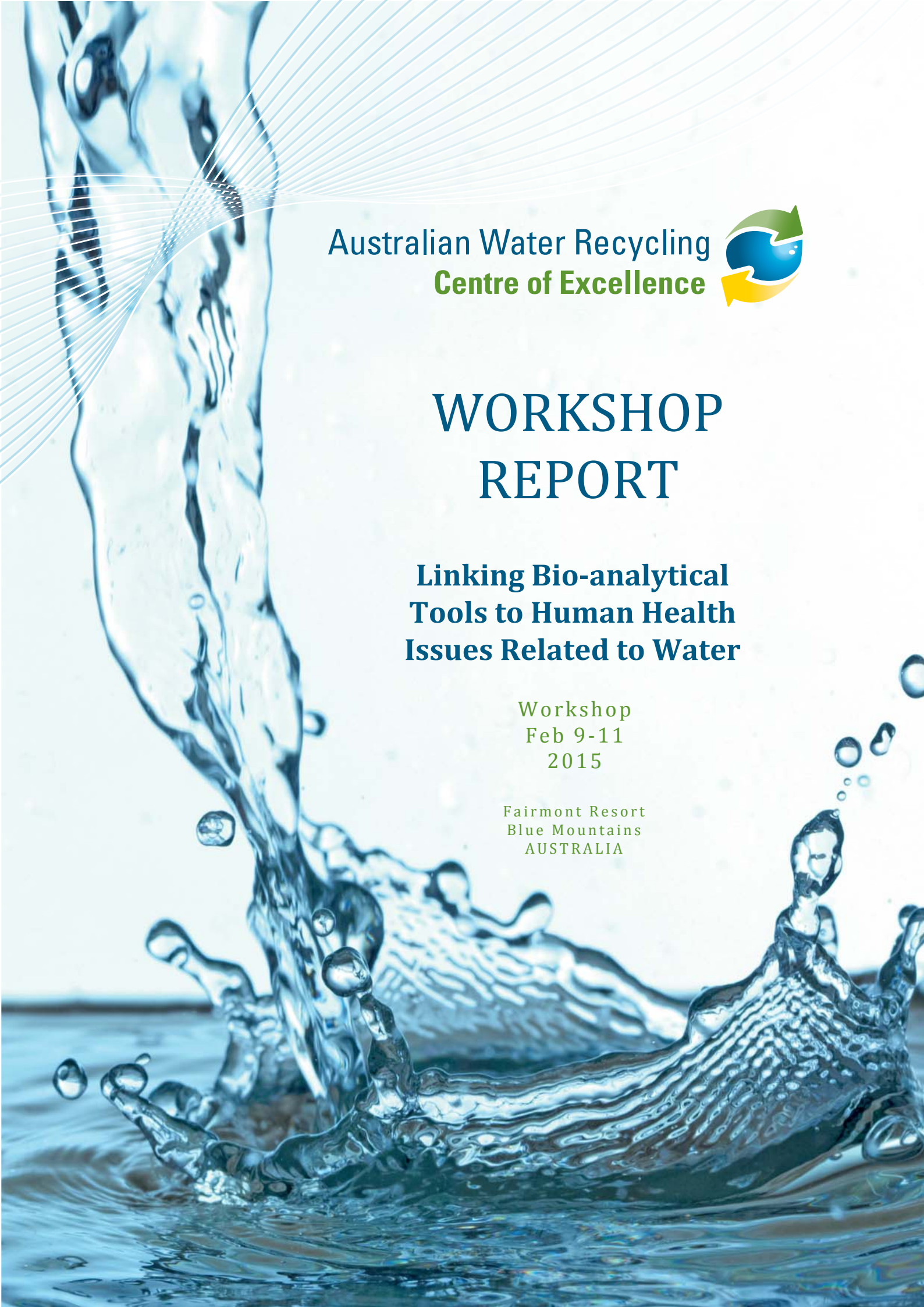

Australian Water Recycling
Centre of Excellence

WORKSHOP REPORT

Linking Bio-analytical Tools to Human Health Issues Related to Water

Workshop
Feb 9-11
2015

Fairmont Resort
Blue Mountains
AUSTRALIA

Project Leader

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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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Workshop Report
Linking Bio-analytical Tools to Human Health Issues Related to Water

Fairmont Resort Blue Mountains, Leura, Australia
9 – 11 February 2015

Paul Greenfield and Ian Law, February 2015

1. Background

Bio-analytical tools and in particular, *in vitro* bioassay methods, play a significant role in the pharmaceutical industry in ensuring the safety and efficacy of new chemical compounds. Over the last decade research has shown that such assays are compatible with water quality assessment and, by combining bioassays with chemical analyses, a significant improvement in water quality assessment may be possible. In particular, bio-analytical tools have the potential to provide a high-throughput platform to address issues of mixture toxicity and transformation product toxicity – both difficult to achieve by chemical analyses alone. In fact, both *in vitro* and *in vivo* bioassays have been used in the USA and Europe since the late 1970s in attempts to characterise drinking water hazards and subsequently some industrial wastewater hazards, with varying levels of success. There remains a gap, however, in linking these assessments to specific human health questions.

Many of the technical difficulties associated with bio-analytical tools are being progressively overcome. How such bioassay results can be meaningfully interpreted in terms of specific human health questions and risks, and, more specifically, how they might be used within a regulatory framework remain unclear. The concept of adverse outcome pathways (AOP) and attempts to link molecular initiating events (MIE) to atypical outcomes can provide some guidance.

The *Australian Water Recycling Centre of Excellence (AWRCoE)* recognized the need to map a path forward that addressed key barriers in linking bio-analytical tools to specific human health outcomes, the absence of which will continue to limit wider acceptance of these techniques for determining environmental health risks generally, and those associated with drinking water more particularly *i.e.* there is a need to clearly outline the practical and regulatory challenges and the research needed to address these challenges.

This recognition led to a 3-day workshop being organized by *AWRCoE* in February 2015 to identify these barriers and map a way forward to address the relevant issues. The workshop involved a small group of international researchers experienced in the development and application of bio-analytical tools, regulators and water industry professionals.

2. Aims and Objectives of the Workshop

The workshop brought together a small number of key stakeholders from the water industry, drinking water regulators and scientists from various fields (toxicology, molecular toxicology, human health, environmental science, risk assessment and bioassay development) to develop a concise road-map of the path to improve the linkages between bio-analytical tools and predictions of human health outcomes, in particular addressing the issue of wider acceptability of bio-analytical tools for determining human health risks associated with drinking water. **Appendix 1** lists the participants and their affiliation.

3. Workshop Focus & Agenda

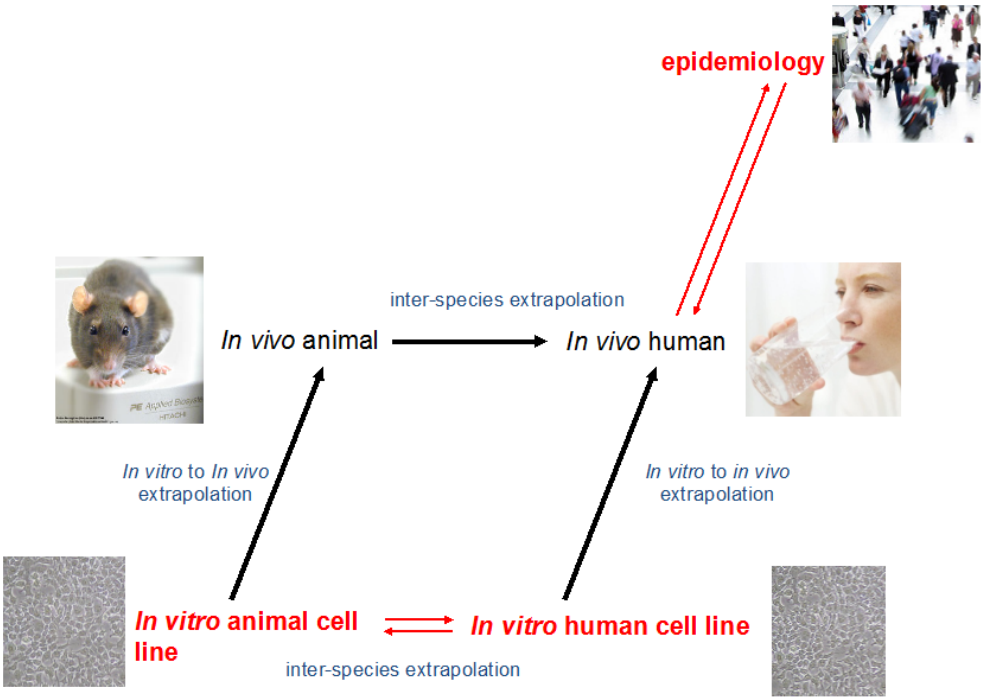
Health effects testing of drinking water began in the 1970s in the US, England, France and Holland. Initially, these efforts focused on both *in vitro* (primarily Ames' test and cell transformation assays) and *in vivo* testing. As potable reuse of wastewater began to be explored in the same time period, several studies were initiated, including the Denver, Potomac Estuary, San Diego, and Tampa projects some of which utilized both *in vivo* and *in vitro* testing. Studies have also been carried out at Windhoek in Namibia, using both *in vitro* and *in vivo* methods with the latter being based on water fleas and fish. Singapore, in the most recent application of health effects testing, used both mice and fish for *in vivo* studies that were carried out over the period 2000-2002 as part of Singapore's NEWater initiative.

The expense of conducting whole animal studies and inability to deploy them in a way to meaningfully analyze discrete samples over time made the *in vivo* testing infeasible. Many investigations of *in vitro* testing were also pursued during this period, largely in academia. While the *in vitro* studies sparked much useful research over the past several decades, direct use of *in vitro* testing has had limited impact on decision making aside from assisting in the identification of compounds (primarily disinfection by-products) for further testing as individual chemicals.

Despite this and much other work, important questions remain – as they relate to human health assessment:

- Can we confidently extrapolate from *in vitro* animal to *in vivo* animal?
- Can we confidently extrapolate from *in vivo* animal to *in vivo* human?
- Can we confidently extrapolate from *in vitro* human to *in vivo* human?

These questions are highlighted in the diagram below and provided an over-arching structure for the workshop discussions.



To provide greater focus on issues relevant to *AWRCoE,* a number of more specific questions were also addressed over the three days, although the key question remained *"What needs to be done to link bio-analytical tools to human health effects (related in particular to water)."*

- What are the learnings from *in vivo* animal research that can be applied to *in vivo* human assessment?
- What are the hurdles that must be addressed in order to move from *in vitro* human research to *in vivo* human assessment, and how can they be addressed?
- What can bio-analytical tools tell us at present and what are the key gaps in establishing a closer nexus with human health issues, particularly those related to water?
- What has to be done to enable us to use bioassays as a determinant of human health risks associated with drinking water?
- What do we need to do to make bio-analytical tools more useful and acceptable to the water industry and their regulators?

An outline of the program for the workshop is provided in **Appendix 2**.

4. Discussion Outcomes

Over the three days, a consensus position was reached on a number of key issues. There remained differences amongst the attendees on the feasibility (in some cases) and on the extent of work required to establish ultimately a more robust link between the results of bioassays and human health and what that meant for the application of bio-analytical skills in the immediate term.

There was broad agreement on the following positions (related to drinking water):

- *In vitro* tools will rapidly replace *in vivo* bio-analytical assays for reasons of cost, timeliness and ethical issues associated with using live animals.
- Bio-analytical tools referencing the quality of drinking water should not be seen as an alternative to chemical analyses; indeed advances in modern chemical analytical technologies are a key component to developing further the human health links to specific bioanalytical tools.
- Bioassays are valuable in informing biological effects of complex mixtures in addition to individual chemicals; something which no chemical analyses are able to do.
- There is widespread concern as to what a positive *in vitro* biological response actually means in terms of saying something meaningful about the human health implications of the water being tested. This “so what” question is particularly an issue for both regulators and water utility operators in considering greater use of bio-analytical tools in the drinking water sector?
- Bio-analytical tools will not lead to less expensive water quality testing; rather they have the potential of improving the value of information for resources spent.
- There was recognition that there were both qualitative and quantitative steps in achieving the goal of validating the proposition that *in vitro* assays are predictive for health effects. The first step deals with the question of whether a particular bioassay produces a result consistent with that causing the health effect and essentially compares false positive and false negative rates. The second step has to do with the relative *in vitro*/*in vivo* dose-response among chemicals that act through that mechanism/mode of action to produce the adverse effect. This is necessary to relate the response to a level of risk and is complicated by the fact that adverse outcomes are not caused by one mechanism/mode of action. While the group recognised the issues and developed a high-level pathway forward, it left to the Opinion Piece and the future Steering Committee to provide relevant details of the steps for particular bioassays.

There was also agreement that bio-analytical assay technologies will continue to develop:

- There are strong drivers of bio-analytical assays in the broad field of toxicology and human health, of which water is only one part, that will continue to lead to improved tools with improved linkages

to human health issues. Many of these advances will come from related disciplines(e.g. omics, robotics, systems biology, bio-informatics).
- Within the water sector, recycled water for drinking (or potable reuse) offers the most acceptable entry point for water utilities and regulators to embrace bio-analytical tools and, in particular, direct potable reuse.
- Another entry point is the replacement of existing *in vivo* assays for reasons outlined above.

Not only is the technology of bio-assays being driven by advances in related fields but it became clear during the workshop that understanding better the approaches used in other areas of toxicology (e.g. US EPA approach to pesticides) provides a framework for advancing the key issue – linking bio-analytical tools to human health outcomes related to drinking water. A meta-pathway, with key intermediate goals to be achieved, was identified as a possible way forward. A Steering Group was established to advance this approach.

While a possible "road map" was identified to establish better links between the results of specific bio-analytical tools and human health related to water, the workshop participants were under no illusions that this would not occur in the short term. The challenges can be captured as follows:

- Institutional issues
  - Utility nervousness versus regulator enthusiasm versus researcher optimism (see **Appendix 3** for a summary of water utility needs and concerns)
  - Regional/Country differences in regulatory environment
  - Regulators are looking for tools that address concerns of the community and health officals, particularly PPCPs, EDCs and DBPs leading to carcinogencity
  - Which bioassays?
- Economic
  - Value for money
- Scientific/technical
  - AOP determination, tox-kinetics
  - Mixtures
  - Re-formulating human health criteria
- Communication of potential and role of bio-analytical tools and the technologies in simple language.

While these challenges are formidable, there was general agreement that there are some more immediate applications of bio-analytical tools which can provide useful information on the performanve of existing water treatment operations or which can convey increased confidence as to the relative safety of particular water sources and streams. Such uses include:
- comparative benchmarking of water quality from various sources or treatment schemes;
- optimizing treatment options;
- detecting toxic transformation products;
- screening "unknowns"; and
- influencing public perception.

Finally, a potential way forward was outlined which involved:
- a coordinated effort with a focused outcome;
- collaboration both within and outside water sector;
- involvement from the beginning of all relevant stakeholders, in particular recognising the different expectations of these stakeholder groups;
- developing a prospectus outlining the path forward and the end-goal and using this to secure additional buy-in and funding.

5. Workshop Outputs

The AWRCoE did not expect this workshop to be a "one-off' exercise with all issues identified and resolved; rather it saw it as the start of a journey that will lead to very focused research being carried out in appropriate locations to agreed timelines to address the hurdles that **have been** identified at this workshop.

To this end, the AWRCoE has identified a number of outcomes from the workshop (apart from this Summary Report and the notes from the Group Discussion sessions, **Appendix 4** of this report), namely:

- A high level (1-3 pages) opinion piece written for a top journal (e.g. Science) outlining the potential, imperative and possible way forward in terms of the scientific and translational questions in linking bio-analytical tools to human health outcomes associated with drinking water, and how these might be resolved. This will need to contextualize the workshop within other international activities. (Responsibility: F. Leusch. Timing: A skeleton of the paper was discussed at the workshop, draft is due by end of April 2015.)
- A concise assessment written particularly for regulators and utility managers, outlining the potential, challenges and way forward for bio-analytical tools as indicators of health issues in the water space will be generated by D. Begbie by May 2015.
- A *Steering Committee* was established with Fred Leusch as the Convenor and with representatives drawn from attendees at the workshop – refer to Appendix 4. This Committee was tasked with the development of a time-line or program clearly identifying the work required to address the hurdles identified during the workshop. The *Steering Committee* will look to augment its number from other interested parties around the world. One of its aims is to produce a concise prospectus outlining the next steps, written in a style to attract key organizations in water-related research, some of whom attended this workshop, to commit to organizing a follow-up workshop within a realistic time-frame. A tentative second meeting in California was agreed to in early 2016? The following were nominated as the Steering Committee for the next phase of this initiative:

  Fred Leusch (Convenor)
  Beate Escher
  Dick Bull
  Melissa Meeker (WRRF)
  Jeff Mosher (NWRI)
  Bob Kavlock (or an alternate from USEPA)
  Mong Hoo Lim (PUB)
  Andrew Humpage
  Klára Hilscherová
  Michael Denison
  Michael Plewa
  Jeff Fisher (USFDA)
  Representative from OECD
  Representative from the Demeau Project in Europe
  Secretarial support initially from Don Begbie (ARWCoE).

Appendix 1: Details of Workshop Attendees

Name	Organisation	Field of expertise	Origin
Siao Yun Chang	Principal Microbiologist, Water Quality Office, PUB, Singapore	Industry	Singapore
Dick Bull	MoBull Consulting, Washington State, USA	Toxicology	USA
Beate Escher	Professor, Environmental Toxicology, Eberhard Karls University, Tübingen and Department of Cell Toxicology, Helmholtz Centre for Environmental Research, UFZ, Leipzig, Germany	Environ Sci - bio + chem	Germany
Bob Kavlock	Deputy Director Science, Office of Research and Development, USEPA	Molecular toxicology	USA
Michael Denison	Professor, Dept. of Environmental Toxicology, Uni of California, Davis, CA USA	Molec toxicol and bioassays	USA
Klára Hilscherová	Senior Scientist, Research Centre for Toxic Compounds in the Environment (RECETOX), Masaryk Uni, Brno, Czech Republic	Water quality & Mech tox	Czech Republic
Michael Plewa	Emeritus Professor of Genetics University of Illinois, Urbana, Ill USA	Molec toxicol and bioassays	USA
Melissa Meeker	Executive Director, WateReuse Research Foundation (WRRF), USA	Research provider	USA
Jeff Mosher	Exec Director NWRI, California, USA	Research Provider	USA
Eric Miguelino	Research Scientist, State Water Research Control Board, California, USA	Research Scientist / Regulator / Industry	USA
Andrew Humpage	Senior Scientist, Australian Water Quality Centre (AWQC), SA Water, South Australia	Industry + Environ Sci - bio	Local
Fred Leusch	Associate Professor, Smart Water Research Centre, Griffith University, Qld Aust	Environ Sci - bio	Local
Anu Kumar	Principal Research Scientist - Contaminant Biogeochemistry and Environmental Toxicology, CSIRO	Aquatic ecotoxicology	Local
Brian Priestly	Director, Australian Centre for Human Health Risk Assessment (ACHHRA), Monash Uni, Vic Aust	Toxicology	Local
Greg Jackson	Director, Water Program, Health Protection Unit, Dept of Health, Qld Aust	Regulator	Local
Richard Theobald	Manager Water Unit, Department of Health, WA Aust	Regulator	Local
David Halliwell	CEO, Water Research Australia, Vic Aust	Research provider	Local
Matti Lang	Director, National Research Centre for Environmental Toxicology (Entox), Qld Aust	Mechanistic Toxicology	Local
Mark O'Donohue	CEO, AWRCoE, Qld Aust	Research provider	Local
Don Begbie	Program Manager R&D, AWRCoE, Qld Aust	Research provider	Local
Judy Blackbeard	Acting Manager Applied Research, Integrated Planning, Melbourne Water, Vic Aust	RAC/PAC, Industry	Local
Paul Greenfield	Chair, International Water Centre, Qld Aust	RAC/PAC	Local
Ian Law	IBL Solutions, Wellington, NSW Aust	RAC/PAC	Local

Appendix 2: Program Timetable

Day 1: Mon 9 Feb	**Agenda Item**
AM	
8.30am	Welcome, housekeeping and format of the Workshop
8.45am	Setting the scene - background and aim of the workshop.
9.15am	Presentations from each attendee on area of expertise and how they can help realize the aim (1 slide, max 6 bullet points)
10.30am	Morning tea/coffee
11am	Presentations from each attendee - continued
PM	
12.30 – 1.15pm	Lunch
1.15pm	Presentations from each attendee - continued
2.15pm	Can we confidently extrapolate from *in vitro* to *in vivo* ? What have we learnt and what are the gaps ?
2.45pm	Afternoon tea/coffee
3.15pm	*Group Session 1*: What can bio-analytical tools tell us at present and what are the key gaps in establishing a closer nexus with human health issues, particularly those related to water?
4.30pm	Presentation of group discussions followed by general discussion
5.30pm	Wrap up of Day 1
6pm	Dinner
8 – 9pm	*Evening Session*: Overview talk on development of guidelines for use in the water industry – reminder of basics, safety factors, how regulatory standards are produced and how they will be applied. What does the water industry need and how can these be applied?
Day 2: Tues 10 Feb	
AM	
7am	Relaxation - Bushwalk, Swim, Gym
10am	Morning Tea/Coffee
10.30am	Overview talk on what the industry needs in terms of operational tools
11am	*Group Session 2*: What do we need to do to make such tools more useful and acceptable for the water industry and their regulators?
PM	
12pm	Presentation of group discussions followed by general discussion
1pm	Lunch
1.45pm	*Group Session 3*: What has to be done to enable us to use bioassays to determine human health risks associated with drinking water?
2.45pm	Presentation of group discussions followed by general discussion
3.45pm	Afternoon Tea/Coffee
4pm	Concept of the "Miracle Night"... if on waking up one morning we find the problem has been fixed, what would the solution look like? Discuss application: recycled water as highest risk. Characteristics of solution: cost effective, quick, quantitative, readily available standards, no/min ethics approval, sensitive, reliable, <1% false +/-ves, lab skills and facilities needed, not tied to one company by IP, detects a variety of high risk endpoints, complements/replaces chemical analysis, etc.
5pm	Presentation of Group discussions followed by general discussion
6pm	Wrap up of Day 2
7pm	Workshop Dinner

Day 3: Wed 11 Feb	
AM	
8.30am	Facilitated discussion regarding ways forward and the gaps that must be addressed in order to achieve the overall aim, including a timeline – noting that there may be more than one acceptable pathway proposed.
9.45am	Discussion of outline of opinion piece for publication and items to be included. Paper is likely to be statement of why no current solution and identification of the currently insoluble issues which should be tackled by research and perhaps hints on how this could be done.
10.30am	Formation of Steering Committee together with agreement on its 'brief'
11.30pm	Wrap up and thank you to attendees
12.00pm	Lunch
12.45pm	Board Bus for Sydney airport & hotels

Appendix 3: What is needed to make bioanalytical tools more useful and acceptable for the water industry and their regulators?

A list of factors that would make bioanalytical tools more attractive to the Water Industry and their Regulators was presented and discussed at the workshop, as follows:

- Very little sample preparation would be needed
- Robust transformed competent human cell line assays that are stable
- A test that is repeatable and can survive inter laboratory trials
- Sensitive
- Almost no false positives and false negatives
- Not too expensive… ideally around $50-100 a test?
- Not so complicated that we need specialist lab staff
- Built in replicates
- Easy to interpret the result … not something that is subjective
- Something that measures a concentration continuum
- Something smart that will indicate what chemical class is causing the problem
- Something that we can link to a human health outcome
- Something that we can explain to the public
- Something our regulators will accept
- And perhaps in 10 years time make it on-line?

Appendix 4: Human Health Workshop Outputs - Group Discussion Notes

Hurdles

- Trigger values leading to operational
- Why
- Regulatory baggage
- Community baggage
- Cost vs benefits achieved
- What does a +ve and /or a –ve result mean
- Time to get familiar with the techniques
- False negatives
- Mixture toxicity
- Mode of Action
    - *in vivo* vs *in vitro*
- Triggers

Group Session 1

What can bioanalytical tools tell us at present and what are the key gaps in establishing a closer nexus with human health issues, particularly those related to water?

Group A

1. ***What do Bioassays tell us at present?***
    - Give an indication of biological activity (mode of action) in a water sample
    - Measure of mixture chemicals
    - Benchmark water samples
        - Through treatment processes
        - From different water sources
        - In different locations
    - Identify generation of toxic transformation products
    - Proof of active research
    - Public perception

2. ***What are the key items or approaches to narrow the "gaps"?***
    - Chronic low dose not addressed
    - Integrating analytical biological and chemistry – generating competent water samples
    - Experience and application of bioassays for water quality assessment
    - Develop new cell based bioassays which help inform cell- and tissue-specific toxicity as well as on activation of apoptotic pathways
        - Improved bioassays
        - Suite (battery) of tests
    - Correlation of operational parameters
    - Safety of the water

Group B

1. *What do Bioassays tell us at present?*

Refer to Bob Kavlock's presentation on the USEPA approach (ToxCast Program) - to *"assess potential human risks posed by exposures to environmental agents over a broad range of doses and compounds and to … use this information in quantitative human health risk assessment"* - to predict human toxicity for a wide range of chemicals.

- ToxCast Phases I and II already tested has over 1000 chemicals with approx 600 *in vitro* assays for approx 1100 endpoints. ToxCast Phase III will test a further 1000 chemicals with approx 100 *in vitro* assays and endpoints. Some 8200 chemicals will be tested under the Tox21 program.
- Automated, high-throughput screening (HTS) assays.
- Provides a Response Curve showing the concentration of chemical that generates AC50 response.
- Have AC50 for over 700 *in vitro* assays.
- Reverse toxicokinetics (TK) using hepatocytes and blood serum can determine the rate of hepatic metabolism (removal) and the serum aggregation (or binding) of the chemicals.
- The biological availability of the parent compounds and the exposure in the environment (usage) enables us to determine the oral equivalent dose needed to be consumed to give an AC50 response.
- Can plot the AC50 values and the distribution of oral equivalent (dose) values for each of the chemicals. Whisker plots of upper and lower limits enable us to determine the margin of exposure and whether the chemical is of concern or not.
- This can lead to the informed testing of chemicals for endocrine activity and a range of Adverse Outcome Pathways (AOP).
- ToxCast data are available online http://actor.epa.gov/actor/faces/ToxMiner/Home.jsp

2. *What are the key items or approaches to narrow the "gaps"?*

Assumptions

- Parent chemical is active and giving the bioanalytical response, not the by-products.
- *In vitro* pathway is equivalent to the *in vivo* pathway.
- Bio-analytical assays cover all the important pathways.

Critical Tox21 Issues (source: Bob Kavlock's presentation)

- Cells don't get disease
- Not all compounds can be screened in HTS
- Incorporation of metabolic capabilities
- Interactions between different cell types
- Range of human variability
- Extrapolation from acute to chronic exposure conditions
- Interpretation of effective *in vitro* concentrations

Gaps

- *In vitro* pathway reliability reflects *in vivo*
- Extrapolation from single present chemical to mixture of chemicals (additive impact)
- Better characterisation of potential source waters
- Lack of integration (or understanding) of role of metabolism – is the response from the chemical or the metabolites.

Group C

1. ***What do Bioassays tell us at present?***
    - Biological response, linked to adverse health effects
    - Depends on bioanalytical methods
    - Some groups are relatively predictive of effects
        - Dioxin bioassays high correlation with TEQs of instrumental analysis based on mammalian technology
        - Ames test potential for mutagenic activity – assumed predictive for human health accepted
        - Endocrine Disruption (ED) – estrogen receptor (ER) / androgen (AR) / thyroid receptor (TR)
            - Positives -> will have biological response in humans with appropriate dose
            - Toxicity not predictable (potency, concentration, persistence)
            - How run assays?
        - Oxidative stress and adaptive stress
        - Predictive of biological response in humans, but not of toxicity

2. ***What are the key items or approaches to narrow the "gaps"?***
    - Biological response, linked to adverse health effects
    - Bioassays directly relevant to human health effects
        - What are the "right" bioassays?
        - Most bioassays more characterise mechanism or identify the chemical

Group D

1. ***What do Bioassays tell us at present?***
    - Depends on bioanalytical methods
    - Useful tool for process control and treatment steps
    - Screening tools for prioritisation
    - As detection tools for a whole range of compounds
        - Of concern/not of concern
        - Eliminates those chemicals not to look at
        - Relative to guideline values
    - Pinpointing certain modes of action, receptors, etc
        - Can screen for these, BUT……
    - Has been a lot of development , but still many gaps

2. ***What are the key items or approaches to narrow the "gaps"?***
    - No real trigger points now

    - Have some rules of thumb
- Acceptance of bio-analytical tools even for lower level outcomes
- Step-wise
    - Consensus of key adverse outcome pathways
    - Consensus on appropriate bio-analytical tools to assess those pathways
- Smaller group of tools
    - Standardisation
    - Validation for human health cell based assays
- Tools to take out into the field or on-line
- Not sure we fully understand false +ve and –ve.
- Extrapolation from concentration/doses producing effects in bioassays to dose/exposure response in whole organisms (animal and human)
- Lack of consistent metrics relating bio-analytical tools to human health
    - Toxic equivalents
- Not a comprehensive set of tools
    - Human cell lines/tissues
    - Human relevant models
    - *In vitro*

Group Session 2

What do we need to do to make such tools more useful and acceptable for the water industry and their regulators?

Extract from an introductory presentation by Dr Judy Blackbeard, Melbourne Water ("Santa's List")

So what would the water industry like to have in bioassays……..

Dear Santa,

Please would you bring us bioassays which have the below characteristics:

- Very little sample preparation would be needed
- Robust, transformed, competent human cell line assays that are stable
- A test that is repeatable and can survive inter-laboratory trials
- Sensitive
- Almost no false positives and false negatives
- Not too expensive … can you manage around the $50-100 a test?
- Not so complicated that we need specialist lab staff
- Built in replicates
- Easy to interpret the result …. Not something that is a bit subjective
- Something that measures a concentration continuum
- Something smart that will indicate what chemical class is causing the problem
- Something that we can link to a human health outcomes
- Something that we can explain to the public
- Something our regulators will accept.
- And perhaps in 10 years time you could make it on-line?

Group A

- Bioassays need to establish the **safety of water**
- May not need full human health to be mapped
- Applications already
    - Public perception
    - Treatment monitoring and validation
- Need to understand how to deal with water quality without a +ve result
    - Implementation framework can vary without variation
    - USA – leave out of specific regulations
    - Aust – trigger for further investment
- Identified needs for drinking water and recycled water
    - See Judy's "Santa's List"
- Role for scientists to demonstrate

Group B

- Increasing nervousness from catchment to product water
    - Catchment /Reservoirs – source characterisation
    - Water Treatment
        - Treatment optimisation
        - Hazard identification (chemical identification)
    - Product water (Advanced Water Treatment)
        - Robust/reliable bioassays with clear interpretations

Group C

Increasing Difficulty ↑				
	Hard			Real-time monitoring of DPR for pathways or chemicals
	Easy			
		Short		Long

Time →

Group D

- Would like a limited suite of bioassay tests that have known links to chemical classes found in water (with a broad range of chemicals picked up)
- Rules for interpreting outcomes of results

- Describe what the outcomes are
- Understanding what +ve tests mean
- Establish a baseline across the supply network to understand the status quo (which is considered safe)
- Need good info on individual chemicals to help interpret above +ve results
- Tests need to be rapid to detect issues quickly to enable response
- Judy's "Santa's List"

Group Plenary

- Staged implementation
    - Low hanging fruit
    - For what? – context
    - Value for money
- Bioassays to identify …. (what are the priorities of the regulators?)
- How do bioassays help
    - characterise source waters
    - design and operation of treatment plants
    - with product assurance (safe water)
- How do bio-analytical tools add to what we do now?
- Need a focus
- Public relations is the main driver
- Evaluation of benefit – chemical vs chem / bio-analytical
- Have additional cost, but offset by additional safety assurance
    - Identify the additional benefits.

Group Session 3

What has to be done to enable us to use bioassays to determine human health risks associated with drinking water?

Group 1 steps/boxes (Genotoxicity)

Stage 1 - Candidate suite of bioassays

- DNA damage (available now)
- Micronuclei (available now)
- Cell cycle (available now)
- Optimising transformation assay (available now, but needs additional work, short term – 3 weeks)
- Omics (future – 5-10 years)

Stage 2 - *In vitro* -> *in vivo* dose

- PBPK modelling (now *in vivo*)
- PBPK (future *in vitro* – 5-10 years)

Stage 3

- Ensure the endpoints being measured are relevant for humans
- Comes out of the Omics work
- Is this endpoint the relevant one?, eg promotion vs genotoxicity vs hormonal

Stage 4 - Mixtures

- Bioassay directed fractionation (TIE) (can do now)
- ToF–MS to identify what is there - the main chemical causing the reaction (can do now)
- Omic signature (modelling)

Stage 5 - Extrapolation to human health guidelines

- Have 6 ADWG chemical guidelines at present.

Group 2 steps/boxes (Adaptive Stress)

A generalised model could be:

- Continually assess new/better assays
- Identify assays for initial screening across the wide range of chemicals likely to be present
    - Improving assays for safety of water
- Focus on a group of bioassays that address a number of pathways to cover most/all chemicals
    - Smart, strategic
    - Aimed at detection
    - Start with the USEPA ToxCast database
- Assess mode of action (or adverse outcome pathways) in terms of potency and stability/longevity of the chemical
    - Chemicals likely to be causing the response
    - Supplement with chemical analysis
- Extrapolation of potency data from bio-analytical tools to assess human exposure and risk
    - Quantitative data
- Setting guideline values, but based on what criteria
    - Concentration of the chemical?
    - Trigger dose level?
    - Other?

Group 3 steps/boxes (EDC -> Estrogenicity)

Basecamp

- *in vitro* – chemical linkages are 80-90% there
- Reference compounds (EEQ)

Determine Trigger Values

- Eg 0.2-3 mg/l EEQ

Guideline

- Develop operational response
- Traffic light approach

Steps to get there

- Map out Adverse Outcome Pathways
    - Include sensitive life stages
    - x + E2 + BPA (single cpr)
- Quantitative
- Identify which assays are good enough

- Toxicokinetic (TK) pathways
    - Mixtures and distribution
    - Response curves

In vitro --> *In vivo* human

- Get the Human Health data

Quick doodle from 3 scientists groups on Tuesday night

Some initial thoughts were presented on how one might design an experiment to:

1. Extrapolate from *in vitro* target concentration to *in vivo* dose

- Establish distribution of F_{ub} and C_{liv}
- Continue approach outlined in Welmore (Tox Sci 2012)
- Can we make it work for mixtures?
- USEPA unable to study all compounds of relevance to water industry
- Is bioavailability a problem? – is FPE a problem?
- What is the right liver model?
    - primary hepatocytes?
    - cell lines?
- How to model renal clearance?
    - Is there an *in vitro* model?
    - Are there transporter assays that could inform renal clearance?
- Is the effect of hormone binding different from binding to albumin?
- Select data rich chemicals of relevance to water – estimate with human/animal doses cf LOAEL and NOAEL
- 2 scales:
    - v1 – use USEPA's validated approach and apply to selected chemicals of relevance to water – 6-12 months
    - v2 – complete approach from scratch – 12-36 months

2. Determine what endpoints are relevant in water quality assessment

3. Extrapolate from a cell-based response to an adverse effect; and

4. Extrapolate TK and AOP designed for single compounds to mixtures in water

- Apply concentration addition as reference model for mixtures with many components as they occur in complex samples
- Focus on bioanalytical characterisation on complex mixtures in source waters
- How to determine relevant endpoints to include?
- Then calculate contribution of pollutants to the detected biological activities
    - Knowns vs Unknowns
    - Agonists vs antagonists
- For TK, can we use 95% percentile value?

Paul's Summation Report

Additional thoughts to capture:

- Regulators are looking for tools that address concerns of the community and health officials, particularly PPCPs, EDCs and DBPs leading to carcogenicity.
- What are we missing that straight chemical analyses can't tell us?
- Constant nagging fear of the unknowns.

Steering Committee Formation

The following were nominated to form a Steering Committee tasked with the development of a time-line or program clearly identifying the work required to address the hurdles identified during the workshop and producing a concise prospectus outlining the next steps, written in a style to attract key organizations in water-related research.

Members

Fred Leusch

Beate Escher

Dick Bull

Melissa Meeker / Jeff Mosher

Bob Kavlock (or an alternate from USEPA)

Mong Hoo Lim, nominee from PUB

Andrew Humpage

Klára Hilscherová

Michael Denison

Michael Plewa

Representative from OECD

Representative from the Demeau Project in Europe

Nominee with specialist skills in TK, eg Jeff Fisher (USFDA)

Fred Leusch was nominated as the Convenor of this group to get the planning started, with secretarial support from Don Begbie.

Timeframe

WRRF meeting in May 2015 will consider future priorities for research. The prospectus should be available for consideration at that time.

The prospectus needs to be available for briefing and consideration at other key research meetings.

The next workshop/meeting event should tie in with another international water event to make it easier for people to travel and attend.

Appendix 5: Glossary of Terms

Linking Bio-analytical Tools to Human Health Issues Related to Water Glossary

Adverse Outcome Pathway	A conceptual framework to link the molecular initiating event (MIE) to the eventual adverse outcome in a whole organism.
Bioanalytical tool	Analytical tools that use a biological detection mechanism to detect contaminants. Historically applied to molecular and enzyme-based techniques (e.g., ELISA), but more recently meant to include cell-based techniques as well.
Bioassay	A test of the potency of a compound or sample on molecules, cells or living organisms used as a detection mechanism.
Estrogenicity	A type of endocrine (hormonal) activity associated with natural "female" sex hormones such estradiol and estrone. Estrogenicity in water can cause feminisation of fish.
General cytotoxicity	A basic toxic response at the cellular level that leads to cell death.
Genotoxicity	Physical damage or change to DNA structure, function or altered DNA repair processes which can lead to mutation or genomic instability and may result in uncontrolled cellular proliferation and eventually cancer.
In-vitro	Using molecules, enzymes, organelles or cells (but not whole organisms).
In-vivo	Using whole (living) organisms.
Molecular Initiating Event	Within the adverse outcome pathways (AOP), the molecular initiating event (MIE) is the first interaction between a toxic chemical and a biological organism that starts the adverse response.
Mutagenicity	Introduction of a heritable change in the DNA sequence ("mutation") or damage to chromosomes. This is a type of genotoxicity, and can lead to uncontrolled cellular proliferation, cancer and other adverse biological effects.
Receptor-mediated effect	A type of specific toxicity, where the toxicant produces a biological response via a receptor molecule (for example, binding to the progesterone receptor to induce a progestagenic response).
Reporter gene assay	A bioassay that has been genetically engineered with an easily detectable reporter protein (such as a fluorescent protein or the enzymes β-galactosidase, luciferase or β-lactamase) linked to a response element specific to the toxic pathway of interest. Induction of the reporter gene (and production of the reporter protein) is proportional to the biological activity of the tested sample.
Toxic equivalent	A way of expressing a bioassay response in chemical language, a toxic equivalent is the concentration of a reference compound required to produce a specific level of response in a bioassay (for example, estradiol equivalent EEQ).