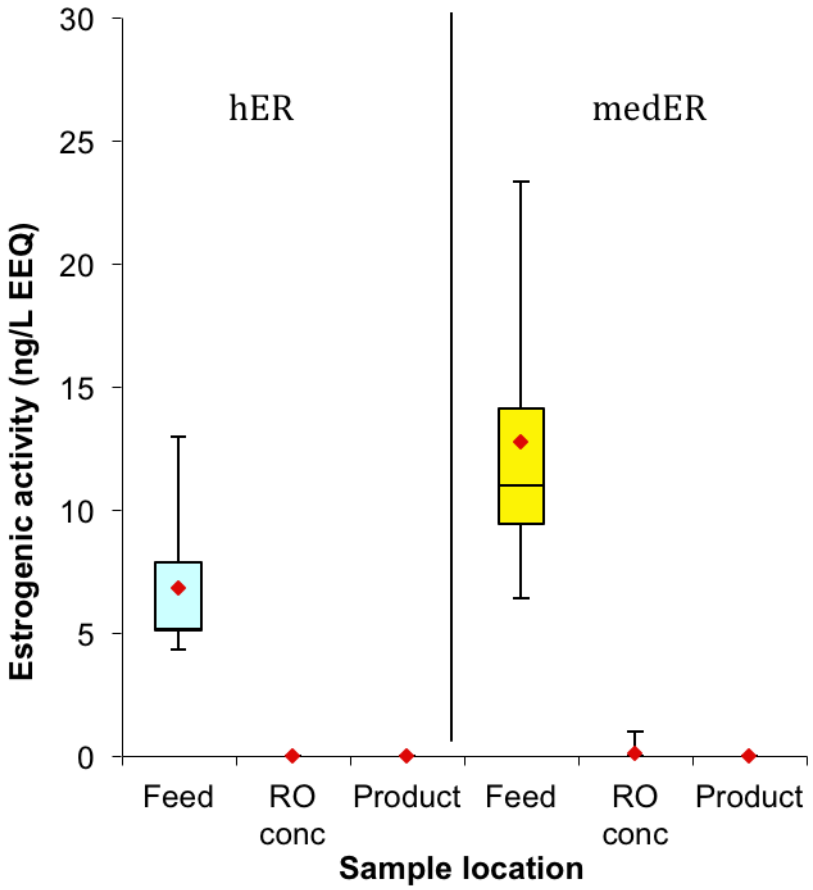


Figure 3.5 Summary of ER assay data (R5-R12)

◆, arithmetic mean; dividing line within data boxes, data median; upper and lower boundaries of boxes, 75th and 25th percentile of data; error bars represent the range.

The data (all fractions pooled) obtained for R5 –R12 suggests that there are estrogenic chemicals in the feedwater (hER up to ~ 13 ng/L EEQ; medER, up to ~ 23 ng/L EEQ; Figure 3.5), but that these chemicals are being removed by the treatment train with no ER activity observed in product water or RO concentrate.

The majority of known AhR ligands are coplanar aromatic dioxin-like compounds. For instance, Hilscherová et al. (2000) reported that the three potential classes of compounds with dioxin-like properties that can bind to the AhR were (1) planar hydrophobic aromatic compounds (such as planar congeners of PCBs and PCDD/PCDFs, polychlorinated naphthalenes (PCNs), and several high molecular weight PAHs); (2) poly- and mixed halogenated and alkylated analogues of class (1) compounds (chlorinated xanthenes and xanthones, and polychlorinated diphenyl toluenes, anisols, anthracenes, fluorenes); and (3) a wide range of non-planar, non-aromatic, lipophilic compounds that are transient inducers and weak AhR ligands (includes natural compounds like indoles, heterocyclic amines, and some pesticides and drugs). Most, if not all, of these chemicals might be expected to be adequately sampled by the Empore™ C18FF disk used in this study.

The data obtained for R5 – R12 suggests that there are chemicals that stimulate the AhR receptor in the feedwater (AhR up to ~ 380 ng/L β-NF EQ; Figure 3.6), but that these chemicals are mostly being removed by the treatment train, with little AhR activity observed in product water or RO concentrate.

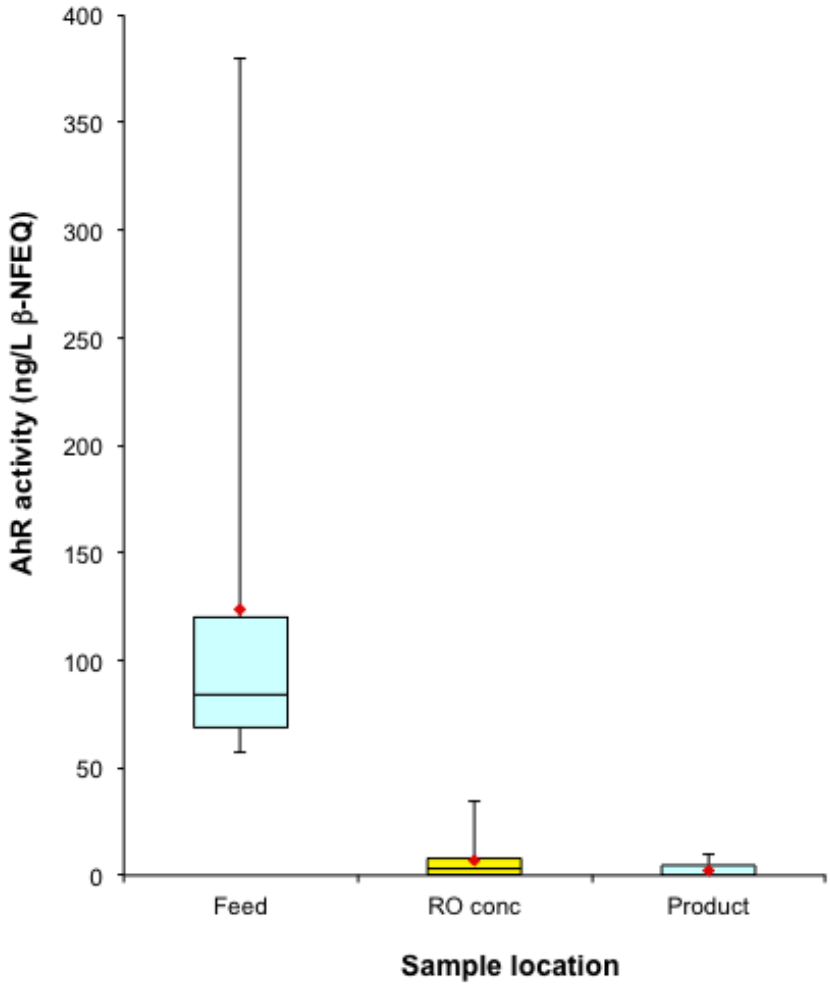


Figure 3.6 Summary of AhR assay data (R5-R12)

◆, arithmetic mean; dividing line within data boxes, data median; upper and lower boundaries of boxes, 75th and 25th percentile of data; error bars represent the range.

The CAR assay responds to a very wide range of xenobiotic chemicals, including chemicals that stimulate the ER and AhR receptors. The CAR data is consistent with that reported for the ER and AhR assays, i.e. that there are chemicals that stimulate the CAR receptor in the feedwater (CAR up to ~ 3.4 µg/L OPEQ; Figure 3.7), but that these chemicals are mostly removed by the treatment train.

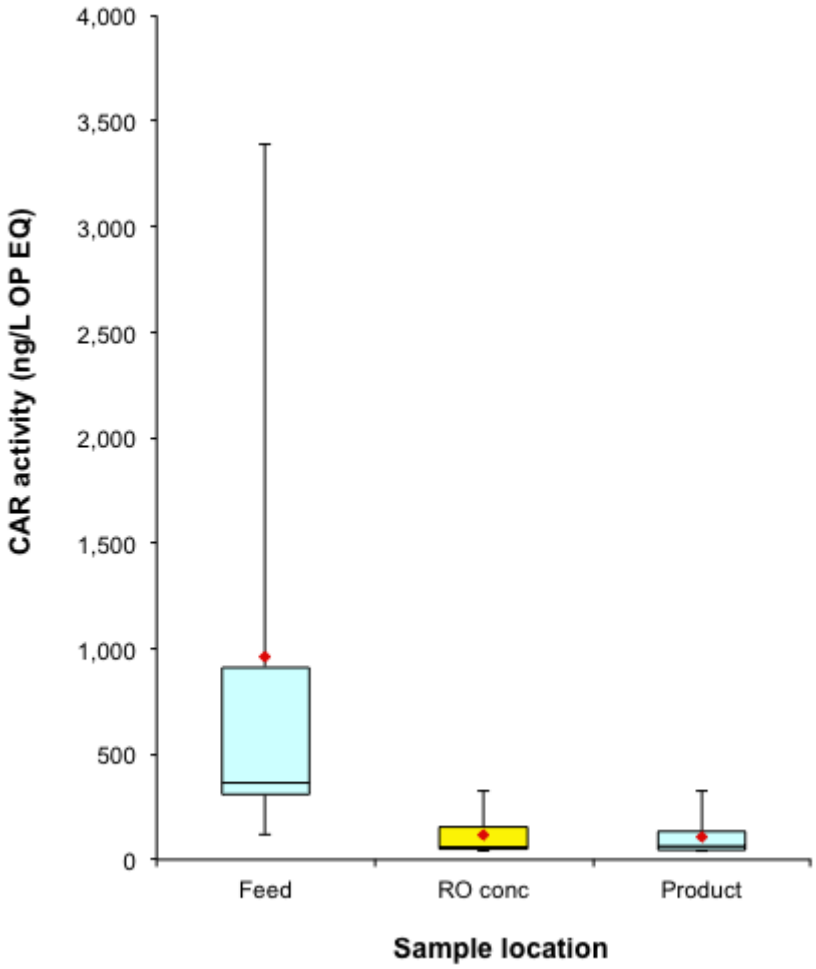


Figure 3.7 Summary of CAR assay data (R5-R12)

◆, arithmetic mean; dividing line within data boxes, data median; upper and lower boundaries of boxes, 75th and 25th percentile of data; error bars represent the range.

3.4 Nitrosamines

The project addressed its fourth objective (*to verify that the demonstration plant is operating effectively by screening feed, barriers' and outlet waters for nitrosamines*) through collaboration with Associate Professor Stuart Khan, UNSW.

N-nitrosamines are TrOCs of rapidly growing health and regulatory concern in drinking water and reclaimed effluent. The formation of N-nitrosamines, especially N-nitrosodimethyl-amine (NDMA), in treated wastewater and environmental waters has been known since 1970's, but the recognition of these chemicals as disinfection by-products is relatively recent. N-nitrosamines can be formed during chloramination, chlorination, ozonation, combination of chlorine and ultraviolet, or advanced oxidation process of drinking waters and wastewaters (NHMRC & NRMMC 2011).

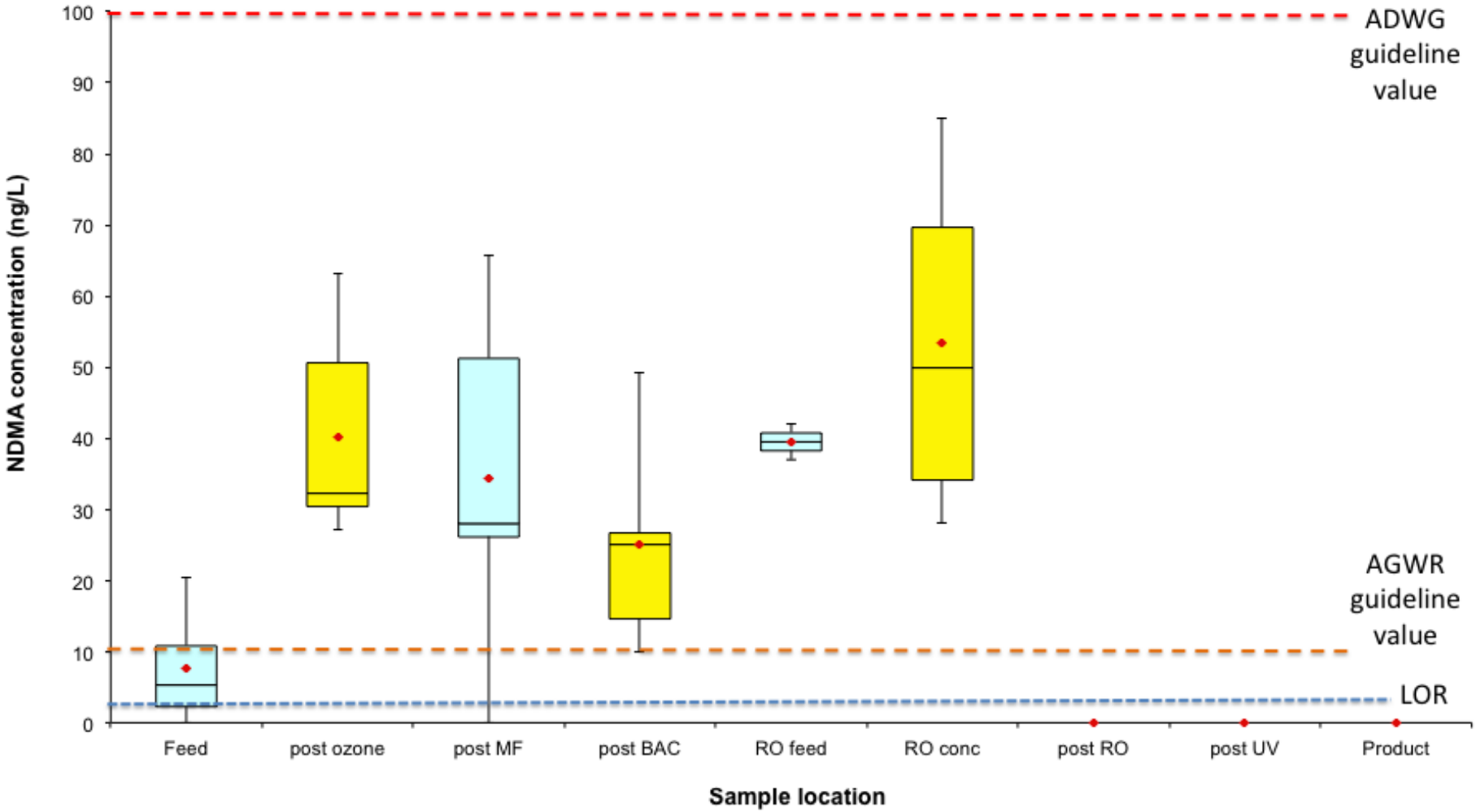


Figure 3.8 Summary of NDMA data for sampling rounds R5 – R10.

◆, arithmetic mean; dividing line within data boxes, data median; upper and lower boundaries of boxes, 75th and 25th percentile of data; error bars represent the range of reported data (concentrations). LOR, limit of reporting (3 ng/L)

N-nitrosamines were among the highest ranked emerging disinfection by-products in a recent prioritization process for future public health regulation. The World Health Organization Guidelines for Drinking Water Quality have recently included a guideline for NDMA of 100 ng/L. Consistent with this, recent Australian Drinking Water Guidelines (ADWG) have also included a health-based guideline value of 100 ng/L for NDMA (NHMRC & NRMMC 2011), whilst the Australian Guidelines for Water Recycling (Augmentation of Drinking Water Supplies) (AGWR; NRMMC, EPHC, NHMRC 2008) has a guideline level of 10 ng/L (will be reviewed in the next revision of the guidelines). It is against the ADWG and AGWR guideline values that our product water data is assessed.

The project conducted six rounds of sampling for N-nitrosamines in influent, post-barriers and discharge water samples (Table 3.1). An analysis of the data suggests that a small amount of NDMA (< 10 ng/L on average) enters the plant in the feed water (Figure 3.8). The concentration of NDMA then increases post-ozone to~ 45 ng/L, but is not observed in the brine concentrate or in the product water.

The other most consistently observed N-nitrosoamine was NDEA, which was observed in the feed water and all barrier samples (maximum concentration 54 ng/L in RO concentrate), including in the product water at up to 10 ng/L. N-nitrosomorpholine was also observed in the feed water and all barrier samples until the RO concentrate (maximum concentration 53 ng/L in RO concentrate), but not observed in the product water.

We can say that our objective with this testing was achieved, because the **final product water quality meets the ADWG and AGWR levels for NDMA**, specifically because all product water concentrations were well below their respective 100 and 10 ng/L limits.

There is no ANZECC & ARCMANZ (2000) marine water quality guideline value for NDMA. However, by using the World Health Organisation's hyper-sensitive approach to assessing the risks of NDMA to aquatic organisms, we can again say that our objective with this testing was achieved because we find that the RQs for the measured nitrosamines are well below 1 (Table 3.6). Moreover, the $\log_{10}$TUs for fish and invertebrates for the measured nitrosamines are well below -3 (Table 3.7). Taking these assessments together, we can conclude that **NDMA releases at the concentrations observed in this study would be unlikely cause adverse effects** on populations of aquatic organisms in the receiving environment.

4 Conclusions and Recommendations

In this study we used two chromatographic-mass spectrometric multi-residue methods to screen TrOCs in feed, environmental discharge (a brine concentrate), and product water. We were able to unambiguously detect almost 80 chemicals in the feed water, but only 20 chemicals in the product water and only 16 chemicals in the environmental discharge (brine concentrate). In that context, we conclude that:

- M**ost of the TroCs were removed from the feed water by the treatment train**.
- **No residue in the product water exceeded its listed Australian Guidelines for Water Recycling (Augmentation of Drinking Water Supplies) (NRMMC, EPHC, NHMRC 2008) level**.
- **No residue in the brine concentrate waste stream exceeded an ANZECC & ARMCANZ (2000) water quality guideline trigger value for marine waters**,

Consequently, we conclude **that releases of the TrOCs observed at the concentrations observed in this study would be unlikely cause adverse effects** on populations of aquatic organisms in the receiving environment or people drinking the product water.

Sample toxicity and receptor activity measurements of the brine concentrate and product water also suggests that most of the toxic and bioactive TrOCs were being removed by the treatment train.

A small amount of NDMA entered the plant in the feed water, with an additional small amount created by the AWTP itself, but no NDMA was observed post-RO in the environmental discharge water (the brine concentrate) or in the product water. The TrOCs team conducted six rounds of sampling for NDMA through collaboration with a reputable university laboratory, and so this observation is considered to be a real effect. However, experts on reverse osmosis both within and outside the project team consider the complete removal of NDMA by the RO system to be a very unusual observation. Consequently, the first major recommendation from the project team is to:

- **Undertake further testing for NDMA across the AWTP's barriers and in the environmental discharge and product water**. Screening should be at least monthly for up to 12 months. Because NDMA levels are very low, samples should be analysed simultaneously by two laboratories recognised for their ability to measure ng/L levels of NDMA. Aggregating the data obtained from two laboratories should assure the industry that any lack of NDMA post-RO is a real effect, perhaps leading to new insights for the removal of NDMA from potable and otherwise recycled water.

The objective of the 'Demonstration of Robust Recycling' project is to demonstrate a robust, low chemical use water recycling process that produces a saline effluent fit for disposal in remote locations with minimal operator involvement. One issue with operation of treatment plants in remote areas is that of the logistical difficulties associated with transport of water samples to distant laboratories for analysis. Grab (or spot) samples are commonly used to characterise chemical residues in water samples. The advantage is that the matrix itself is analysed and concentrations can be easily related to toxicity values for assessing exceedances of regulatory

threshold values (TVs) as well as for probabilistic risk assessment. The disadvantage of grab samples is that they may miss a residue peak if they are taken too infrequently. In that context, the second major recommendation from this project team is:

- **To trial time integrative passive sampling as a means for cost-effective monitoring of chemical concentrations in feed, environmental discharge and product waters**.

Passive sampling devices allow measurement of an average, pseudo-bioavailable concentration over a long period of time (typically in the order of several weeks). In principle, passive samplers can be calibrated so that the time-weighted average concentrations of TrOCs can be determined after the sampler has been exposed in the field. Moreover, many passive samplers are small enough to be sent to and from a treatment facility by post. These attributes may facilitate the abilities of remote communities to ensure adequate removal of TrOCs from an AWTP.

Once the AWTP is commissioned at Davis Station the AAD may need to provide reassurance of adequate TrOCs removal. In that context, the project team recommends that **a determination of an indicator list be based on the number and type of chemicals found at Davis Station and the relative risk of their being observed in the feed to the AWTP**. Once the AAD has determined which surrogate (performance) TrOCs it wishes to monitor, and the method it wishes to use (i.e. spot vs. passive vs. on-line sampling) then the number and type of analytical screens can be determined.

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Appendices

Appendix A: Micro-contaminant assessment

Background

Recycling of water for recreational and potable use is a key issue in securing water supply and providing community resilience to variable water supply and climatic events. The pathogen status of recycled water is a key regulatory issue. It dominates the design, technology choice and operational practice of recycled water facilities. However, as well as pathogens such as bacteria, virus, helminths and protozoa, municipal wastewaters may contain a large number of chemicals. More than 100,000 chemicals are registered at EINECS (European inventory of existing chemical substances) and around 30,000 to 70,000 chemicals are used daily (European Commission, 2011).

Traditionally, wastewater treatment facilities are designed to reduce environmental nutrients such as nitrogen, phosphorus and readily assimilated carbon rich chemicals to levels that, upon discharge to receiving waters, ensure no detrimental eutrophication effects in the environment. However, there are some chemicals that are directly toxic to organisms living in receiving waters, while others may elicit more subtle effects, including genotoxic or endocrine disrupting (EDCs) outcomes. Managing the effects of such contaminants ultimately requires information on both effluent toxicity and chemical concentrations. Equally, both of these issues are important to community re-use of waste water, particularly direct or indirect recycle to potable. The Australian Drinking Water Guidelines (ADWG; NHMRC & NRMMC 2011) and Australian Guidelines for Water Recycling (AGWR; NRMMC, EPHC & NHMRC 2008) provide an overview of the maximum recommended concentration of a range of chemicals (344 in total) that fall into a variety of categories including disinfection by-products [DBP's; 18 chemicals], pesticides [160 chemicals], pharmaceuticals and personal care products (PPCP's; 82 chemicals], industrial chemicals [41 chemicals or classes of chemicals], antioxidants [5 chemicals], chelating agents [4 chemicals], flame retardants [4 chemicals], fragrances [7 chemicals], plasticizers [4 chemicals], surfactants [3 chemicals], sterols [3 chemicals], phytochemicals [1 chemical], and hormones [12 chemicals]. Some chemicals are prescribed in both the ADWG and AGWR and the maximum recommended levels often differ.

The design of the Advanced Water Treatment Plant (AWTP) for Davis Station, whilst primarily focussed on pathogen removal, specifically considered the removal of micro-contaminants in its design. Micro-contaminant reduction in both the product water and in the wastewater discharged is of interest. In particular, a ceramic membrane with active ozone followed by biological activated carbon was chosen specifically prior to reverse osmosis, UV and chlorine treatments to avoid the use of chloramine for membrane protection, produce disinfection by-products that are predominately highly charged and easily removed by reverse osmosis and break down of organic compounds. The barrier configuration chosen for the AWTP is unique and although the role of barriers such as ozonation and reverse osmosis have been studied in detail in isolation, the expected combined micro-contaminant removal effect of the AWTP configuration was unknown and needed to be tested.

The wide structural variety of organic chemicals found in wastewaters has historically meant many analytical methods have had to be used to cover the large number of known chemicals, with the concomitant financial implications associated with conducting multiple quantitative tests. This is an

issue even for large recycling facilities, where the expense of operating or outsourcing the operation of the necessary analytical equipment are combined with a wide range of site sampling, sample stabilization and sample transport protocols. Such analytical programs become even more problematic for small and/or remote facilities where both the analytical cost per unit of water produced becomes prohibitive and the deployment of expertise to site is also costly (relative to large facilities). Indeed, in the case of the AWTP, there are times of the year where deployment of expertise to site is impossible. Bearing these factors in mind, it is evident, that the cost of regular (i.e. weekly or monthly) measurement of all chemicals listed in the ADWG or AGWR would preclude the recycling to potable of water from small and remote facilities.

Preliminary screening of samples using rapid assessment tools that allow sample batching and simple sampling and stabilization protocols is an increasingly attractive prospect for waterways managers and is considered as essential to this project. Rapid screening does not preclude the innovative use of more traditional chemical analytical techniques, such as gas chromatography-mass spectrometry (GC-MS). For instance, Kadokami et al. (2005) developed a new method combining a mass-structure database with GC-MS to create a system (Automated Identification and Quantification System: AIQS-DB) that can screen samples for 940 semi-volatile trace organic chemicals (TrOCs), including numerous halogenated and non-halogenated hydrocarbons; polycyclic aromatic hydrocarbons (PAHs); polychlorinated biphenyl compounds (PCBs); a range of pharmaceutical and personal care products (PPCPs); and agricultural compounds (see Table A3). Regular additions to the database means the number of TrOCs will grow considerably and there is a good opportunity to show significant overlap with the ADWG and AGWR lists into the future. Importantly, the analytical technique involves a single sample preparation and analytical step. Kadokami and his team have also developed a multi-residue method for liquid chromatography linked to time of flight mass spectroscopy (LC-TOF-MS) analysis that can screen samples for 265 non-volatile compounds, including 180 agricultural compounds and 70 pharmaceuticals (antibiotics, antidepressants, beta blockers, analgesics, etc.). The complete range of chemicals analysed in this screen is provided in Table A4.

The AIQS-DB method identifies and quantifies chemical substances by using a combination of retention times, mass spectra, and internal standard calibration curves registered in the database. In order to obtain accurate results, a GC-MS and LC-TOF-MS has to be adjusted to designated conditions that closely match the instrumental conditions when the database was constructed. For GC-MS analysis, the results obtained from performance check standards are evaluated against three criteria (Kadokami et al. 2004; 2005): spectrum validity, inertness of column and inlet liner, and stability of response. When the results for performance check standards satisfy the criteria, the difference between the predicted and actual retention times is less than 3 s, and chemical concentrations obtained are comparable to those obtained by conventional internal standard methods (Kadokami et al. 2005; 2009). The method detection limits (MDL) for most of the target substances from the GC-MS, as estimated from concentration ratio and the instrument detection limit (IDL), are from 0.01 to 0.1 µg/L

The LC-MS AIQS-DB method has been newly developed with Time-of-Flight-mass spectrometry. High resolution, high sensitivity, and new TOF-MS methodology has made a multi-screen of non-volatile TrOCs possible. By utilising 125 model compounds with a new solid-phase extraction method, the method detection limits of 70% of model compounds is in the range 2.5-5 ng/L.

Not all of the chemicals listed in the AIQS screen database are relevant to the screening of wastewater. A typical example for 120 compounds is shown in Table A1. The table also summarises the occurrence of these chemicals in WWTP effluents in Victoria (39 sites; Allinson, unpublished data) and candidate chemicals for indicators of treatment performance (from Drewes et al. 2008). It is interesting to note that of the 120 compounds listed, 62 have been detected in the screen of WWTPs in Victoria, 28 are listed as indicator compounds by Drewes et al. (2008) but, as noted earlier, there is only an overlap of 15 compounds between those detected in WWTP effluents and the indicator compounds of Drewes et al. (2008).

The 64 indicator compounds recommended by Drewes et al. (2008) are shown in Table A2, cross-referenced to the presence in the AIQS-DB method (GC-MS and/or LC-TOF-MS).

The 12 indicator compounds recommended by Water Corporation (2013) are shown in Table A3, cross-referenced to the presence in the AIQS-DB method (GC-MS and/or LC-TOF-MS).

In this study we used two chromatographic-mass spectrometric multi-residue methods to screen for ~1250 TrOCs in the AWTP feed, post-barrier and product water (see Tables A4 and A5). Approximately half of the TrOCs in the ADWG and AGWR guidelines (~100 and 80 chemicals, respectively), approximately half the TrOCs in Water Corporation (2013) list, and approximately one quarter of the TrOCs in Drewes et al. (2008) list are in one of (or both of) the two multi-residue screens, suggesting that even though we chose not to screen for a pre-selected list of indicator chemicals, the multi-residue methods still provided a representative indicator subset of chemicals found in WWTP effluent.

Table A1. GC-MS AIQS Chemical List - Relevant to Wastewater 1. MDL: Method Detection Limit; 2. Allinson, 2012 survey, unpublished data; 3. WRRF Report (Drewes, et al. 2008). ref. Table 8.1; 4. NDMA is under consideration for entry.

Category	Compounds	[1]MDL (μg/L)	[2]Occurrence in VIC WWTP effluent	[3]Indicator Candidates in WRRF Report
Ph-analgesic	Acetylsalicylic acid (Aspirin)	0.01	no	yes
Ph-antiepileptic	Carbamazepine	0.01	yes	yes
Ph-antifungal	Crotamiton	0.01	yes	no
Ph-insecticide	Diethyltoluamide (DEET)	0.01	yes	yes
Ph-analgesic	Ethenzamide	0.01	no	no
Ph-anti-inflammatory	Fenoprofen	0.01	no	yes
Ph-analgesic	Ibuprofen	0.01	no	yes
Ph-topical-analgesic	L-Menthol	0.01	no	no
Ph-anti-inflammatory	Mefenamic acid	0.01	no	yes
Ph-anti-histamine	Methapyrilene	0.01	no	no
Ph-anti-inflammatory	Naproxen	0.01	no	yes
Ph-analgesic	Phenacetin	0.01	no	no
Ph-analgesic	Propyphenazone	0.01	no	yes
Ph-topical	Squalane	0.025	yes	no
Ph-antiseptic	Thymol	0.01	yes	no
Ph-antibacterial	Triclosan	0.01	no	yes
stimulant	Caffeine	0.01	yes	yes
stimulant	Nicotine	0.01	no	no
cosmetics/fragrance	Acetophenone	0.01	no	no
cosmetics/fragrance	1-Nonanol	0.01	no	no
cosmetics/fragrance olvent	Octanol	0.01	no	no
cosmetics/fuel additive/solvent	2-Methyl-2,4-pentandiol	0.01	no	no
cosmetics/fuel	Benzyl alcohol	0.01	no	no
fragrance	Diphenyl ether	0.025	no	no
fragrance	2-Heptanol	0.01	no	no
fragrance	Butanoic acid, butyl ester	0.01	no	no
fragrance	Phenylethyl alcohol	0.01	no	no
fragrance/solvent	Anthraquinone	0.025	no	no
perfumes	Isosafrole	0.01	no	no
perfumes/solvent	alpha-Terpineol	0.025	no	no
surfactant	4-n-Octylphenol	0.01	no	yes
surfactant	4-tert-Octylphenol	0.01	yes	yes
surfactant	4-n-Heptylphenol	0.01	yes	no
surfactant	3-&4-Methylphenol	0.02	yes	no
surfactant	Phenol	0.01	yes	yes
surfactant	Nonylphenol	0.01	yes	yes
plasticizer	Bisphenol A	0.01	yes	yes
plasticizer	1,1,1-Trichloro-2-methyl-2-propanol	0.01	no	no
plasticizer	2-Ethyl-1-hexanol	0.01	no	no
plasticizer	Bis(2-ethylhexyl)sebacate	0.01	no	no
plasticizer	Bis(2-ethylhexyl)phthalate (DEHP)	0.01	yes	yes
plasticizer	Butyl benzyl phthalate	0.01	yes	no
plasticizer	Di-n-butyl phthalate	0.01	yes	no
plasticizer	Di-n-octyl phthalate	0.01	no	no
plasticizer	Di(2-ethylhexyl)adipate	0.01	no	no
plasticizer	Dicyclohexyl phthalate	0.01	yes	no
plasticizer	Diethyl phthalate	0.01	yes	no

Table A1 (continued)

Category	Compounds	[1]MDL (μg/L)	[2]Occurrence in VIC WWTP effluent	[3]Indicator Candidates in WRRF Report
plasticizer	Diisobutyl phthalate	0.025	yes	no
plasticizer	Dimethyl phthalate	0.01	yes	no
plasticizer	Dipentyl phthalate	0.01	no	no
plasticizer	Dipropyl phthalate	0.01	no	no
flame retardant	1,2,5,6,9,10-Hexabromocyclododecane	0.01	no	no
flame retardant	2,2',4,4',5,5'-Hexabromobiphenyl (BB-153)	0.01	no	no
flame retardant	2,2',5,5'-Tetrabromobiphenyl (BB-52)	0.01	no	no
flame retardant	2,2'-Dibromobiphenyl (BB-4)	0.01	no	no
flame retardant	2,2',4,4',5,5'-Hexabromodiphenyl ether (BDE-153)	0.01	no	no
flame retardant	2,2',4,4'-Tetrabromodiphenyl ether (BDE-47)	0.01	no	no
flame retardant	2,4-Dibromodiphenyl ether (BDE-7)	0.01	no	no
flame retardant	Tributyl phosphate	0.01	yes	no
flame retardant	Tris(1,3-dichloro-2-propyl) phosphate (TDCPP)	0.025	no	yes
flame retardant	Tris(2-chloroethyl) phosphate (TCEP)	0.01	yes	yes
flame retardant	Tris(2-chloroethyl)phosphite	0.01	no	no
flame retardant	Tricresyl phosphate	0.025	no	no
flame retardant	Tris(2-ethylhexyl) phosphate (TEHP)	0.025	no	yes
flame retardant	1,2,5,6,9,10-Hexabromocyclododecane	0.01	no	no
flame retardant	2,2',4,4',5,5'-Hexabromobiphenyl (BB-153)	0.01	no	no
flame retardant	2,2',5,5'-Tetrabromobiphenyl (BB-52)	0.01	no	no
flame retardant	2,2'-Dibromobiphenyl (BB-4)	0.01	no	no
flame retardant	2,2',4,4',5,5'-Hexabromodiphenyl ether (BDE-153)	0.01	no	no
flame retardant	2,2',4,4'-Tetrabromodiphenyl ether (BDE-47)	0.01	no	no
DBPs	1,4-Dichlorobenzene	0.01	no	yes
DBPs	N-Nitrosodiethylamine (NDEA)	0.01	no	no
DBPs	[4]N-Nitrosodimethylamine (NDMA)	-	-	yes
insecticide	3-Hydroxycarbofuran	0.01	yes	no
insecticide	Allethrin	0.01	yes	no
insecticide	Bendiocarb	0.01	yes	no
insecticide	Carbaryl	0.01	yes	no
insecticide	Ethiofencarb	0.01	yes	no
insecticide	Fenobucarb	0.01	yes	no
insecticide	Methidathion	0.01	yes	no
insecticide	Omethoate	0.01	yes	no
insecticide	p,p'-DDD	0.01	yes	no
insecticide	Piperonyl butoxide	0.01	yes	no
insecticide	Propoxur	0.01	yes	no
insecticide	Thiocyclam	0.01	yes	no
insecticide	Trichlorfon	0.01	yes	no
herbicide	Atrazine	0.01	yes	yes
herbicide	Benfuresate	0.01	yes	no
herbicide	Benoxacor	0.01	yes	no
herbicide	Bensulide	0.01	yes	no
herbicide	Cinmethylin	0.01	yes	no
herbicide	Dichlobenil	0.01	yes	no
herbicide	Hexazinone	0.01	yes	no
herbicide	Methyl dymron	0.01	yes	no

Table A1 (continued)

Category	Compounds	[1]MDL (μg/L)	[2]Occurrence in VIC WWTP effluent	[3]Indicator Candidates in WRRF
herbicide	Metolachlor	0.01	yes	yes
herbicide	Pebulate	0.01	yes	no
herbicide	Propham	0.01	yes	no
herbicide	Simazine	0.01	yes	yes
herbicide	Terbcarb	0.01	yes	no
herbicide	Terbutryn	0.01	yes	yes
fungicide	2-Phenylphenol (OPP)	0.01	yes	no
fungicide	Chloroneb	0.01	yes	no
fungicide	Oxadixyl	0.01	yes	no
fungicide	Simeconazole	0.01	yes	no
fungicide	Spiroxamine	0.01	yes	no
fungicide	Thiabendazole	0.01	yes	no
tyre leachate	1,3-Dicyclohexylurea	0.01	no	no
tyre leachate	2-(Methylthio)-benzothiazol	0.025	yes	yes
tyre leachate	2-Acetyl-5-methylthiophene	0.01	no	no
tyre leachate	2-Cyclohexen-1-one	0.01	no	no
tyre leachate	2-Mercaptobenzothiazole	0.01	no	yes
tyre leachate	2-Methoxyphenol	0.025	no	no
tyre leachate	2-Methylbenzothiazole	0.025	no	no
tyre leachate	2(3H)-Benzothiazolone	0.01	yes	no
tyre leachate	Acetamide, N-(2-phenylethyl)-	0.01	yes	no
tyre leachate	Acetamide, N-phenyl-	0.01	no	no
tyre leachate	Benzaldehyde, 4-hydroxy-3,5-dimethoxy-	0.01	no	no
tyre leachate	Benzamide, N-phenyl-	0.01	no	no
tyre leachate	Benzothiazole	0.01	yes	yes
tyre leachate	Cyclohexanamine, N-cyclohexyl-	0.01	no	no
tyre leachate	Cyclohexanol	0.01	no	no
tyre leachate	Ethanol, 2-phenoxy-	0.01	yes	no
tyre leachate	Formamide, N-cyclohexyl-	0.01	no	no
tyre leachate	Phenol, 2,6-dimethoxy-	0.01	no	no
tyre leachate	Phenol, 4-(phenylamino)-	0.01	yes	no
tyre leachate	Phthalimide	0.01	no	no
tyre leachate	Urea, N,N-diethyl-	0.01	no	no

Table A2. The 64 TRoCs suggested by Drewes, et al. (2008) **as indicator chemicals**

Indicator Compound	$logK_{ow}$	GC	LC	AIQS	Category	Sub-category
Chloroform	1.97	GC (unknown)			DBP	
NDMA	-0.64	GC-MS/MS			DBP	
TDCPP (Tris[1,3-dichloro-2-propyl]phosphate)	1.79	GC-MS, GC-MS/MS	LC-MS/MS	GC-MS	HHC	flame retardant
Dichlorprop	3.43	GC-MS			HHC	herbicide
Mecoprop	3.13	GC-MS			HHC	herbicide
Estriol (E3)	2.94	GC-MS/MS	LC-MS/MS		hormone	
Estrone (E1)	3.69	GC-MS/MS	LC-MS/MS		hormone	
Acetaminophen	0.34		LC-MS/MS	LC-MS	pharm	analgesic
Diclofenac	3.28		LC-MS/MS		pharm	analgesic
Hydrocodone	2.00		LC-MS/MS		pharm	analgesic
Ibuprofen	3.97	GC-MS	LC-MS/MS	GC-MS	pharm	analgesic
Ketoprofen	3.12	GC-MS		LC-MS	pharm	analgesic
Naproxen	3.18	GC-MS	LC-MS/MS	GC-MS	pharm	analgesic
Salicylic acid	2.26	GC-MS			pharm	analgesic
Dilantin	2.47		LC-MS/MS		pharm	anticonvulsant
Meprobamate	0.70		LC-MS/MS		pharm	antianxiety
Ciprofloxacin	1.31				pharm	antibiotic
Erythromycin-H2O	2.83		LC-MS/MS	LC-MS	pharm	antibiotic
Ofloxacin	1.49				pharm	antibiotic
Sulfamethoxazole	0.89		LC-MS/MS	LC-MS	pharm	antibiotic
Trimethoprim	0.79		LC-MS/MS		pharm	antibiotic
Fluoxetine	4.35		LC-MS/MS	LC-MS	pharm	antidepressant
Norfluoxetine			LC-MS/MS		pharm	antidepressant
Carbamazepine	2.67	GC-MS	LC-MS/MS	GC-MS	pharm	antiepileptic
Primidone	-0.84	GC-MS			pharm	antiepileptic
Atenolol	0.56	GC-MS		LC-MS	pharm	beta blocker
Metoprolol	1.79	GC-MS/MS		LC-MS	pharm	beta blocker
Propranolol	3.10	GC-MS/MS		LC-MS	pharm	beta blocker
Iopromide	-3.24		LC-MS/MS		pharm	iodinated X-ray media
Gemfibrozil	4.39	GC-MS	LC-MS/MS		pharm	lipid regulator
Atorvastatin	6.36		LC-MS/MS		pharm	lowers cholesterol
Atorvastatin (o-hydroxy)			LC-MS/MS		pharm	lowers cholesterol
Atorvastatin (p-hydroxy)			LC-MS/MS		pharm	lowers cholesterol
Simvastatin hydroxy acid	4.68		LC-MS/MS		pharm	lowers cholesterol
Phenylphenol (o-)	2.94				PPCP	antimicrobial
Triclocarban	5.74				PPCP	antimicrobial
Triclosan	5.80	GC-MS/MS	LC-MS/MS	GC-MS	PPCP	antimicrobial
Isobutylparaben	3.28				PPCP	antimicrobial
Propylparaben	2.93				PPCP	antimicrobial
Butylated hydroxyanisole (BHA)	3.50				PPCP	antioxidant
EDTA	-0.43	GC-NPD,			PPCP	complexing metal
TCEP (Tris[2-chloroehtyl] phosphate)	0.48	GC-MS, GC-MS/MS	LC-MS/MS	GC-MS	PPCP	flame retardant
TCPP (Tris[2-chloroisopropyl] phosphate)	1.52	GC/MS			PPCP	flame retardant

DBP: disinfection byproduct; HHC: household chemical; pharm: pharmaceutical; PPCP: pharmaceutical and personal care products

Table A2 (continued)

Indicator Compound	logKow	GC	LC	AIQS	Category	Sub-category
Acetyl cedrene	5.17	GC-MS			PPCP	fragrance
Benzyl acetate	1.93	GC-MS			PPCP	fragrance
Benzyl salicylate	4.00	GC-MS			PPCP	fragrance
Bucinal (p-t-)	4.07	GC-MS			PPCP	fragrance
Galaxolide (HHCB)	5.95	GC-MS			PPCP	fragrance
Hexyl salicylate	5.06	GC-MS			PPCP	fragrance
Hexylcinnamaldehyde	5.33	GC-MS			PPCP	fragrance
Isobornyl acetate	3.60	GC-MS			PPCP	fragrance
Methyl dihydrojasmonate	2.50				PPCP	fragrance
Methyl ionine (g-)	4.41	GC-MS			PPCP	fragrance
Methyl salicylate	2.23	GC-MS			PPCP	fragrance
Musk ketone	3.86	GC-MS			PPCP	fragrance
Musk xylene	3.83	GC-MS			PPCP	fragrance
OTNE	5.29	GC-MS			PPCP	fragrance
Terpineol	3.33	GC-MS			PPCP	fragrance
Tonalide (AHTN)	6.37	GC-MS			PPCP	fragrance
Caffeine	-0.07	GC-MS, GC-MS/MS	LC/MS-MS	GC-MS	PPCP	stimulant
Bisphenol A	3.32	GC-MS, GC-MS/MS		GC-MS	PPCP	plasticizer
Nonylphenol	5.71	GC-MS		GC-MS	PPCP	surfactant
Indolebutyric acid (3-indolebutyric acid)	2.30				PPCP	plant growth regulator
DEET	1.96	GC-MS/MS	LC-MS/MS	GC-MS	PPCP	insecticide

DBP: disinfection byproduct; HHC: household chemical; pharm: pharmaceutical; PPCP: pharmaceutical and personal care products

Table A3. The 12 TrOCs suggested by Water Corporation (2013) **indicator chemicals**

RWQI	logKow	Guideline Value	Chemical Group	Method	
				Water Corporation (2013)	This project
		ng/L			
Estrone	3.7	30	Hormones	SPE-LC-MS/MS	
N-nitrosodimethylamine (NDMA)	-0.6	100	DBP	SPE-GC-MS isotope dilution	GC-MS/MS
Octachlorodibenzo-p-dioxin	8.4	9000	TrOC	HRGC-HRMS	
Trifluralin	5.3	50000	Pesticides	SPE-GC-MS	AIQS GC-MS
		ug/L			
1,4-Dioxane	-0.3	50	Organic chemicals, surfactants	SPE (headspace)-GC-MS	
1,4-dichlorobenzene	3.4	40	Organic chemicals, VOCs	SPE-GC-MS	AIQS GC-MS
2,4,6-trichlorophenol	3.6	20	Phenols	SPE-GC-MS	AIQS GC-MS
Carbamazepine	2.7	100	PPCP	SPE-LC-MS/MS (4 methods)	AIQS GC-MS and LC-MS
Chloroform	1.8	200	DBP	Purge & trap GC-MS	
Diclofenac	4.1	1.8	PPCP	SPE-LC-MS/MS (4 methods)	
EDTA	-0.4	250	Organic chemicals	LLE and derivatization GC-MS, SRM-CE/MS	
Fluorene	4.2	140	Organic chemicals, PAHs	SPE-GC-MS	AIQS GC-MS

Table A4: Summary of the names, sources and uses of the 940 chemicals in the GC-MS-database method

Chemical	Use/Origin	Source
1-Acetoxy-2-methoxyethane	solvent	industry
1-Chloronaphthalene	PCN	industry
1-Methylnaphthalene	PAH	industry
1-Methylphenanthrene	PAH	industry
1-Naphthol	intermediate for dyes	industry
1-Naphthylamine	reagent	business/household
1-Nitronaphthalene	PAH	industry
1-Nitropyrene	PAH	industry
1-Nonanol	cosmetics/fragrance	business/household/traffic
1-Phenylnaphthalene	PAH	industry
1,1,1-Trichloro-2-methyl-2-propanol	plasticizer	business/household
1,2-Dibromo-3-chloropropane	intermediate in organic synthesis	industry
1,2-Dichlorobenzene	solvent	industry
1,2-Dimethylnaphthalene	PAH	industry
1,2,3-Trichlorobenzene	in organic synthesis/as solvent	industry
1,2,3-Trichloronaphthalene	PCN	industry
1,2,3-Trimethoxybenzene	other	industry
1,2,3,4,5,6,7-Heptachloronaphthalene	PCN	industry
1,2,3,4,5,6,8-Heptachloronaphthalene	PCN	industry
1,2,3,4,5,8-Hexachloronaphthalene	PCN	industry
1,2,3,4,6,7-Hexachloronaphthalene	PCN	industry
1,2,3,5-Tetrachloronaphthalene	PCN	industry
1,2,3,5,7-Pentachloronaphthalene	PCN	industry
1,2,3,5,7,8-Hexachloronaphthalene	PCN	industry
1,2,3,5,8-&1,2,3,6,8-Pentachloronaphthalene	PCN	industry
1,2,4-Trichlorobenzene	in organic synthesis/ as solvent	industry
1,2,4,5-Tetrabromobenzene	other	industry
1,2,4,5-Tetrachlorobenzene	intermediate in organic synthesis	industry
1,2,4,5,6-Pentachloronaphthalene	PCN	industry
1,2,4,5,6,8-&1,2,4,5,7,8-Hexachloronaphthalene	PCN	industry
1,2,4,5,8-Pentachloronaphthalene	PCN	industry
1,2,4,6,8-Pentachloronaphthalene	PCN	industry
1,2,4,7,8-Pentachloronaphthalene	PCN	industry
1,2,5,6,9,10-Hexabromocyclododecane	fire retardant	business/household
1,2,5,7-&1,2,4,6-&1,2,4,7-Tetrachloronaphthalene	PCN	industry
1,2,5,8-&1,2,6,8-Tetrachloronaphthalene	PCN	industry
1,3-Dichloro-2-propanol	solvent	industry
1,3-Dichlorobenzene	Solvent/ in organic synthesis	industry
1,3-Dicyclohexylurea	leaching from tyre	business/household
1,3-Dimethylnaphthalene	PAH	industry
1,3-Dinitrobenzene	intermediate in organic synthesis	industry
1,3,5-Trichlorobenzene	in organic synthesis/ solvent	industry
1,3,5-Trinitrobenzene	vulcanization/reagent	business/household
1,3,7-&1,4,6-Trichloronaphthalene	PCN	industry
1,4-&1,6-Dichloronaphthalene	PCN	industry
1,4-&2,3-Dimethylnaphthalene	PAH	industry
1,4-Benzenediol	developing fluid	business/household
1,4-Dichlorobenzene	insecticidal fumigant	business/household
1,4-Dinitrobenzene	intermediate in organic synthesis	industry
1,4,5-Trichloronaphthalene	PCN	industry
1,4,5,8-Tetrachloronaphthalene	PCN	industry
1,4,6,7-Tetrachloronaphthalene	PCN	industry
1,5-Dichloronaphthalene	PCN	industry
1,8-Dimethylnaphthalene	PAH	industry
2-(Methylthio)-benzothiazol	leaching from tire	business/household
2-Acetyl-5-methylthiophene	leaching from tire	business/household
2-Acetylaminofluorene	reagent	business/household
2-Amino-4,6-dinitrotoluene	explosive	industry
2-Amino-6-nitrotoluene	other	industry
2-Anisidine	intermediate for dyes	industry
2-Bromo-4,6-dichloroaniline	reagent	business/household
2-Bromochlorobenzene	other	industry
2-Butoxyethanol	solvent	industry
2-Chloro-6-methylphenol	other	industry
2-Chloroaniline	intermediate for dyes	industry
2-Chloronaphthalene	PCN	industry
2-Chlorophenol	by-product of chlorination/ intermediate in organic synthesis	industry

Table A4 (continued)

Chemical	Use/Origin	Source
2-Cyclohexen-1-one	leaching from tire	business/household
2-Ethyl-1-hexanol	plasticizer	business/household/traffic
2-Heptanol	fragrance	business/household
2-Hydroxy-4-methoxy-4'-methyl-benzophenone	other	industry
2-Isopropylnaphthalene	PAH	industry
2-Mercaptobenzothiazole	leaching from tire	business/household/traffic
2-Methoxyphenol	leaching from tire	business/household
2-Methyl-2,4-pentandiol	cosmetics/fuel additive/solvent	business/household
2-Methyl-4,6-dinitrophenol	intermediate for dyes/pesticide/	industry
2-Methylaniline	intermediate for dyes	industry
2-Methylbenzothiazole	leaching from tire	business/household/traffic
2-Methylnaphthalene	PAH	industry
2-Methylphenanthrene	PAH	industry
2-Methylphenol	disinfectant	business/household
2-Naphthol	intermediate in organic synthesis	industry
2-Naphthylamine	reagent	business/household
2-Nitroaniline	intermediate in organic synthesis	industry
2-Nitroanisole	intermediate in organic synthesis	industry
2-Nitronaphthalene	PAH	industry
2-Nitrophenol	intermediate in organic synthesis/exhaust gas of automobile	Industry
2-Nitrotoluene	intermediate in organic synthesis	industry
2-Phenylnaphthalene	PAH	industry
2-Phenylphenol (OPP)	fungicide	agriculture
2-sec-Butylphenol	intermediate in organic synthesis	industry
2-tert-Butyl-4-methoxyphenol	antioxidant	business/household
2-*tert*-Butylphenol	intermediate in organic synthesis	industry
2,2'-Dibromobiphenyl (BB-4)	fire retardant	business/household
2,2',4,4'-Tetrabromodiphenyl ether (BDE-47)	fire retardant	business/household
2,2',4,4',5,5'-Hexabromobiphenyl (BB-153)	fire retardant	business/household
2,2',4,4',5,5'-Hexabromodiphenyl ether (BDE-153)	fire retardant	business/household
2,2',5,5'-Tetrabromobiphenyl (BB-52)	fire retardant	business/household
2,3-&3,4-Dimethylaniline	intermediate in organic synthesis	industry
2,3-Benzofluorene	PAH	industry
2,3-Dichloroaniline	reagent	business/household
2,3-Dichloronitrobenzene	intermediate in organic synthesis	industry
2,3-Dichlorophenol	reagent	business/household
2,3,4-Trichlorophenol	intermediate for pesticides/ preservative	industry
2,3,4,5,6-Pentachloro-p-terphenyl	other	industry
2,3,4,6-Tetrachlorophenol	fungicide	agriculture
2,3,5-Trichlorophenol	intermediate for pesticides/ preservative	industry
2,3,5,6-&2,3,4,5-Tetrachlorophenol	other	industry
2,3,5,6-Tetrachloro-p-terphenyl	other	industry
2,3,6-Trichlorophenol	intermediates in the synthesis of dyes, pigments, and phenolic resins	industry
2,3,6,7-&1,2,4,8-Tetrachloronaphthalene	PCN	industry
2,4-&2,5-Dichloro-p-terphenyl	other	industry
2,4-Diamino-6-nitrotoluene	explosive	industry
2,4-Dibromodiphenyl ether (BDE-7)	fire retardant	business/household
2,4-Dichloroaniline	reagent/intermediate in organic synthesis	business/household
2,4-Dichloronitrobenzene	intermediate in organic synthesis	industry
2,4-Dichlorophenol	reagent, by-product of chlorination	business/household
2,4-Dimethylphenol	intermediate in organic synthesis	industry
2,4-Dinitroaniline	intermediate for dyes	industry
2,4-Dinitrophenol	intermediate in organic synthesis	industry
2,4-Dinitrotoluene	intermediate in organic synthesis	industry
2,4,4',6-Tetrachloro-p-terphenyl	other	industry
2,4,5-Trichlorophenol	intermediate for pesticides / preservative	industry
2,4,6-Tri-tert-butylphenol	other	industry
2,4,6-Tribromoaniline	reagent	business/household
2,4,6-Tribromophenol	intermediate for resin	industry
2,4,6-Trichloro-p-terphenyl	other	industry
2,4,6-Trichloroaniline	reagent/intermediate in organic synthesis	business/household
2,4,6-Trichlorophenol	by-product of chlorination/intermediate for pesticides	industry
2,4,6-Trinitrotoluene	explosive	industry
2,5-Dichloro-o-terphenyl	other	industry
2,5-Dichloronitrobenzene	intermediate in organic synthesis	industry

Table A4 (continued)

Chemical	Use/Origin	Source
2,5-Dichlorophenol	intermediate for pesticides	industry
2,5-Dimethylaniline	intermediates in the synthesis of dyes	industry
2,6-&1,7-Dichloronaphthalene	PCN	industry
2,6-Di-t-butyl-4-ethylphenol	antioxidant	business/household
2,6-Di-tert-butyl-4-benzoquinone	antioxidant	business/household
2,6-Diamino-4-nitrotoluene	explosive	industry
2,6-Diaminotoluene	intermediate in organic synthesis	industry
2,6-Dibromo-4-chloroaniline	reagent	business/household
2,6-Dichloro-4-nitroaniline	intermediate in organic synthesis	industry
2,6-Dichlorobenzamid	herbicide	agriculture
2,6-Dichlorophenol	by-product of chlorination/intermediate for trichlorophenol	industry
2,6-Diisopropylnaphthalene	PAH	industry
2,6-Dimethylaniline	intermediate in organic synthesis	industry
2,6-Dimethylnaphthalene	PAH	industry
2,6-Dimethylphenol	intermediate for resin	industry
2,6-Dinitrotoluene	intermediate in organic synthesis	industry
2(3H)-Benzothiazolone	leaching from tire	business/household
3- & 4-tert-Butylphenol	antioxidant	business/household/traffic
3-&4-Chlorophenol	by-product of chlorination/ intermediate in organic synthesis	industry
3-&4-Methylphenol	disinfectant	business/household
3-&4-Nitroanisole	intermediate in organic synthesis	industry
3-Anisidine	other	industry
3-Bromochlorobenzene	other	industry
3-Chloronitrobenzene	intermediate in organic synthesis	industry
3-Hexanol, 4-ethyl-	other	industry
3-Hydroxycarbofuran 1	insecticide	agriculture
3-Hydroxycarbofuran 2	insecticide	agriculture
3-Methoxy-1-butyl acetate	intermediate for resin/solvent	industry
3-Methylcholanthrene	PAH	industry
3-Methylphenanthrene	PAH	industry
3-Methylpyridine	intermediate in organic synthesis /sorbent	industry
3-Nitroaniline	intermediate in organic synthesis	industry
3-Nitrofluoranthene	PAH	industry
3-Nitrophenanthrene	PAH	industry
3-Nitrotoluene	intermediate in organic synthesis	industry
3-Toluidine	intermediate for dyes	industry
3,3'-Dichlorobenzidine	intermediate for dyes	industry
3,4-Dichloroaniline	intermediate for dyes and pesticides	industry
3,4-Dichlorophenol	intermediate in organic synthesis	industry
3,4,5-Trichlorophenol	intermediates in the synthesis of dyes, pigments, and phenolic resins	industry
3,5-di-tert-Butyl-4-hydroxybenzaldehyde	antioxidant/leaching from tire	business/household
3,5-Dichlorophenol	reagent	business/household
3,5-Dimethylaniline	intermediate for dyes	industry
3,5-Dimethylphenol	intermediate in organic synthesis	industry
3,6-Dimethylphenanthrene	PAH	industry
4-Amino-2-nitrotoluene	other	industry
4-Amino-2,6-dinitrotoluene	explosive	industry
4-Aminobiphenyl	reagent	business/household
4-Anisidine	intermediate for dyes	industry
4-Bromo-2,6-dichloroaniline	reagent	business/household
4-Bromophenol	natural product	other
4-Bromophenylphenyl ether	reagent	business/household
4-Chloro-2-nitroaniline	intermediate in organic synthesis	industry
4-Chloro-3-methylphenol	fungicide, paint	agriculture
4-Chloro-o-terphenyl	other	industry
4-Chloro-p-terphenyl	other	industry
4-Chloroaniline	intermediate for dyes and pesticides	industry
4-Chloronitrobenzene	intermediate in organic synthesis	industry
4-Chlorophenylphenyl ether	dielectric fluid	industry
4-Cymene	solvent	industry
4-Dimethylaminoazobenzene	reagent	business/household
4-Methyl-2,6-di-t-butylphenol	antioxidant	business/household
4-Methyl-3-nitrophenol	other	industry
4-n-Butylphenol	intermediate for liquid crystal	industry
4-n-Heptylphenol	PPCPs	business/household/traffic

Table A4 (continued)

Chemical	Use/Origin	Source
4-n-Hexylphenol	intermediate in organic synthesis	industry
4-n-Nonylphenol	co-stabilizer	industry
4-n-Octylphenol	nonionic detergent metabolite	business/household
4-n-Pentylphenol	dyes tuff intermediates/ rubber chemicals/ surfactants	business/household
4-Nitroaniline	intermediate in organic synthesis	industry
4-Nitrophenanthrene	PAH	industry
4-Nitrophenol	intermediate in organic synthesis/ fungicide	industry
4-Nitrotoluene	intermediate in organic synthesis	industry
4-Phenylphenol	intermediate in organic synthesis	industry
4-sec-Butylphenol	other	business/household
4-tert-Octylphenol	nonionic detergent metabolite	business/household
4,4'-Methylene-bis(2-chloroaniline)	intermediate for resin	industry
4,5-Methylene-phenanthrene	PAH	industry
5-Bromoindole	other	industry
5-Chloro-2-methyl aniline	intermediate for dyes	industry
5-Nitro-o-toluidine	intermediate for dyes	industry
6-Benzylaminopurine	other pesticide	agriculture
6-Nitrochrysene	PAH	industry
7-Nitrobenz(*a*)anthracene	PAH	industry
7,12-Dimethylbenz(*a*)anthracene	PAH	industry
9-Methylphenanthrene	PAH	industry
9-Nitroanthracene	PAH	industry
9-Nitrophenanthrene	PAH	industry
a-HCH	insecticide	agriculture
Acenaphthene	PAH	industry
Acenaphthylene	PAH	industry
Acephate	insecticide	agriculture
Acetamide, N-(2-phenylethyl)-	leaching from tire	business/household
Acetamide, N-phenyl-	leaching from tire	business/household
Acetamiprid	insecticide	agriculture
Acetochlor	herbicide	agriculture
Acetophenone	cosmetics/fragrance	business/household/traffic
Acrinathrin	other pesticide	agriculture
Alachlor	herbicide	agriculture
Aldoxycarb (deg)	insecticide	agriculture
Aldrin	insecticide	agriculture
Allethrin 1	insecticide	agriculture
Allethrin 2 & Bioallethrin 1	insecticide	agriculture
Allidochlor	herbicide	agriculture
alpha-Terpineol	perfumes/solvent	business/household
Ametryn	herbicide	agriculture
Amino-chlornitrofen	herbicide	agriculture
Amitraz	other pesticide	agriculture
Amitraz (deg)	other pesticide	agriculture
Aniline	intermediate in organic synthesis/ leaching from tyre	Industry
Anilofos	herbicide	agriculture
Anthracene	PAH	industry
Anthraquinone	fragrance/solvent	business/household
Arachidic acid methyl ester	fatty acid methy ester	business/household
Arachidonic acid methyl ester	fatty acid methy ester	business/household
Aspirin	PPCPs	business/household
Atrazine	herbicide	agriculture
Azaconazole	fungicide	agriculture
Azamethiphos	insecticide	agriculture
Azinphos-ethyl	insecticide	agriculture
Azinphos-methyl	insecticide	agriculture
Azoxystrobin	fungicide	agriculture
b-HCH	insecticide	agriculture
Behenic acid methyl ester	fatty acid methy ester	business/household
Benalaxyl	fungicide	agriculture
Bendiocarb	insecticide	agriculture
Benfluralin	herbicide	agriculture
Benfuresate	herbicide	agriculture
Benoxacor	herbicide	agriculture
Bensulide	herbicide	agriculture
Bentazone	herbicide	agriculture

Table A4 (continued)

Chemical	Use/Origin	Source
Benzaldehyde, 4-hydroxy-3,5-dimethoxy-	leaching from tire	business/household
Benzamide, N-phenyl-	leaching from tire	business/household
Benzanthrone	intermediate in organic synthesis	industry
Benzidine	intermediate for dyes	industry
Benzo(a)anthracene	PAH	industry
Benzo(a)pyrene	PAH	industry
Benzo(*c*)phenenthrene	PAH	industry
Benzo(*e*)pyrene	PAH	industry
Benzo(*ghi*)perylene	PAH	industry
Benzo(*j&b*)fluoranthene	PAH	industry
Benzo(*k*)fluoranthene	PAH	industry
Benzothiazole	leaching from tire	business/household
Benzyl alcohol	cosmetics/fuel additive/solvent/leaching from tire	business/household
Benzyl chloride	intermediate in organic synthesis	industry
beta-Sitosterol	phytosterol	
Bifenazate	insecticide	agriculture
Bifenox	herbicide	agriculture
Bifenthrin	insecticide	agriculture
Bioresmethrin	insecticide	agriculture
Biphenyl	intermediate in organic synthesis	industry
Bis(2-chloroethoxy)methane	intermediate in organic synthesis	industry
Bis(2-ethylhexyl) sebacate	plasticizer	business/household
Bis(2-ethylhexyl)phthalate	plasticizer	business/household
Bisphenol A	intermediate for resin/antioxidant	business/household
Bitertanol	fungicide	agriculture
Bromacil	herbicide	agriculture
Bromobutide	herbicide	agriculture
Bromophos	insecticide	agriculture
Bromopropylate	other pesticide	agriculture
Bromuconazole-1	fungicide	agriculture
Bromuconazole-2	fungicide	agriculture
Bupirimate	fungicide	agriculture
Buprofezin	insecticide	agriculture
Butachlor	herbicide	agriculture
Butafenacil	herbicide	agriculture
Butamifos	herbicide	agriculture
Butanoic acid, butyl ester	fragrance	business/household
Butyl benzyl phtalate	plasticizer	business/household
Butylate	herbicide	agriculture
Cadusafos	insecticide	agriculture
Cafenstrole	herbicide	agriculture
Caffeine	PPCPs	business/household
Campesterol	phytosterol	
Captafol	fungicide	agriculture
Captan	fungicide	agriculture
Carbamazepine	PPCPs	business/household
Carbaryl	insecticide	agriculture
Carbazole	intermediate in organic synthesis	industry
Carbetamide	herbicide	agriculture
Carbofuran	insecticide	agriculture
Carbophenothion	insecticide	agriculture
Carboxin	fungicide	agriculture
Carfentrazone-ethyl	herbicide	agriculture
Chinomethionat	fungicide	agriculture
Chlorethoxyfos	insecticide	agriculture
Chlorfenapyr	insecticide	agriculture
Chlorfenson	insecticide	agriculture
Chlorfenvinphos E	insecticide	agriculture
Chlorfenvinphos Z	insecticide	agriculture
Chloridazon	herbicide	agriculture
Chlorimuron-ethyl	herbicide	agriculture
Chlormephos	insecticide	agriculture
Chlornitrofen (CNP)	herbicide	agriculture
Chlorobenzilate	other pesticide	agriculture
Chloroneb	fungicide	agriculture
Chlorothalonil (TPN)	fungicide	agriculture
Chlorpropham	herbicide	agriculture

Table A4 (continued)

Chemical	Use/Origin	Source
Chlorpropylate	insecticide	agriculture
Chlorpyrifos	insecticide	agriculture
Chlorpyrifos-methyl	insecticide	agriculture
Chlorthal-dimethyl	herbicide	agriculture
Cholestane	animal sterol	
Cholestanol	animal sterol	
Cholesterol	animal sterol	
Chrysene & Triphenylene	PAH	industry
Cinmethylin	herbicide	agriculture
cis-10-Heptadecenoic acid methyl ester	fatty acid methy ester	business/household
cis-11-Eicosenoic acid methyl ester	fatty acid methy ester	business/household
cis-11,14-Eicosadienoic acid methyl ester	fatty acid methy ester	business/household
cis-11,14,17-Eicosatrienoic acid methyl ester	fatty acid methy ester	business/household
cis-13,16-Docosadienoic acid methyl ester	fatty acid methy ester	business/household
cis-4,7,10,13,16,19-Docosahexaenoic acid methyl ester	fatty acid methy ester	business/household
cis-5,8,11,14,17-Eicosapentaenoic acid, methyl ester	fatty acid methy ester	business/household
cis-8,11,14-Eicosatrienoic acid methyl ester	fatty acid methy ester	business/household
cis-Chlordane	insecticide	agriculture
cis-Nonachlor	insecticide	agriculture
Clofentezine	other pesticide	agriculture
Clomazone	herbicide	agriculture
Clomeprop	herbicide	agriculture
Coprostanol	facal sterol	
Coprostanone	animal sterol	
Coumaphos	insecticide	agriculture
Crimidine	insecticide	agriculture
Crotamiton	PPCPs	business/household
Cyanazine	herbicide	agriculture
Cyanofenphos	insecticide	agriculture
Cyanophos, CYAP	insecticide	agriculture
Cycloate	herbicide	agriculture
Cyclohexanamine, N-cyclohexyl-	leaching from tire	business/household
Cyclohexanol	leaching from tire	business/household
Cyclopentanone, 2-methyl-	fragrance/synthetic intermediate	industry
Cyflufenamid	fungicide	agriculture
Cyfluthrin 1	insecticide	agriculture
Cyfluthrin 2	insecticide	agriculture
Cyfluthrin 3	insecticide	agriculture
Cyfluthrin 4	insecticide	agriculture
Cyhalofop Butyl	herbicide	agriculture
Cyhalothrin 1	insecticide	agriculture
Cyhalothrin 2	insecticide	agriculture
Cypermethrin 1	insecticide	agriculture
Cypermethrin 2	insecticide	agriculture
Cypermethrin 3	insecticide	agriculture
Cypermethrin 4	insecticide	agriculture
Cyproconazole	fungicide	agriculture
Cyprodinil	fungicide	agriculture
Cyromazine	insecticide	agriculture
d-HCH	insecticide	agriculture
DCIP (Bis(2-chloroisopropyl)ether)	insecticide	agriculture
DDVP	insecticide	agriculture
Deltamethrin	insecticide	agriculture
Demeton-S-methyl	insecticide	agriculture
Demeton-S-methylsulphon	insecticide	agriculture
Desmedipham	herbicide	agriculture
Di-n-butyl phthalate	plasticizer	business/household
Di-n-octyl phthalate	plasticizer	business/household
Di(2-ethylhexyl)adipate	plasticizer	business/household
Dialifos	insecticide	agriculture
Diazinon	insecticide	agriculture
Diazinon oxon	insecticide	agriculture
Dibenzo(*a,h*)anthracene	PAH	industry
Dibenzofuran	intermediate in organic synthesis	industry
Dibenzothiophene	petroleum	business/household
Dibenzylether	solvent	industry

Table A4 (continued)

Chemical	Use/Origin	Source
Dibutylamine	intermediate in organic synthesis	industry
Dichlobenil	herbicide	agriculture
Dichlofenthion, ECP	insecticide	agriculture
Dichlofluanid	fungicide	agriculture
Dichlofluanid metabolite	fungicide	agriculture
Dichlone	fungicide	agriculture
Diclobutrazol	fungicide	agriculture
Diclocymet 1	fungicide	agriculture
Diclocymet 2	fungicide	agriculture
Diclofop-methyl	herbicide	agriculture
Diclomezine	fungicide	agriculture
Dicloran	fungicide	agriculture
Diclosulam	herbicide	agriculture
Dicofol	other pesticide	agriculture
Dicofol-deg	other pesticide	agriculture
Dicrotophos	insecticide	agriculture
Dicyclohexyl phthalate	plasticizer	business/household
Dicyclopentadiene	intermediate for resin	industry
Dieldrin	insecticide	agriculture
Diethofencarb	fungicide	agriculture
Diethyl phthalate	plasticizer	business/household
Diethyl-p-nitrophenyl phosphate	insecticide, metabolite of parathion	agriculture
Diethyltoluamide	PPCPs	business/household
Difenoconazole 1	fungicide	agriculture
Difenoconazole 2	fungicide	agriculture
Difenzoquat metilsulfate	herbicide	agriculture
Diflufenican	herbicide	agriculture
Diisobutyl phthalate	plasticizer	business/household
Dimepiperate	herbicide	agriculture
Dimethametryn	herbicide	agriculture
Dimethenamid	herbicide	agriculture
Dimethipin	herbicide	agriculture
Dimethoate	insecticide	agriculture
Dimethomorph E	fungicide	agriculture
Dimethomorph Z	fungicide	agriculture
Dimethyl phthalate	plasticizer	business/household
Dimethylterephthalate	intermediate for resin	industry
Dimetylvinphos 1	insecticide	agriculture
Dimetylvinphos 2	insecticide	agriculture
Diniconazole	fungicide	agriculture
Dinoseb	insecticide	agriculture
Diofenolan 1	insecticide	agriculture
Diofenolan 2	insecticide	agriculture
Dioxabenzofos(Salithion)	insecticide	agriculture
Dipentyl phthalate	plasticizer	business/household
Diphenamid	herbicide	agriculture
Diphenyl ether	fragrance	business/household
Diphenylamine	intermediate in organic synthesis	industry
Diphenyldisulfide	other	industry
Diphenylmethane	PAH	industry
Dipropyl phthalate	plasticizer	business/household
Disulfoton	insecticide	agriculture
Ditalimfos	fungicide	agriculture
Dithiopyr	herbicide	agriculture
e-Caprolactam	intermediate for fiber	industry
Edifenphos	fungicide	agriculture
Elaidic acid methyl ester	fatty acid methy ester	business/household
Endosulfan I	insecticide	agriculture
Endosulfan II	insecticide	agriculture
Endosulfan sulfate	insecticide	agriculture
Endrin	insecticide	agriculture
Endrin aldehyde	insecticide	agriculture
Endrin ketone	insecticide	agriculture
Epicoprostanol	facal sterol	
EPN	insecticide	agriculture
EPN oxon	insecticide	agriculture
EPTC	herbicide	agriculture
Ergosterol	phytosterol	

Table A4 (continued)

Chemical	Use/Origin	Source
Erucic acid methyl ester	fatty acid methy ester	business/household
Esfenvalerate 1	insecticide	agriculture
Esfenvalerate 2	insecticide	agriculture
Esprocarb	herbicide	agriculture
Ethalfluralin	herbicide	agriculture
Ethanol, 2-phenoxy-	leaching from tire/ solvent/ intermediate in organic synthesis	business/household
Ethenzamide	PPCPs	business/household
Ethiofencarb	insecticide	agriculture
Ethion	insecticide	agriculture
Ethofumesate	herbicide	agriculture
Ethoprophos	insecticide	agriculture
Ethoxyquin	fungicide	agriculture
Ethychlozate	other pesticide	agriculture
Ethyl methanesulfonate	reagent	business/household
Ethylcarbamate	reagent	business/household
Etobenzanid	herbicide	agriculture
Etofenprox	insecticide	agriculture
Etoxazole	other pesticide	agriculture
Etoxazole metabolite	insecticide	agriculture
Etridiazole (Echlomezol)	fungicide	agriculture
Etrimfos	insecticide	agriculture
Famoxadone	fungicide	agriculture
Famphur	insecticide	agriculture
Fenamidone	fungicide	agriculture
Fenamiphos	other pesticide	agriculture
Fenarimol	fungicide	agriculture
Fenbuconazole	fungicide	agriculture
Fenbuconazole lactone A	fungicide	agriculture
Fenbuconazole lactone B	fungicide	agriculture
Fenchlorphos	insecticide	agriculture
Fenitrothion (MEP)	insecticide	agriculture
Fenitrothion oxon	insecticide	agriculture
Fenobucarb	insecticide	agriculture
Fenoprofen	PPCPs	business/household
Fenothiocarb	other pesticide	agriculture
Fenoxanil	fungicide	agriculture
Fenoxaprop-ethyl	herbicide	agriculture
Fenoxycarb	insecticide	agriculture
Fenpropathrin	other pesticide	agriculture
Fenpropimorph	fungicide	agriculture
Fensulfothion	other pesticide	agriculture
Fenthion	insecticide	agriculture
Fenvalerate 1	insecticide	agriculture
Fenvalerate 2	insecticide	agriculture
Ferimzone	fungicide	agriculture
Fipronil	insecticide	agriculture
Flamprop-methyl	herbicide	agriculture
Fluacrypyrim	other pesticide	agriculture
Fluazinam	fungicide	agriculture
Flucythrinate 1	insecticide	agriculture
Flucythrinate 2	insecticide	agriculture
Fludioxonil	fungicide	agriculture
Flufenoxuron dec2	insecticide	agriculture
Flufenoxuron dec3	insecticide	agriculture
Flumiclorac-pentyl	herbicide	agriculture
Flumioxazin	herbicide	agriculture
Fluoranthene	PAH	industry
Fluorene	PAH	industry
Fluquinconazole	fungicide	agriculture
Fluridone	herbicide	agriculture
Flusilazole	fungicide	agriculture
Flusilazole metabolite	fungicide	agriculture
Flusulfamide	fungicide	agriculture
Fluthiacet-methyl	herbicide	agriculture
Flutolanil	fungicide	agriculture
Flutriafol	fungicide	agriculture

Table A4 (continued)

Chemical	Use/Origin	Source
Fluvalinate 1	insecticide	agriculture
Fluvalinate 2	insecticide	agriculture
Folpet	fungicide	agriculture
Fonofos	insecticide	agriculture
Formamide, N-cyclohexyl-	leaching from tire	business/household
Fosthiazate 1	other pesticide	agriculture
Fosthiazate 2	other pesticide	agriculture
Fthalide	fungicide	agriculture
Furametpyr	fungicide	agriculture
Furametpyr metabolite	fungicide	agriculture
Furilazole	herbicide	agriculture
g-HCH	insecticide	agriculture
gamma-Linolenic acid methyl ester	fatty acid methy ester	business/household
Halfenprox	other pesticide	agriculture
Heneicosanoic acid methyl ester	fatty acid methy ester	business/household
Heptachlor	insecticide	agriculture
Heptachlor epoxide (B)	insecticide	agriculture
Hexachlorobenzene	fungicide	agriculture
Hexachlorobenzene	by-product	industry
Hexachlorobutadiene	solvent	industry
Hexachlorocyclopentadiene	intermediate in organic synthesis	industry
Hexachloroethane	intermediate in organic synthesis	industry
Hexachloropropylene	solvent	industry
Hexaconazole	fungicide	agriculture
Hexazinone	herbicide	agriculture
Hexythiazox	other pesticide	agriculture
Hymexazol	fungicide	agriculture
Ibuprofen	PPCPs	business/household
Imazalil	fungicide	agriculture
Imazamethabenz-methyl	herbicide	agriculture
Imibenconazole	fungicide	agriculture
Indanofan	herbicide	agriculture
Indeno(1,2,3-cd)pyrene	PAH	industry
Indoxacarb	insecticide	agriculture
Iprobenfos (IBP)	fungicide	agriculture
Iprodione	fungicide	agriculture
Iprodione metabolite	fungicide	agriculture
Isazofos	insecticide	agriculture
Isocarbophos	insecticide	agriculture
Isofenphos	insecticide	agriculture
Isofenphos oxon	insecticide	agriculture
Isophorone	solvent/paint	industry
Isoprocarb	insecticide	agriculture
Isopropalin	herbicide	agriculture
Isoprothiolane	fungicide	agriculture
Isosafrole	perfumes	business/household
Isoxadifen-ethyl	herbicide	agriculture
Isoxathion	insecticide	agriculture
Isoxathion oxon	insecticide	agriculture
Kresoxim methyl	fungicide	agriculture
L-Menthol	PPCPs	business/household
Lenacil	herbicide	agriculture
Leptophos	insecticide	agriculture
Lignoceric acid, methyl ester	fatty acid methy ester	business/household
Linoleic acid methyl ester	fatty acid methy ester	business/household
Linolelaidic acid methyl ester	fatty acid methy ester	business/household
Linolenic acid methyl ester	fatty acid methy ester	business/household
Longifolene	other	industry
m-Aminophenol	intermediate for dyes	industry
m-Phenylenediamine	intermediate for dyes	industry
m-Terphenyl	storage and transfer agents/ intermediate for resin	industry
Malathion	insecticide	agriculture
MCPA-thioethyl (Phenothiol)	herbicide	agriculture
MCPB-ethyl	herbicide	agriculture
Mecarbam	insecticide	agriculture
Mefenacet	herbicide	agriculture

Table A4 (continued)

Chemical	Use/Origin	Source
Mefenamic Acid	PPCPs	business/household
Mefenoxam (Metalaxyl-M)	fungicide	agriculture
Mefenpyr-diethyl	herbicide	agriculture
Mepanipyrim	fungicide	agriculture
Mepronil	fungicide	agriculture
Metalaxyl	fungicide	agriculture
Methacrifos	insecticide	agriculture
Methamidophos	insecticide	agriculture
Methapyrilene	PPCPs	business/household
Methidathion	insecticide	agriculture
Methiocarb	insecticide	agriculture
Methomyl oxime	other pesticide	agriculture
Methoprene	insecticide	agriculture
Methoxychlor	insecticide	agriculture
Methyl decanoate	fatty acid methy ester	business/household
Methyl dodecanoate	fatty acid methy ester	business/household
Methyl dymron	herbicide	agriculture
Methyl heptadecanoate	fatty acid methy ester	business/household
Methyl hexanoate	fatty acid methy ester	business/household
Methyl myristate	fatty acid methy ester	business/household
Methyl octanoate	fatty acid methy ester	business/household
Methyl palmitate	fatty acid methy ester	business/household
Methyl palmitoleate	fatty acid methy ester	business/household
Methyl parathion	insecticide	agriculture
Methyl pentadecanoate	fatty acid methy ester	business/household
Methyl tridecanoate	fatty acid methy ester	business/household
Methyl undecanoate	fatty acid methy ester	business/household
Metolachlor	herbicide	agriculture
Metominostrobin E	fungicide	agriculture
Metominostrobin Z	fungicide	agriculture
Metribuzin	herbicide	agriculture
Metribuzin DA	herbicide	agriculture
Metribuzin DADK	herbicide	agriculture
Metribuzin DK	herbicide	agriculture
Mevinphos 1	insecticide	agriculture
Mevinphos 2	insecticide	agriculture
Molinate	herbicide	agriculture
Monocrotophos	insecticide	agriculture
Myclobutanil	fungicide	agriculture
n-Butylacrylate	intermediate for resin	industry
n-C10H22	petroleum	business/household
n-C11H24	petroleum/plant	business/household
n-C12H26	petroleum	business/household
n-C13H28	petroleum/plant	business/household
n-C14H30	petroleum	business/household
n-C15H32	petroleum/plant	business/household
n-C16H34	petroleum	business/household
n-C17H36	petroleum/plant	business/household
n-C18H38	petroleum	business/household
n-C19H40	petroleum/plant	business/household
n-C20H42	petroleum	business/household
n-C21H44	petroleum/plant	business/household
n-C22H46	petroleum	business/household
n-C23H48	petroleum/plant	business/household
n-C24H50	petroleum	business/household
n-C25H52	petroleum/plant	business/household
n-C26H54	petroleum	business/household
n-C27H56	petroleum/plant	business/household
n-C28H58	petroleum	business/household
n-C29H60	petroleum/plant	business/household
n-C30H62	petroleum	business/household
n-C31H64	petroleum/plant	business/household
n-C32H66	petroleum	business/household
n-C33H68	petroleum/plant	business/household
n-C9H20	petroleum/plant	business/household
N-Ethylaniline	intermediate for dyes	industry

Table A4 (continued)

Chemical	Use/Origin	Source
N-Ethylmorpholine	solvent	industry
N-Methylaniline	intermediate in organic synthesis	industry
N-Nitroquinoline-N-oxide	reagent	business/household
N-Nitroso-di-n-butylamine	reagent	business/household
N-Nitrosodiethylamine	Gasoline & lubricant additive; antioxidant; stabilizer in plastics	business/household
N-Nitrosomorpholine	solvent/intermediate in organic synthesis	industry
N-Nitrosopiperidine	reagent	business/household
N-Nitrosopyrrolidine	reagent	business/household
N-Phenyl-1-naphthylamine	antioxidant	business/household
N-Phenyl-2-naphthylamine	antioxidant	business/household
N,N-Dimethylaniline	intermediate for dyes	industry
Naled	insecticide	agriculture
Naphthalene	PAH	industry
Napropamide	fungicide	agriculture
Naproxen	PPCPs	business/household
Nereistoxin oxalate deg.	insecticide	agriculture
Nervonic acid methyl ester	fatty acid methy ester	business/household
Nicotine	PPCPs	business/household
Nicotinonitrile	intermediate for pesticides	industry
Nitralin	herbicide	agriculture
Nitrobenzene	intermediate in organic synthesis	industry
Nitrofen (NIP)	herbicide	agriculture
Nitrothal-isopropyl	fungicide	agriculture
Nonylphenol	nonionic detergent metabolite	business/household
Norflurazon	herbicide	agriculture
Novaluron-deg	insecticide	agriculture
o-Terphenyl	storage and transfer agents/intermediate for resin	industry
o,p'-DDD	insecticide	agriculture
o,p'-DDE	insecticide	agriculture
o,p'-DDT	insecticide	agriculture
Octachloronaphthalene	PCN	industry
Octanol	cosmetics/fragrance/solvent	business/household
Oleic acid methyl ester	fatty acid methy ester	business/household
Omethoate	insecticide	agriculture
Oryzalin	herbicide	agriculture
Oxabetrinil	herbicide	agriculture
Oxadiazon	herbicide	agriculture
Oxadixyl	fungicide	agriculture
Oxpoconazole-formyl	fungicide	agriculture
Oxpoconazole-fumalate	fungicide	agriculture
Oxychlordane	insecticide	agriculture
Oxyfluorfen	herbicide	agriculture
p-Phenylenediamine	intermediate for dyes/developing fluid	industry
p-Terphenyl	storage and transfer agents	industry
p,p'-DDD	insecticide	agriculture
p,p'-DDE	insecticide	agriculture
p,p'-DDT	insecticide	agriculture
Paclobutrazol	other pesticide	agriculture
Parathion	insecticide	agriculture
PCB #1	PCB	industry
PCB #101	PCB	industry
PCB #104	PCB	industry
PCB #105	PCB	industry
PCB #110	PCB	industry
PCB #114	PCB	industry
PCB #118	PCB	industry
PCB #119	PCB	industry
PCB #123	PCB	industry
PCB #126	PCB	industry
PCB #128	PCB	industry
PCB #138&158	PCB	industry
PCB #149	PCB	industry
PCB #15	PCB	industry
PCB #151	PCB	industry
PCB #153&168	PCB	industry
PCB #155	PCB	industry

Table A4 (continued)

Chemical	Use/Origin	Source
PCB #156	PCB	industry
PCB #157	PCB	industry
PCB #167	PCB	industry
PCB #169	PCB	industry
PCB #170	PCB	industry
PCB #171	PCB	industry
PCB #177	PCB	industry
PCB #178	PCB	industry
PCB #18	PCB	industry
PCB #180	PCB	industry
PCB #183	PCB	industry
PCB #187	PCB	industry
PCB #188	PCB	industry
PCB #189	PCB	industry
PCB #19	PCB	industry
PCB #191	PCB	industry
PCB #194	PCB	industry
PCB #199	PCB	industry
PCB #201	PCB	industry
PCB #202	PCB	industry
PCB #205	PCB	industry
PCB #206	PCB	industry
PCB #208	PCB	industry
PCB #209	PCB	industry
PCB #22	PCB	industry
PCB #28	PCB	industry
PCB #3	PCB	industry
PCB #33	PCB	industry
PCB #37	PCB	industry
PCB #4&10	PCB	industry
PCB #44	PCB	industry
PCB #49	PCB	industry
PCB #52	PCB	industry
PCB #54	PCB	industry
PCB #70	PCB	industry
PCB #74	PCB	industry
PCB #77	PCB	industry
PCB #8	PCB	industry
PCB #81	PCB	industry
PCB #87	PCB	industry
PCB #95	PCB	industry
PCB #99	PCB	industry
Pebulate	herbicide	agriculture
Penconazole	fungicide	agriculture
Pencycron	fungicide	agriculture
Pendimethalin	herbicide	agriculture
Pentachlorobenzene	by-product	industry
Pentachloroethane	other	industry
Pentachloronitrobenzene (Quintozene)	fungicide	agriculture
Pentachlorophenol	herbicide	agriculture
Pentamethylbenzene	other	industry
Pentoxazone	herbicide	agriculture
Permethrin 1	insecticide	agriculture
Permethrin 2	insecticide	agriculture
Perylene	PAH	industry
Phenacetin	PPCPs	business/household
Phenanthrene	PAH	industry
Phenazine	other	industry
Phenmedipham deg.	herbicide	agriculture
Phenol	disinfectant	business/household
Phenol, 2,6-dimethoxy-	leaching from tire	business/household
Phenol, 4-(phenylamino)-	leaching from tire	business/household
Phenothiazine	intermediate in organic synthesis	industry
Phenothrin 1	insecticide	agriculture
Phenothrin 2	insecticide	agriculture
Phenoxathiin	other	industry
Phenoxazine	other	industry
Phenthoate	insecticide	agriculture

Table A4 (continued)

Chemical	Use/Origin	Source
Phenylethyl alcohol	fragrance/leaching from tire	business/household
Phorate	insecticide	agriculture
Phosalone	insecticide	agriculture
Phosmet	insecticide	agriculture
Phosphamidon	insecticide	agriculture
Phthalimide	leaching from tire	business/household
Picolinafen	herbicide	agriculture
Piperonyl butoxide	insecticide	agriculture
Piperophos	herbicide	agriculture
Pirimicarb	insecticide	agriculture
Pirimiphos-methyl	insecticide	agriculture
Pretilachlor	herbicide	agriculture
Probenazole	other pesticide	agriculture
Prochloraz	fungicide	agriculture
Procymidone	fungicide	agriculture
Profenofos	insecticide	agriculture
Prohydrojasmon	other pesticide	agriculture
Prometryn	herbicide	agriculture
Propachlor	herbicide	agriculture
Propamocarb	fungicide	agriculture
Propanil	herbicide	agriculture
Propanoic acid, 2-methyl-, 2-methylpropyl ester	flavouring	business/household
Propaphos	insecticide	agriculture
Propargite 1	other pesticide	agriculture
Propargite 2	other pesticide	agriculture
Propazine	herbicide	agriculture
Propetamphos	insecticide	agriculture
Propham	herbicide	agriculture
Propiconazole 1	fungicide	agriculture
Propiconazole 2	fungicide	agriculture
Propoxur	insecticide	agriculture
Propyphenazone	PPCPs	business/household
Propyzamide	herbicide	agriculture
Prothiofos	insecticide	agriculture
Pyraclofos	insecticide	agriculture
Pyraclostrobin	fungicide	agriculture
Pyraflufen ethyl	herbicide	agriculture
Pyrazophos	fungicide	agriculture
Pyrazoxyfen	herbicide	agriculture
Pyrene	PAH	industry
Pyrethrin 1	insecticide	agriculture
Pyrethrin 2	insecticide	agriculture
Pyrethrin 3	insecticide	agriculture
Pyrethrin 4	insecticide	agriculture
Pyributicarb	herbicide	agriculture
Pyridaben	insecticide	agriculture
Pyridaphenthion	insecticide	agriculture
Pyridate	herbicide	agriculture
Pyrifenox E	fungicide	agriculture
Pyrifenox Z	fungicide	agriculture
Pyrimethanil	fungicide	agriculture
Pyrimidifen	other pesticide	agriculture
Pyriminobac-methyl E	herbicide	agriculture
Pyriminobac-methyl Z	herbicide	agriculture
Pyriproxyfen	insecticide	agriculture
Pyroquilon	fungicide	agriculture
Quinalphos	insecticide	agriculture
Quinoclamine	herbicide	agriculture
Quinoline	intermediate in organic synthesis	industry
Quinoline, 2,7-dimethyl-	other	industry
Quinoxyfen	fungicide	agriculture
Quizalofop-ethyl	herbicide	agriculture
Safrole	intermediate in organic synthesis/preservative	industry
Silafluofen	insecticide	agriculture
Simazine	herbicide	agriculture
Simeconazole	fungicide	agriculture
Simetryn	herbicide	agriculture

Table A4 (continued)

Chemical	Use/Origin	Source
Spirodiclofen	other pesticide	agriculture
Spiroxamine 1	fungicide	agriculture
Spiroxamine 2	fungicide	agriculture
Squalane	PPCPs	business/household
Stearic acid methyl ester	fatty acid methy ester	business/household
Stigmasterol	phytosterol	
Sulfentrazone	herbicide	agriculture
Sulfotep	insecticide	agriculture
Sulprofos	insecticide	agriculture
Swep	herbicide	agriculture
TCMTB	fungicide	agriculture
Tebuconazole	fungicide	agriculture
Tebufenpyrad	other pesticide	agriculture
Tebupirimfos	insecticide	agriculture
Tecloftalam	other pesticide	agriculture
Tecnazene	fungicide	agriculture
Tefluthrin	insecticide	agriculture
Temephos	insecticide	agriculture
Terbacil	herbicide	agriculture
Terbcarb (MBPMC)	herbicide	agriculture
Terbufos	insecticide	agriculture
Terbutryn	herbicide	agriculture
Tetrachlorvinphos	insecticide	agriculture
Tetraconazole	fungicide	agriculture
Tetradifon	other pesticide	agriculture
Tetramethrin-1	insecticide	agriculture
Tetramethrin-2	insecticide	agriculture
Tetryl	explosive	industry
Thenylchlor	herbicide	agriculture
Thiabendazole	fungicide	agriculture
Thiamethoxam deg.	insecticide	agriculture
Thifluzamide	fungicide	agriculture
Thiobencarb	herbicide	agriculture
Thiocyclam	insecticide	agriculture
Thiometon	insecticide	agriculture
Thymol	PPCPs	business/household
Tolclofos-methyl	fungicide	agriculture
Tolfenpyrad	insecticide	agriculture
Tolylfluanid	fungicide	agriculture
Tolylfluanid metabolite	fungicide	agriculture
Tralomethrin-deg	insecticide	agriculture
trans-Chlordane	insecticide	agriculture
trans-Decahydronaphthalene	solvent	industry
trans-Nonachlor	insecticide	agriculture
Tri-allate	herbicide	agriculture
Triadimefon	fungicide	agriculture
Triadimenol 1	fungicide	agriculture
Triadimenol 2	fungicide	agriculture
Triazophos	insecticide	agriculture
Tribenuron-methyl	herbicide	agriculture
Tribufos	other pesticide	agriculture
Tributyl phosphate	fire retardant	business/household
Trichlamid	fungicide	agriculture
Trichlorfon	insecticide	agriculture
Triclopyr	herbicide	agriculture
Triclosan	PPCPs	business/household
Tricosanoic acid methyl ester	fatty acid methyl ester	business/household
Tricresyl phosphate	fire retardant/plasticizer	business/household
Tricyclazole	fungicide	agriculture
Tridemorph	fungicide	agriculture
Trifloxystrobin	fungicide	agriculture
Triflumizole	fungicide	agriculture
Trifluralin	herbicide	agriculture
Trimethyl phosphate	solvent	industry
Triphenylmethane	intermediate for dyes	industry
Tris(1,3-dichloro-2-propyl) phosphate	fire retardant	business/household
Tris(2-chloroethyl) phosphate	fire retardant	business/household
Tris(2-chloroethyl)phosphite	fire retardant	business/household

Table A4 (continued)

Chemical	Use/Origin	Source
Tris(2-ethylhexyl) phosphate	fire retardant/plasticizer	business/household
Tris(4-chlorophenyl)methane	other	industry
Tris(4-chlorophenyl)methanol	other	industry
Uniconazole P	other pesticide	agriculture
Urea, N,N-diethyl-	leaching from tire	business/household
Vinclozolin	fungicide	agriculture
XMC	insecticide	agriculture
Xylylcarb	insecticide	agriculture
Zoxamide	fungicide	agriculture

Table A5 Summary of the 265 chemicals in the LC-TOF-MS-database method

Compounds	CAS RN	Type
4,4'-Oxybis-benzenamine	101-80-4	Industrial
4,4'-methylenebis(N,N-dimethylaniline)	101-61-1	Industrial
3,3-dimethoxybenzidine	119-90-4	Industrial
4,4'-Diaminodiphenyl-methane	101-77-9	Industrial
Triphenylphosphate	115-86-6	Industrial
2-(Di-n-butylamino)ethanol	102-81-8	Industrial
Pymetrozin	123312-89-0	Pesticides
Avermectin B1a	65195-55-3	Pesticides
Azoxystrobin	131860-33-8	Pesticides
Boscalid	188425-85-6	Pesticides
Carbendazim	10605-21-7	Pesticides
Carpropamid	104030-54-8	Pesticides
Cyazofamid	120116-88-3	Pesticides
Cyflufenamid	180409-60-3	Pesticides
Cyprodinil	121552-61-2	Pesticides
Dimethirimol	5221-53-4	Pesticides
Dimethomorph(E)	110488-70-5 (isomer)	Pesticides
Dimethomorph(Z)	110488-70-5 (isomer)	Pesticides
Epoxiconazole	106325-08-0	Pesticides
Ethoxyquin	91-53-2	Pesticides
Fenamidone	161326-34-7	Pesticides
Fenarimol	60168-88-9	Pesticides
Fenhexamid	126833-17-8	Pesticides
Ferimzone(E)	89269-64-7 (isomer)	Pesticides
Ferimzone(Z)	89269-64-7 (isomer)	Pesticides
Furametpyr	123572-88-3	Pesticides
Hexaconazole	79983-71-4	Pesticides
Imazalil	35554-44-0	Pesticides
Iprodione	36734-19-7	Pesticides
Iprovalicarb	140923-17-7	Pesticides
Mepanipyrim	110235-47-7	Pesticides
Mepanipyrim_metabolite		Pesticides
Oxycarboxin	5259-88-1	Pesticides
Pencycuron	66063-05-6	Pesticides
Prochloraz	67747-09-5	Pesticides
Propamocarb	24579-73-5	Pesticides
Pyraclostrobin	175013-18-0	Pesticides
Simeconazole	149508-90-7	Pesticides
Thiabendazole	148-79-8	Pesticides
Tricyclazole	41814-78-2	Pesticides
Triflumizole	68694-11-1	Pesticides
Triflumizole_metabolite		Pesticides
Triticonazole	131983-72-7	Pesticides
Adenochrome semicarbazone	8050-86-0	Pesticides
Alachlor	15972-60-8	Pesticides

Table A5 (continued)

Compounds	CAS RN	Type
Anilofos	64249-01-0	Pesticides
Asulam	3337-71-1	Pesticides
Azimsulfuron	120162-55-2	Pesticides
Bensulfuron-methyl	83055-99-6	Pesticides
Bensulide	741-58-2	Pesticides
Benzobicyclon	156963-66-5	Pesticides
Benzobicyclon metabolite		Pesticides
Benzofenap	82692-44-2	Pesticides
Butafenacil	134605-64-4	Pesticides
Cafenstrole	125306-83-4	Pesticides
Chloridazon	1698-60-8	Pesticides
Chlorimuron-ethyl	90982-32-4	Pesticides
Chloroxuron	1982-47-4	Pesticides
Chlorsulfuron	64902-72-3	Pesticides
Cinosulfuron	94593-91-6	Pesticides
Clodinafop	114420-56-3	Pesticides
Clofencet	129025-54-3	Pesticides
Clomeprop	84496-56-0	Pesticides
Cloquintocet-mexyl	99607-70-2	Pesticides
Cumyluron	99485-76-4	Pesticides
Cyclosulfamuron	136849-15-5	Pesticides
Diclosulam	145701-21-9	Pesticides
Diuron	330-54-1	Pesticides
Dymron	42609-52-9	Pesticides
Esprocarb	85785-20-2	Pesticides
Ethametsulfuron-methyl	97780-06-8	Pesticides
Ethoxysulfuron	126801-58-9	Pesticides
Fenoxaprop-ethyl	66441-23-4 (racemate)	Pesticides
Fentrazamide	158237-07-1	Pesticides
Flazasulfuron	104040-78-0	Pesticides
Florasulam	145701-23-1	Pesticides
Fluazifop	69335-91-7	Pesticides
Flufenacet	142459-58-3	Pesticides
Flumetsulam	98967-40-9	Pesticides
Fluridone	59756-60-4	Pesticides
Fomesafen	72178-02-0	Pesticides
Foramsulfuron	173159-57-4	Pesticides
Halosulfuron-methyl	100784-20-1	Pesticides
Imazaquin	81335-37-7	Pesticides
Indanofan	133220-30-1	Pesticides
Iodosulfuron-methyl-sodium	144550-36-7	Pesticides
Isouron	55861-78-4	Pesticides
Isoxaflutole	141112-29-0	Pesticides
Lactofen	77501-63-4	Pesticides
Linuron	330-55-2	Pesticides

Table A5 (continued)

Compounds	CAS RN	Type
Methabenzthiazuron	18691-97-9	Pesticides
Metosulam	139528-85-1	Pesticides
Metsulfuron-methyl	74223-64-6	Pesticides
Monolinuron	1746-81-2	Pesticides
Naproanilide	52570-16-8	Pesticides
Naptalam	132-66-1	Pesticides
Oryzalin	19044-88-3	Pesticides
Oxaziclomefone	153197-14-9	Pesticides
Penoxsulam	219714-96-2	Pesticides
Propaquizafop	111479-05-1	Pesticides
Propoxycarbazone-sodium	181274-15-7	Pesticides
Pyrazolynate (Pyrazolate)	58011-68-0	Pesticides
Pyrazosulfuron-ethyl	93697-74-6	Pesticides
Pyriftalid	135186-78-6	Pesticides
pyriminobac-methyl(E)	147411-69-6	Pesticides
Quizalofop-ethyl	76578-14-8	Pesticides
Sethoxydim	74051-80-2	Pesticides
Siduron	1982-49-6	Pesticides
Sulfentrazone	122836-35-5	Pesticides
Sulfosulfuron	141776-32-1	Pesticides
Tebuthiuron	34014-18-1	Pesticides
Tepraloxydim	149979-41-9	Pesticides
Thifensulfuron-methyl	79277-27-3	Pesticides
Tralkoxydim 1	87820-88-0	Pesticides
Tralkoxydim 2		Pesticides
Triasulfuron	82097-50-5	Pesticides
Tribenuron methyl	101200-48-0	Pesticides
Tribenuron-methyl	101200-48-0	Pesticides
Trifloxysulfuron-sodium	199119-58-9	Pesticides
2,3,5-Trimethacarb	12407-86-2	Pesticides
Acephate	30560-19-1	Pesticides
Acephate	30560-19-1	Pesticides
Acetamiprid	135410-20-7	Pesticides
Aldicarb	116-06-3	Pesticides
Aldicarb sulfone	1646-88-4	Pesticides
Aramite	140-57-8	Pesticides
Azamethiphos	35575-96-3	Pesticides
Azinphos-methyl	86-50-0	Pesticides
Bendiocarb	22781-23-3	Pesticides
Benfuracarb	82560-54-1	Pesticides
Butocarboxim	34681-10-2	Pesticides
Butocarboxim sulfoxide	34681-24-8	Pesticides
Carbaryl	63-25-2	Pesticides
Carbofuran	1563-66-2	Pesticides
Carbosulfan	55285-14-8	Pesticides

Table A5 (continued)

Compounds	CAS RN	Type
Chlorfluazuron	71422-67-8	Pesticides
Chromafenozide	143807-66-3	Pesticides
Clofentezine	74115-24-5	Pesticides
Clothianidin	210880-92-5	Pesticides
Cycloprothrin	63935-38-6	Pesticides
Diflubenzuron	35367-38-5	Pesticides
Dioxacarb	6988-21-2	Pesticides
Ethiofencarb	29973-13-5	Pesticides
Fenobucarb	3766-81-2	Pesticides
Fenoxycarb	79127-80-3	Pesticides
Fenpyroximate	111812-58-9	Pesticides
Fenthion oxon sulfone	14086-35-2	Pesticides
Fenthion oxon sulfoxide	6552-13-2	Pesticides
Fenthion sulfone	3761-42-0	Pesticides
Fenthion sulfoxide	3761-41-9	Pesticides
Fipronil	120068-37-3	Pesticides
Furathiocarb	65907-30-4	Pesticides
Hexythiazox	78587-05-0	Pesticides
Imidacloprid	138261-41-3	Pesticides
Indoxacarb	144171-61-9	Pesticides
Isoprocarb	2631-40-5	Pesticides
Methamidophos	10265-92-6	Pesticides
Methiocarb	2032-65-7	Pesticides
Methomyl	16752-77-5	Pesticides
Methoxyfenozide	161050-58-4	Pesticides
Metolcarb	1129-41-5	Pesticides
Monocrotophos	6923-22-4	Pesticides
Nitenpyram	120738-89-8	Pesticides
Oxamyl	23135-22-0	Pesticides
Phoxim	14816-18-3	Pesticides
Pirimicarb	23103-98-2	Pesticides
Promecarb	2631-37-0	Pesticides
Propoxur	114-26-1	Pesticides
Spinosad A	131929-60-7	Pesticides
Tebufenozide	112410-23-8	Pesticides
Terbucarb	1918-11-2	Pesticides
Tetrachlorvinphos	22248-79-9	Pesticides
Thiabendazole metabolite	948-71-0	Pesticides
Thiacloprid	111988-49-9	Pesticides
Thiamethoxam	135719-23-4	Pesticides
Thiodicarb	59669-26-0	Pesticides
Thiofanox-sulfone	39184-59-3	Pesticides
Thiofanox-sulfoxide	39184-27-5	Pesticides
Triflumuron	64628-44-0	Pesticides
Vamidothion	2275-23-2	Pesticides

Table A5 (continued)

Compounds	CAS RN	Type
XMC	2655-14-3	Pesticides
xylylcarb	2425-10-7	Pesticides
Imibenconazole	86598-92-7	Pesticides
Thidiazuron	51707-55-2	Pesticides
Forchlorfenuron	68157-60-8	Pesticides
Scopolamine	51-34-3	Pharmaceuticals
Atenolol	29122-68-7	Pharmaceuticals
Salbutamol	18559-94-9	Pharmaceuticals
Clenbuterol	37148-27-9	Pharmaceuticals
Propranolol	525-66-6	Pharmaceuticals
Acetaminophen	103-90-2	Pharmaceuticals
Ethenzamide	938-73-8	Pharmaceuticals
Phenacetin	62-44-2	Pharmaceuticals
Testosterone	58-22-0	Pharmaceuticals
Lidocaine	137-58-6	Pharmaceuticals
Metoprolol	37350-58-6	Pharmaceuticals
Sotalol	3930-20-9	Pharmaceuticals
Losartan	114798-26-4	Pharmaceuticals
Salinomycin	53003-10-4	Pharmaceuticals
Sulfanilamide	63-74-1	Pharmaceuticals
Cefotaxime	63527-52-6	Pharmaceuticals
Roxithromycin	80214-83-1	Pharmaceuticals
Sulfamerazine	127-79-7	Pharmaceuticals
Thiamphenicol	15318-45-3	Pharmaceuticals
Tylosin	1401-69-0	Pharmaceuticals
Ampicillin	69-53-4	Pharmaceuticals
Azithromycin	83905-01-5	Pharmaceuticals
Erythromycin	114-07-8	Pharmaceuticals
Lincomycin	154-21-2	Pharmaceuticals
Clarithromycin	81103-11-9	Pharmaceuticals
Sulfamethizole	144-82-1	Pharmaceuticals
Sulfamethoxazole	723-46-6	Pharmaceuticals
Sulfamonomethoxine	1220-83-3	Pharmaceuticals
Sulfadimethoxine	122-11-2	Pharmaceuticals
Sulfadiazine	68-35-9	Pharmaceuticals
Dexamethasone	50-02-2	Pharmaceuticals
Prednisolone	50-24-8	Pharmaceuticals
Antipyrine	60-80-0	Pharmaceuticals
PropyphenazoLe	479-92-5	Pharmaceuticals
Ketoprofen	22071-15-4	Pharmaceuticals
Ranitidine	66357-35-5	Pharmaceuticals
Promethazine	60-87-7	Pharmaceuticals
Disopyramide	3737-09-5	Pharmaceuticals
Verapamil	52-53-9	Pharmaceuticals
Griseofulvin	126-07-8	Pharmaceuticals

Table A5 (continued)

Compounds	CAS RN	Type
Fluvoxamine	54739-18-3	Pharmaceuticals
Paroxetine	61869-08-7	Pharmaceuticals
Fluoxetine	54910-89-3	Pharmaceuticals
Sulpiride	15676-16-1	Pharmaceuticals
Metoclopramide	364-62-5	Pharmaceuticals
Haloperidol	52-86-8	Pharmaceuticals
Diphenidol	972-02-1	Pharmaceuticals
Chlorpromazine	50-53-3	Pharmaceuticals
Epinastine	80012-43-7	Pharmaceuticals
Chlorpheniramine maleate	113-92-8	Pharmaceuticals
Diltiazem	42399-41-7	Pharmaceuticals
Carazolol	57775-29-8	Pharmaceuticals
Fenofibrate	49562-28-9	Pharmaceuticals
Ifosfamide	3778-73-2	Pharmaceuticals
Cyclophosphamide	50-18-0	Pharmaceuticals
Mepirizole	18694-40-1	Pharmaceuticals
Etodolac	41340-25-4	Pharmaceuticals
Dextromethorphan	125-71-3	Pharmaceuticals
Pirenzepine	28797-61-7	Pharmaceuticals
Terbutaline	23031-25-6	Pharmaceuticals
Theophylline	58-55-9	Pharmaceuticals
2-Quinoxalinecarboxylic acid	879-65-2	Pharmaceuticals
Cefalexin	15686-71-2	Pharmaceuticals
Acetohexamide	968-81-0	Pharmaceuticals
Dicyclohexylamine	101-83-7	Pharmaceuticals
Cimetidine	51481-61-9	Pharmaceuticals
Metformin	657-24-9	Pharmaceuticals
Tolbutamide	64-77-7	Pharmaceuticals
Cotinine	486-56-6	Pharmaceuticals
Ormetoprim	6981-18-6	Pharmaceuticals
Oleandomycin	3922-90-5	Pharmaceuticals
Spiramycin	8025-81-8	Pharmaceuticals
Tolperisone	728-88-1	Pharmaceuticals
Ifenpridil	23210-56-2	Pharmaceuticals
Amitriptyline	50-48-6	Pharmaceuticals
Pentoxifylline	6493-05-6	Pharmaceuticals
Dipyridamole	58-32-2	Pharmaceuticals
Sulfapyridine	144-83-2	Pharmaceuticals
Cefuroxime	55268-75-2	Pharmaceuticals

Experimental Methods

Sample preparation for GC-MS multiscreen

A glass fibre filter (GMF 150 47 mm; Whatman) was placed on top of an SPE disk (Empore SDB-XC 47 mm; 3M) and this extraction system conditioned with 10mL of dichloromethane, 10mL of acetone, 10mL of methanol followed by 20mL of deionised (MilliQ) water. Grab water samples (1 L) were adjusted to acidic pH with phosphate buffer (1 mol/L KH_2PO_4-KOH pH7) and loaded onto the filter/extraction disks at the rate of <100mL/min followed by rinse with 20mL of MilliQ water. The glass fibre disks and SPE disks were then dried on a hot plate (35^o). Thereafter, the disks were soaked in 5mL of acetone for one minute, and the solvent eluted through the disks. This soaking/elution process was then repeated with another 5 mL aliquot of acetone, before being repeated with 5mL of dichloromethane. The eluants were combined, and the mixture evaporated to < 1 mL under a stream of nitrogen, after which 10mL of hexane was added, the mixture dehydrated using sodium sulfate, and the eluent mixture concentrated to less than 1mL. Prior to GC-MS analysis a mixture of internal standards solutions (4-Chlorotoluene-d4, 1,4-Dichlorobenzene-d4, Naphtalene-d8, Acenaphthene-d10, Phenanthrene-d10, Fluoranthene-d10, Chrysene-d12, and Perylene-d12) were added to the sample (100µL of 10 µg/L, 1µg each) and sample was made up to 1mL with hexane.

Sample preparation for LC-TOF-MS multiscreen

A water sample (200 mL) was pH adjusted with 1mL of phosphate buffer (1M, pH7.0) then filtered through a GF/C glass fiber filter. A combined SPE cartridges (Sep Pak PS-2 and AC-2; Waters) was preconditioned with dichloromethane (5 mL), methanol (5 mL) and Milli-Q water (10 mL). The filtered water sample was then passed through the cartridges at a flow rate of 10 mL/min, after which the cartridge was rinsed with 10 mL of Milli-Q water. After that, the cartridges were dried by nitrogen for 40 min to remove water and finally the desired analytes were eluted with 5 mL of methanol and 3 mL of DCM. The glass fiber filter was extracted twice with methanol (3 mL each) by immersing the filters in methanol in a plastic tube, and using sonication (Ultra sonic cleaner USK-3R); the solutions were then added to the previously eluted samples. The sample mixture was evaporated under nitrogen to a final volume of 60 µL, and then spiked with 40 µL of 5 µg/L internal standard (mixture of methomyl-d_3, pirimicarb-d_6, imazalil-d_5). The concentrates (100% methanol solution) were filtered through a syringe filter into a glass vial (1mL of deactivated glass). The plastic tubes were then rinsed with 400 µL of Milli-Q water and the water was passed through the same syringe filter into the same glass vial (0.5mL of a final volume, resulting in a 20% methanol solution). Finally, the vials were placed in an auto-sampler and measured by LC-TOF-MS.

Measurement

Total ion current chromatograms obtained by, for instance, a GC-MS-Scan were treated with an identification and quantification system with a GC–MS database (AIQS-DB) (Kadokami et al., 2005), that determined the concentrations of the 940 semi-volatile organic compounds (Appendix Table A4). SIM mode was also applied for selected compound screening. LC-TOF-MS databse (AIQS-DB) was also developed that determined the concentration of the 300 non-volatile organic compounds (Appendix Table A5).

GC-MS specification

GC-MS: Shimadzu GCMS-QP 2010 Plus
Column: J&W DB-5 ms (5% phenyl-95% methylsilicone) fused silica capillary column, 30 m X 0.25 mm i.d., 0.25 mm film
Temperature: Column: temperature programmed: 2 min at 40°C, 8°C/min to 310°C, 5 min at 310°C; Injector: 250°C; Transfer line: 300°C; Ion source: 200°C
Injection method: splitless, 1 min for purge-off time
Carrier gas: He
Linear velocity: 40 cm/s, constant flow mode
Ionization method: EI
Tuning method: target tuning for US EPA method 625
Measurement method: SIM/Scan
Scan range: 45 amu to 600 amu
Scan rate: 0.3 s/scan

LC-TOF-MS specification

LC: Agilent 1200

Column: GL Science ODS-4 2.1×150mm (3µm)
Mobile Phase: A 5mmol CH_3COONH_4 in H_2O; B 5mmol CH_3COONH_4 in CH_3OH
Gradient Profile: A95:B5 (0min) - A5:B95 (30min-50min)
Column Temperature: 40 ^oC
Flow Rate: 0.3 mL/min

MS: Agilent 6220 MSD

Ionization: ESI-Positive
Fragmentor Voltage: 100 V
VCap Voltage: 3500 V
Drying gas flow: 10L/min at 325 ^oC
Measurement mode: Scan
Scan range (m/z): 50-1000

Appendix B: Yeast based recombinant receptor-reporter gene bioassays (yeast bioassays)

Urban municipal wastewaters may contain a large number of organic chemicals. More than 100,000 chemicals are registered at EINECS (European inventory of existing chemical substances) and around 30,000 to 70,000 chemicals are used daily (European Commission, 2011). Some of these chemicals, when discharged to receiving waters, may prove directly toxic to organisms living therein, while others may elicit more subtle effects, including eliciting genotoxic or endocrine disrupting effects (EDCs). Managing the effects of such contaminants ultimately requires information on effluent toxicity and chemical concentrations. Yeast bioassays are high-throughput cell-based *in vitro* toxicity testing methods that were developed to target some levels of the toxicity pathway. Recent improvements of sample preparation methods and more sensitive bioassay endpoints have enabled evaluation of high quality water such as purified recycled water and drinking water. A battery of yeast bioassays covers a range of mode of actions and recipients (human and fish) to be tested. In conjunction with baseline toxicity tests (bioluminescence inhibition test with photobacteria *Vibrio fischeri*), yeast bioassays provide a comprehensive picture of the biologically-active chemicals present in a sample, indicative of specified endpoints relevant for human and / or environmental health.

Table B1: The function of nuclear receptors utilized in yeast bioassays

Nuclear receptor	Function	Target mode of action	Inducing chemicals
hER (human estrogen receptor)	Endocrine system	Estrogenicity	Natural estrogens, estrogen mimicking chemicals
medER (medaka estrogen receptor)	Endocrine system	Estrogenicity	Natural estrogens, estrogen mimicking chemicals
AhR (aryl hydrocarbon receptor)	Induction of cytochrome P450 (CYP1A1)	Dioxin-like activity, Aryl hydrocarbon receptor activation	PAHs, Dioxins
CAR (constitutive androstane receptor)	Protective role against toxicity induced by bile acid, regulation of physiological functions	Xenobiotic detection	Various pharmaceuticals

Testing the removal of all micro-contaminants/micro-pollutants is not practical or realistic, although the concentration of individual contaminants is expected to be very low. Bioassays are proven to be more sensitive than chemical analysis in evaluating the removal of organic micro-pollutants by reverse osmosis or other advanced treatment technologies by showing the observed mixed toxicity of chemicals that could fall below the quantification limit of chemical analysis (Cao et al 2009; Escher et al. 2009, 2011; Leusch et al. 2014a, 2014b; Macova et al. 2010, 2011; Stalater et al. 2011; Tang et al. 2014). Many cell based *in vitro* bioassays are available. A comprehensive test battery should include general cytotoxicity tests such as photobacteria toxicity test, estrogen receptor assays, and xenobitotic induction testing. The battery of yeast bioassays for this project included:

- Estrogen receptors (hER, medER): receptor mediated effects = estrogenic effects (endocrine disruption)

- Aryl hydrocarbon receptor (AhR) and Constitutive androstane receptor (CAR): Induction of xenobiotic metabolism pathways

Estrogenicity was tested by hER and medER yeast bioassays transduced with the ligand binding domains of nuclear receptors and co-activators for detecting and measuring the activity of chemicals; detection of xenobiotics was tested by AhR and CAR yeast bioassays. While the induction of xenobiotic metabolism may not lead to cytotoxicity, it is an indicator of the presence of chemical pollutants. Assays were conducted on feed, product and discharge water from the AWTP according to the monitoring program.

Experimental

Sample preparation for bioassays

A water sample (1L) was pH adjusted with 10mL of acetic acid methanol solution (1:9:90 acetic acid : water : methanol). A glass fibre filter (GMF 150 47 mm; Whatman) was placed on top of an SPE disk (Empore SDB-XC 47 mm; 3M) and this extraction system conditioned with 10mL of methanol followed by 20mL of deionised (MilliQ) water. Water samples (1 L) were loaded onto the filter/extraction disks and washed with 20mL of MilliQ water. The glass fibre disks and SPE disks were then dried on a hot plate (35^{o}). Thereafter, the glass fibre disks were sonicated in 6 mL of methanol for five minute (repeat this), and the solvent was collected and combined the centrifuged (at 3000 rpm for 20 min.) residue from filters. The combined methoanl was used to elute Empore disks. The eluants evaporated to dryness under a stream of nitrogen. Dried samples were reconstituted with 1 mL of 10% acetone / dichloromethane mix (A/D mix) then vortex and ultrasonic for 5 min. (repeat this), then loaded thison to Florisil (FL) cartridge (Varian Bond Elut-FL, 500 mg, 3mL; CA, USA) conditioned with 2 mL of A/D mix (eluent was collected). The tube contained sample was washed with 1mL of A/D mix and loaded onto the FL cartridge while eluent was collected, another 2 mL of A/D mix was added (total 4 mL of eluent was collected - FL-A/D fraction). Methanol fraction was collected after loading 1 mL x 2 + 3 mL methanol (total 4mL) by washing the tube by vortex (FL-Me fraction). Both fractions were evaporated under nitrogen stream.

Photobacteria (PB) toxicity test

Dried samples were reconstituted with 100 μL of DMSO then 20 μL of sample was combined with 480 μL T medium (T med; peptone, 0.4 g; glycerol 3.5 g; NaCl 20 g; $MgSO_4 \cdot 7H_2O$, 29 g; KCl, 0.9 g; K_2HPO_4, 0.1 g; 1M MOPS buffer solution, 4.5 mL) and was then ready for toxicity testing after vortex. Photobacterium cells cultured in a mixture of marine broth and T med was diluted with modified T med (m-T med; glucose instead of glycerol). Microplate (96 wells) was prepared by adding m-T med (60 μL) to all wells by an auto-dispensing system (Nichiryo NSP-7000 Multi-channel Auto Sampling System, Nichiryo Co., Tokyo, Japan). Five samples and one control were run on each plate, with aliquots of each sample (60 μL), added to two, neighbouring wells of the 1st row of the plate. An aliquot was removed from each row to next row except for the last row to dilute 2-fold. Thereafter, bacterium solution (60 μL) was added to all wells, the plate shaken (30s). Bioluminescence intensity was measured by a microplate luminometor. The ICR50 values are reported according to a concentration ratio (C.R.), which is effectively how much the sample would have had to be diluted to inhibit luminescence in 50% of the photobacteria. In short, the lower the ICR50 reported, the higher the toxicity of the sample (and *vice versa*, i.e. the higher the ICR50, the lower the toxicity).

Measurement of estrogenicity with hER and medER yeast bioassay

Recombinant yeast cells were cultured (30°C, overnight) in a modified SD (Sabouraud Dextrose) (MSD) medium (0.88% glucose lacking tryptophan and leucine). The yeast solution cell density was measured (595 nm) and adjusted to around 0.18 readings for constant cell density by diluting with MSD medium. Microplate (96 wells) was prepared by adding MSD solution (60 μL) to all wells by an auto-dispensing system (Nichiryo NSP-7000 Multi-channel Auto Sampling System, Nichiryo Co., Tokyo, Japan). Six samples were run on each plate, with aliquots of each sample (60 μL), added to two, neighbouring wells of the 1st row of the plate. An aliquot was removed from each row to next row except for the last row to dilute 2-fold. Thereafter, yeast solution (60 μL) was added to all wells, the plate shaken (30s) and then incubated (30˚C, 4 h). Enzymatic digestion was conducted by adding 50 μL of mixture of Lysis solution (zymolyase 100T / Z buffer) into each well by the auto-dispenser. Then the plate was incubated (37˚C, 1 h) after vortex (40s). After incubation, 80 μL of GS substrate solution (Galacton-Star substrate with reaction buffer diluent; Applied Biosystems) for inducing chemiluminescence was then added to each well, and the plate incubated (30˚C, 10 min). The chemiluminescence produced by released β-galactosidase was measured with a microplate luminometer (Luminescencer-JNR AB-2100, ATTO Bioinstruments, Tokyo, Japan). Agonist activity was recorded as the EC x 10 (defined as the concentration of test solution producing a chemiluminescent signal 10 times that of the blank (negative) control). Positive control is 17β-estradiol in both hER and medER assays.

Measurement of xenobiotic induction with AhR and CAR yeast bioassay

Recombinant AhR yeast cells were cultured (30˚C, overnight) in a modified SD (Sabouraud Dextrose) (MSD) medium (lacking tryptophan). CAR yeast cells were cultured as hER and medER method. The AhR yeast solution was centrifuged (2000 rpm, 20 min) and replaced media to MSD (1.5% galactose + leucine) for AhR assay. The yeast solution cell density was measured (595 nm) and adjusted to around 0.18 readings for constant cell density by diluting with MSD medium. Microplate (96 wells) was prepared by adding MSD solution (60 μL) to all wells by an auto-dispensing system (Nichiryo NSP-7000 Multi-channel Auto Sampling System, Nichiryo Co., Tokyo, Japan). Six samples were run on each plate, with aliquots of each sample (60 μL), added to two, neighbouring wells of the 1st row of the plate. An aliquot was removed from each row to next row except for the last row to dilute 2-fold. Thereafter, yeast solution (60 μL) was added to all wells, the plate shaken (30s) and then incubated (30˚C, 4 h). Enzymatic digestion was conducted by adding 50 μL of mixture of Lysis solution (zymolyase 100T / Z buffer) into each well by an auto-dispenser. Then the plate was incubated (37˚C, 1 h) after vortex (40s). After incubation, 80 μL of GS substrate solution (Galacton-Star substrate with reaction buffer diluent; Applied Biosystems) for inducing chemiluminescence was then added to each well, and the plate incubated (30˚C, 10 min). The chemiluminescence produced by released β-galactosidase measured with a microplate luminometer (Luminescencer-JNR AB-2100, ATTO Bioinstruments, Tokyo, Japan). Agonist activity was recorded as the EC x 10 (defined as the concentration of test solution producing a chemiluminescent signal 10 times that of the blank (negative) control). Positive controls were β–naphthoflavone in AhR and p-*tert*-octylphenol in CAR assays.

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Appendix C Health guideline values

Table C1: Health Guideline Values from ADWG (Australian Drinking Water Guidelines) and AGWR (Australian Guidelines for Water Recycling) and AIQS GC-MS/LC-TOF-MS method of detection limits (μg/L)

Chemical Names	ADWG	AGWR	AIQS GC/LC MDL
Disinfection By-Products			
2-chlorophenol	300		0.01
2,4-Dichlorophenol	200	200	0.01
2,4,6-Trichlorophenol	20	20	0.01
2,6-Dichlorophenol			0.01
Bromoacetic acid		10	
Bromochloroacetonitrile		0.35	
Bromodichloromethane		0.7	
Bromoform		6	
Chlorinated furanones (MX)		100	
Chloroacetic acid	150		
Chloroform		200	
Dibromochloromethane		100	
Dichloroacetic acid	100	100	
Dichloroacetonitrile		2	
N-nitrosodiethylamine (NDEA)		0.01	0.01
N-nitrosodimethylamine (NDMA)	0.1	0.01	
Trichloroacetic acid	100	100	
Trihalomethanes (thMs) (total)	250		
Pesticides			
1,3-Dichloropropene	100		
2-Phenylphenol		1000	0.01
2,2-DPA	500		
2,4-D (2,4-Dichlorophenoxyacetic acid)	30	30	
2,4,5-T	100		
4-Nitrophenol		30	0.01
4,4'-DDE (44DDE; p,p'-DDE)		20	0.01
4,4'-DDT (44DDT; p,p'-DDT)	9		0.01
Acephate	8		0.05
Alachlor (Lasso)			0.01
Aldicarb	4		0.01
Aldrin & Dieldrin (combined)	0.3		0.01
Ametryn	70		
Amitraz	9		0.01
Amitrole	0.9		
Asulam	70		0.01
Atrazine	20	40	0.01
Azinphos-methyl	30	3	0.01
Benomyl	90		
Bentazone	400		0.01
Bioresmethrin	100		0.01
Bromacil	400		0.01
Bromophos-ethyl	10	10	
Bromoxynil	10		
Captan	400		0.01
Carbaryl	30		0.01
Carbendazim	90	100	0.01
Carbofuran	10		0.01
Carbophenothion	0.5		
Carboxin	300		0.01

Table C1 (continued)

Chemical Names	ADWG	AGWR	AIQS GC/LC MDL
[(Carboxymethyl)imino bis(ethylenenitrilo)]		5	
Chlorantraniliprole	6000		
Carfentrazone-ethyl	100		0.01
Chlordane	2	1	
Chlorfenvinphos	2		0.01
Chlorothalonil	50		0.01
Chloroxuron	10		0.01
Chlorpyrifos	10	10	0.01
Chlorpyrifos-methyl		10	0.01
Chlorsulfuron	200		0.1
Clopyralid	2000		
Cyfluthrin, Beta-cyfluthrin	50		0.01
Cypermethrin	200	0.5	0.01
Cyprodinil	90		0.01
Deltamethrin	40		0.01
Demeton-S		0.15	
Diazinon	4	3	0.01
Dicamba	100		
Dichlobenil	10		
Dichloroprop / Dichlorprop-P	100		
Dichlorvos	5	1	
Diclofop-methyl	5		0.01
Dicofol	4		
Difenzoquat	100		
Diflubenzuron	70		
Dimethoate	7	50	0.01
Diphenamid	300		0.01
Diquat	7		
Disulfoton	4		0.01
Diuron	20	30	0.01
EDB	1		
Endosulfan	20		0.01
Endosulfan sulfate		30	0.01
Endothal	100		
EPTC	300		0.01
Esfenvalerate	30		0.01
Ethion	4	3	0.01
Ethoprophos (Mocap)	1	1	0.01
Etridiazole	100		0.025
Fenamiphos	0.5		0.01
Fenarimol	40		0.01
Fenitrothion	7		0.01
Fenoprop	10		
Fensulfothion	10		0.01
Fenthion	7	0.5	0.01
Fenvalerate	60		0.01
Fipronil	0.7		0.01
Flamprop-methyl	4		0.01
Fluometuron	70		
Fluproponate	9		
Formothion	50		
Fosamine	30		
Glyphosate	1000		
Haloxyfop	1		
Heptachlor	0.3		0.01

Table C1 (Continued)

Chemical Names	ADWG	AGWR	AIQS GC/LC MDL
Hexaflurate	30		
Hexazinone	400		0.01
Imazapyr	9000		
Iprodione	100		0.01
Lindane (g-HCH)	10	20	0.01
Malathion		900	0.01
Mancozeb	9		
MCPA	40		
Metaldehyde	20		
Metham	1		
Methidathion	6		0.01
Methiocarb	7		0.01
Methomyl	20		0.01
Methoxychlor	300		0.01
Methyl bromide	1		
Metiram	9		
Metolachlor	300	300	0.01
Metribuzin	70		0.01
Metsulfuron-methyl	40		0.1
Mevinphos	5		0.01
Molinate	4		0.01
Monocrotophos	2		0.01
N,N-diethyltoluamide (DEET)		2500	0.01
Napropamide	400		0.01
Nicarbazin	1000		
Nitralin	500		0.25
Norflurazon	50		0.01
Omethoate	1		0.01
Oryzalin	400		0.01
Oxamyl	7		0.01
Paraquat	20		
Parathion	20	10	0.01
Parathion-methyl (methyl parathion)	0.7	100	0.01
Pebulate	30		0.01
Pendimethalin	400		0.01
Pentachlorophenol (PCP)	10	10	0.01
Permethrin	200		0.01
Picloram	300		
Piperonyl butoxide	600		0.01
Pirimicarb	7		0.01
Pirimiphos methyl	90		0.01
Pirimiphos-ethyl	0.5		
Polihexanide	700		
Profenofos	0.3		0.01
Propachlor	70		0.01
Propanil	700		0.01
Propargite	7		0.01
Propazine	50		0.01
Propiconazole	100		0.01
Propyzamide	70		0.01
Pyrasulfotole	40		
Pyrazophos	20		0.01
Pyroxsulam	4000		

Table C1 (Continued)

Chemical Names	ADWG	AGWR	AIQS GC/LC MDL
Quintozene	30		
Simazine	20	20	0.01
Spirotetramat	200		
Sulprofos	10		0.1
Temephos	400		0.01
Terbacil	200		0.01
Terbufos	0.9		0.01
Terbuthylazine	10		
Terbutryn	400		0.01
Tetrachlorvinphos	100		0.01
Thiobencarb	40		0.01
Thiometon	4		0.01
Thiophanate	5	5	
Thiram	7		
Toltrazuril	4		
Triadimefon	990		0.01
Trichlorfon	7		0.01
Triclopyr	20		0.01
Trifluralin	90	50	0.01
Vernolate	40		
α-BHC (α-HCH)		20	0.01
β-BHC (b-HCH)		20	0.01
PPCPs			
1,7-Dimethylxanthine (Paraxanthine)	0.7	0.01	
2,5-Dihydroxybenzoic acid		7	
5-Methyl-1H-benzotriazole		0.007	0.01
Acetophenone		400	0.01
Alprazolam		0.25	0.01
Amoxycillin		1.5	
Anhydroerythromycin A		17.5	
Antipyrine (phenazone)		1000	0.01
Aspirin (Acetylsalicylic acid)		29	0.01
Atorvastatin		5	0.01
Azithromycin		3.9	0.01
Betaxolol		10	0.01
Bezafibrate		300	0.01
Bisoprolol		0.63	0.01
Caffeine		0.35	0.01
Carazolol		0.35	0.01
Carbamazepine		100	0.01
Cefaclor		250	
Cephalexin		35	0.05
Chloroamphenicol		175	
Chlorophene		0.35	
Chlorotetracycline		105	
Cimetidine		200	0.01
Ciproflaxin		250	
Clarithromycin		250	0.01
Clenbuterol		15	
Clindamycin		300	
Clofibric acid		750	
Codeine		50	
Cotinine		10	0.01
Cyclophosphamide		3.5	0.01

Table C1 (Continued)

Chemical Names	ADWG	AGWR	AIQS GC/LC MDL
Dehydronifedipine		20	
Demeclocycline		300	
Diatrizoate Sodium		350	
Diatrizoic acid		350	
Diclofenac		1.8	
Diltiazem		60	0.01
Dipyrone (vet)		525	
Doxycycline		10.5	
Enalaprilat		1.3	
Enrofloxacin		22	0.25
Erythromycin		17.5	0.01
Fenoprofen		450	
Fluoxetine (Prozac)		10	0.01
Gemfibrozil		600	
Ibuprofen		400	0.01
Indomethacin		25	
Iohexol		720	
Iopamidol		400	
Iopromide		750	
Isophosphamide		3.5	
Ketoprofen		3.5	0.01
Metformin (1,1- Dimethylbiguanide)		250	0.01
Metoprolol		25	0.01
Monensin		35	
Nadolol		20	
Naladixic acid		1000	
Naproxen		220	0.05
Norflaxin		400	
Paracetamol (acetaminophen)		175	0.025
Penicillin G		1.5	0.025
Penicillin V		1.5	
Phenol		150	0.01
Propranolol		40	0.01
Roxithromycin		150	0.01
Salbutamol		3	0.1
Salicylic acid		105	
Sulfamethazine		35	
Sulfamethizole		35	0.01
Sulfamethoxazole		35	0.025
Sulfamethoxine		35	
Sulfasalazine		500	
Temazepam		5	
Terbutaline		4.5	
Terramycin (oxytetracycline)		105	
Tetracycline (TCLN)		105	
Timolol		10	
Tolfenamic acid (vet)		17.5	
Triclosan		0.35	0.01
Trimethoprim		70	
Tylosin		1050	0.1
Valium (Diazepam)		2.5	
Industrial			
1,1-Dichloroethene	30	30	
1,2-Dichlorobenzene	1500		0.01

Table C1 (Continued)

Chemical Names	ADWG	AGWR	AIQS GC/LC MDL
1,2-Dichloroethane	3		
1,2-Dichloroethene	60		
1,4-Dichlorobenzene	40		0.01
3,3',4,4'-Tetrachlorobiphenyl (PCB77)		0.000016	0.01
2,3,3',4,4'-Pentachlorobiphenyl (PCB105)	0.000016	0.01	
2,3,3',4,4',5-Hexachlorobiphenyl	0.000016	0.01	
2,3',4,4',5-Pentachlorobiphenyl (PCB118)	0.000016	0.01	
2,4,5,3',4',5'-Hexachlorobiphenyl	0.000016	0.01	
2,7-Dichlorodibenzo-p-dioxin (DCDD)		0.000016	
3,4,5,3',4',5'-Hexachlorobiphenyl	0.000016	0.01	
4-Chlorophenol		10	
4-Cumylphenol		0.35	
4-Methylphenol (p- cresol)		600	
Anthracene		150	0.01
Benzene	1		
Benzo(a)pyrene	0.01	0.01	0.01
Benzyl chloride		0.2	0.01
Bromochloromethane		40	
Chlorobenzene	300		
Dibutyltin (DBT)		2	
Dichloromethane (methylene chloride)	4	4	
Ethylbenzene	300		
Ethylenediamine tetraacetic acid (EDTA)	250		
Hexachlorobutadiene	0.7		0.01
Monobutyltin (MBT)		0.7	
N-nitrosomorpholine (NMOR)		0.001	
Naphthalene		70	0.01
Octachlorodibenzo-p-dioxin (OCDD)		0.000016	
Phenanthrene		150	0.01
Phthalic anhydride		7000	
Pyrene		150	0.01
Styrene (vinylbenzene)	30		
Tetrachloroethene	50		
Toluene	800		
Tributyltin (TBT)		1	
Tributyltin oxide	1		
Trichlorobenzenes (total)	30		
Vinyl chloride	0.3		
Xylene	600		
Antioxidants			
2,6-Di-tert-butyl-1,4-benzoquinone	0.014	0.025	0.025
2,6-Di-tert-butylphenol	2		
Butylated hydroxyanisole	18000		
Butylated hydroxytoluene (2,6-Di-tert-Butyl-p-Cresol) (BHT)	1000		0.025
Chelating agents			
Ethylenediaminetetraacetic acid (EDTA)	250		
Nitrilotriacetic acid (NTA)		200	
Propylenedinitrilotetraacetic acid (PDTA)	0.7		
Fyrol FR 2 (tri(dichlorisopropyl)	1	0.025	
Flame retardants			
Tributyl phosphate		0.5	0.01

Table C1 (Continued)

Chemical Names	ADWG	AGWR	AIQS GC/LC MDL
Triphenyl phosphate		1	
Tris(2-chloroethyl)phosphate (TCEP)		1	0.01
Tri(butyl cellosolve) phosphate (ethanol,2-	50		
Fragrance			
2,4,6-Trinitro-1,3-dimethyl-5-tert-butylbenzene	350		
4-Acetyl-6-t-butyl-1,1-dimethylindan	7		
6-Acetyl-1,1,2,4,4,7-hexamethyltetraline	4		
Galaxolide		18000	
Musk ketone		350	
Musk tibetene		0.35	
Pentamethyl-4,6- dinitroindane		0.35	
Plasticizers			
Bisphenol A		200	0.01
Di-n-butyl phthalate		35	0.01
Di(2-ethylhexyl) adipate			0.01
Di(2-ethylhexyl) phthalate	10		0.01
Surfactants			
4-Nonylphenol (4NP)		500	0.01
4-tert-Octylphenol		59	0.01
Nitrilotriacetic acid	200		
Sterols			
Cholesterol		7	0.1
Coprostanol		0.7	0.01
Stigmastanol		1000	0.1
Phytochemical			
Coumarin		0.5	
Hormones			
Androsterone		14	
Testosterone		7	
17α-estradiol		0.175	
17α-ethinyl estradiol		0.0015	
17β-estradiol		0.175	
Equilenin		0.03	
Equilin		0.03	
Estriol		0.03	
Estrone		0.03	
Mestranol		0.0025	
Norethindrone		0.25	
Progesterone		105	