Application of Bioassays for Monitoring Chemicals in Water from a Human Health Standpoint

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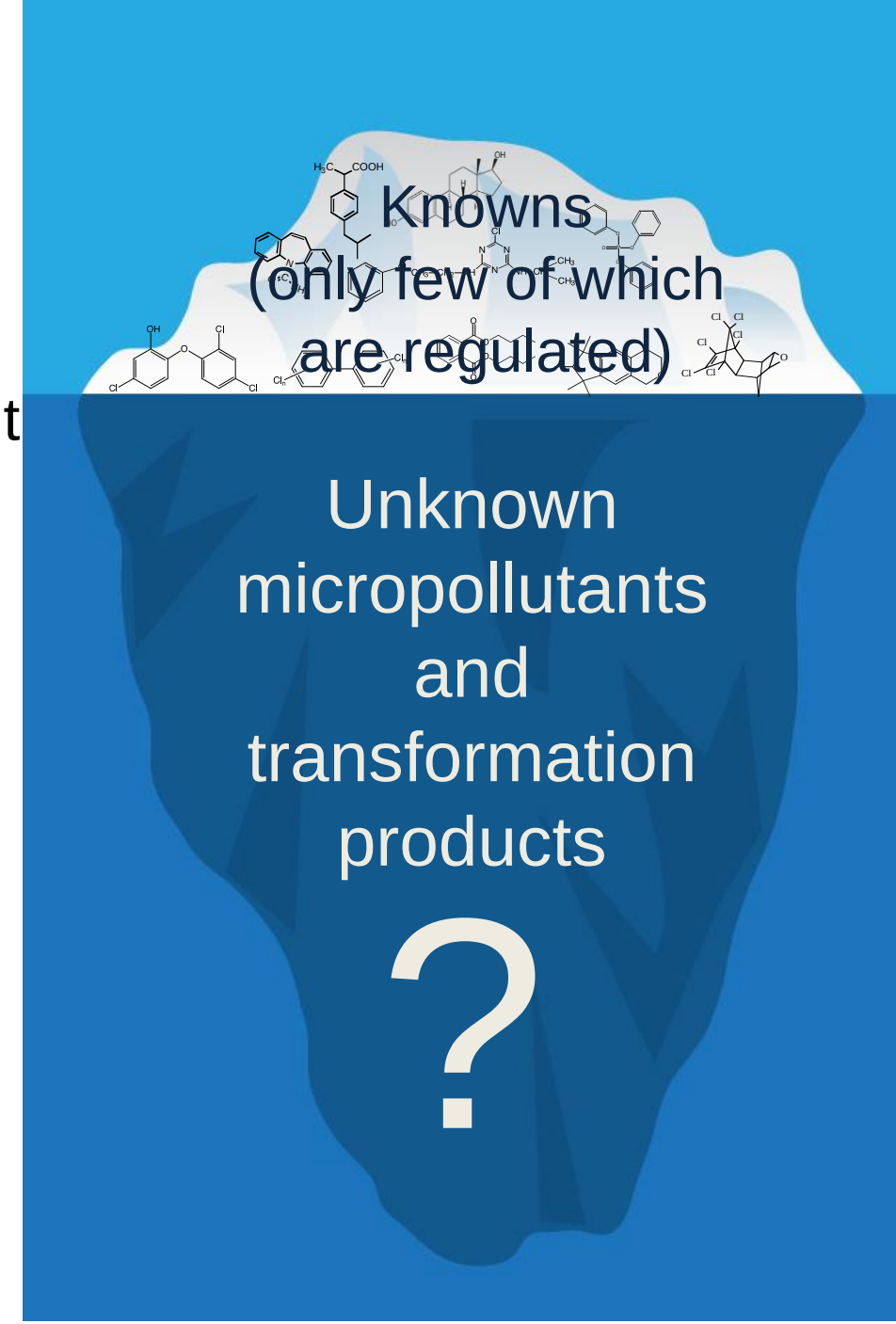
Outline

- Tox 21 and Chemical Measurements
- Adverse Outcome Pathway concept and use to development Bioanalytical assays
- Application of Bioanalytical assays to water assessment
  - Results of 3 International Workshops
  - Short term vs. Long term goals
  - Examples
    - Outcome from recommendations of State Water Board (Anderson et al. 2010)

Chemicals in the Environment

- many chemicals
  - > 87,261,915 ORGANIC AND INORGANIC SUBSTANCES TO DATE CAS #
  - > 100000 in the environment
  - low concentrations
- mixture effects
  - “Something from nothing”
- transformation products



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Feature

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Environmental Designer Drugs: When Transformation May Not Eliminate Risk

David M. Cwiertny,*[,†] Shane A. Snyder,[‡,§] Daniel Schlenk,[∥] and Edward P. Kolodziej*[,⊥,#]

Article

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Occurrence of Halogenated Transformation Products of Selected Pharmaceuticals and Personal Care Products in Secondary and Tertiary Treated Wastewaters from Southern California

Daryl N. Bulloch,[†] Eric D. Nelson,*[,‡] Steve A. Carr,[‡] Chris R. Wissman,[‡] Jeffrey L. Armstrong,[§] Daniel Schlenk,[∥] and Cynthia K. Larive*[,†]

Environmental Science & Technology

Article

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Analytical and Biological Characterization of Halogenated Gemfibrozil Produced through Chlorination of Wastewater

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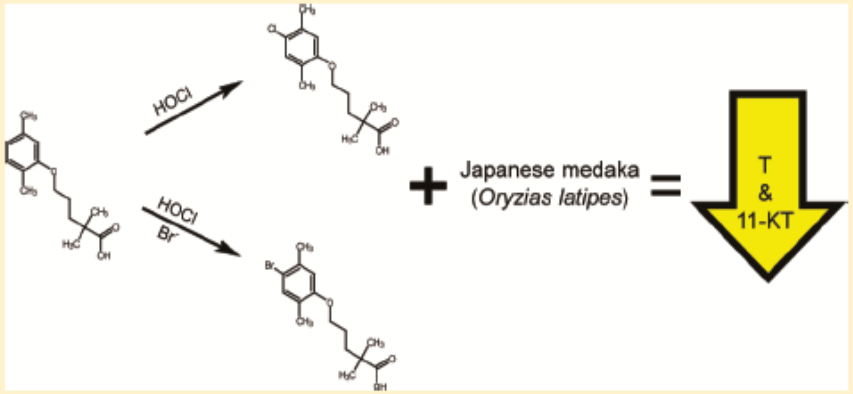
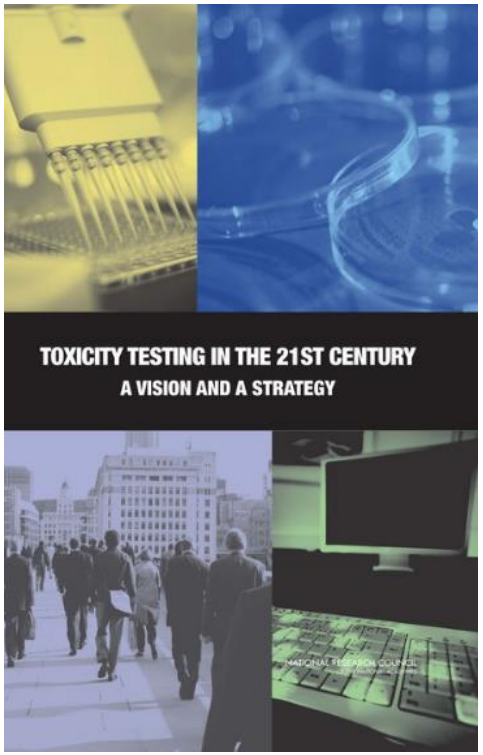
Supporting Information

ABSTRACT: The cholesterol-lowering pharmaceutical gemfibrozil is a relevant environmental contaminant because of its frequency of detection in U.S. wastewaters at concentrations which have been shown to disrupt endocrine function in aquatic species. The treatment of gemfibrozil solutions with sodium hypochlorite yielded a 4′-chlorinated gemfibrozil analog (chlorogemfibrozil). In the presence of bromide ion, as is often encountered in municipal wastewater, hypobromous acid generated through a halogen exchange reaction produced an additional 4′-brominated gemfibrozil product (bromogemfibrozil). Standards of chloro- and bromogemfibrozil were synthe-



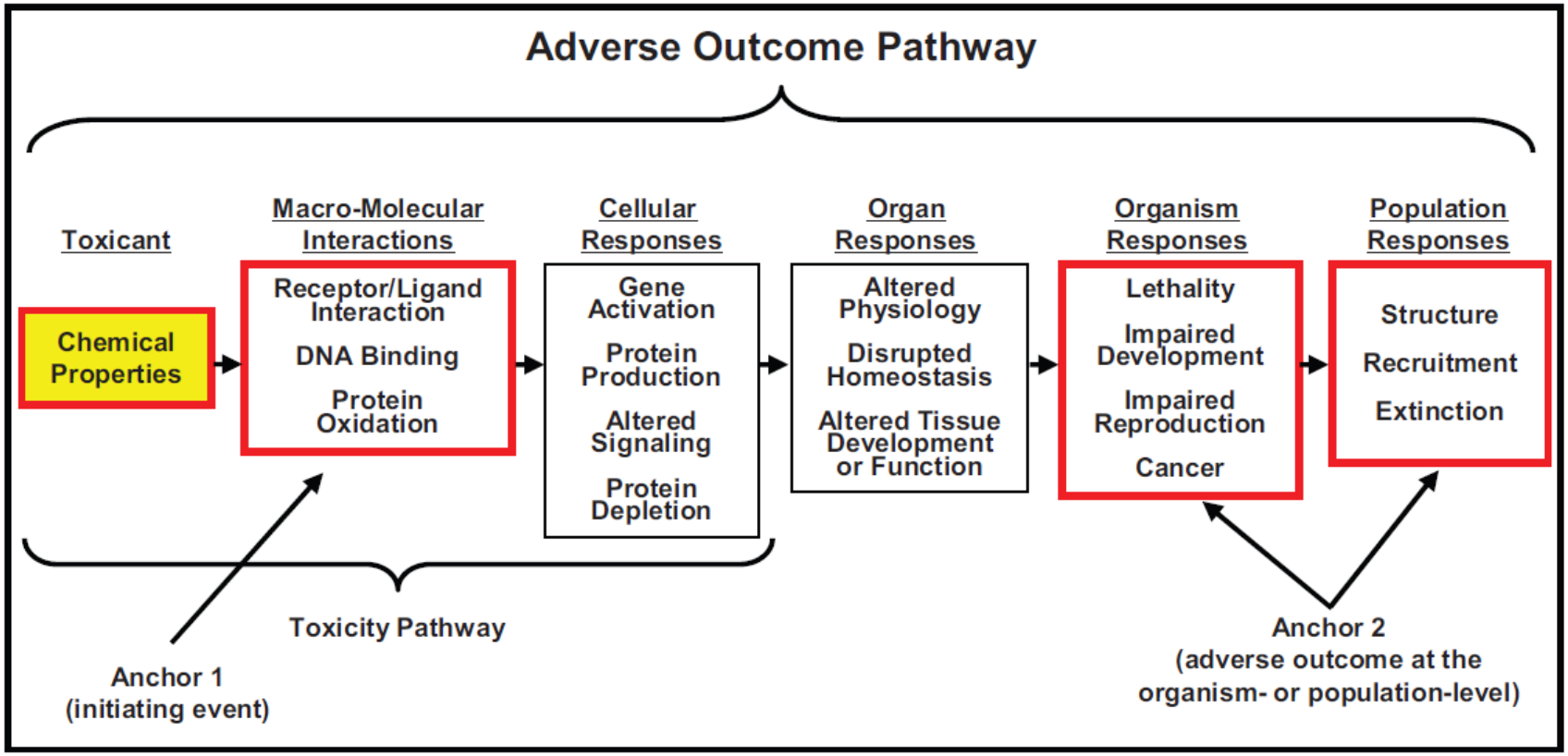
Toxicity testing the 21st century

- NRC 2007 vision for the future of toxicity testing → Tox21 initiative and ToxCast
- Over the past decade, significant effort to map molecular and cellular initiating events that lead to adverse health outcomes
- Has led to great advances in our understanding of mechanistic toxicology



Adverse outcome pathway

- Link between initiating event at molecular/cellular level and whole organism response



Ankley *et al* (2008) *Environ Toxicol Chem* 29(3): 730-741

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To request author access to the wiki, please follow the instructions here: http://www.saaop.org/AccessPage.html.

Wiki Down Time! The AOP-Wiki will be undergoing a major upgrade beginning November 27, 2016. Starting on this date, the wiki will be closed for editing until the upgrade is complete. Users will have constant access to all information in read-only mode. No new user accounts can be created during the down time. The upgrade is anticipated to last approximately one week, but it may be completed sooner. At the latest, read/write access to the new version of the AOP-Wiki will be restored by December 4, 2016.

Welcome to the Collaborative Adverse Outcome Pathway Wiki (AOP-Wiki)

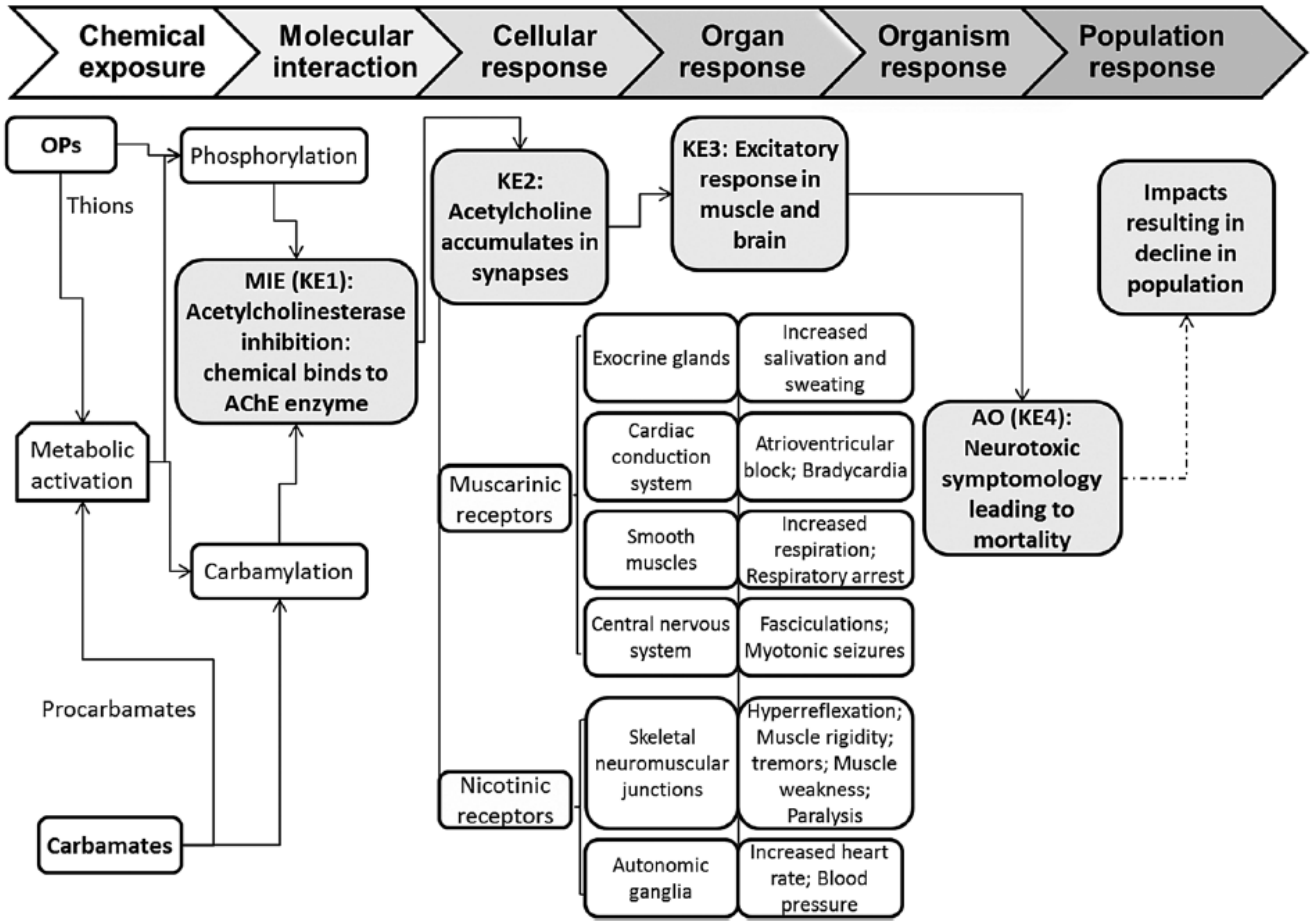
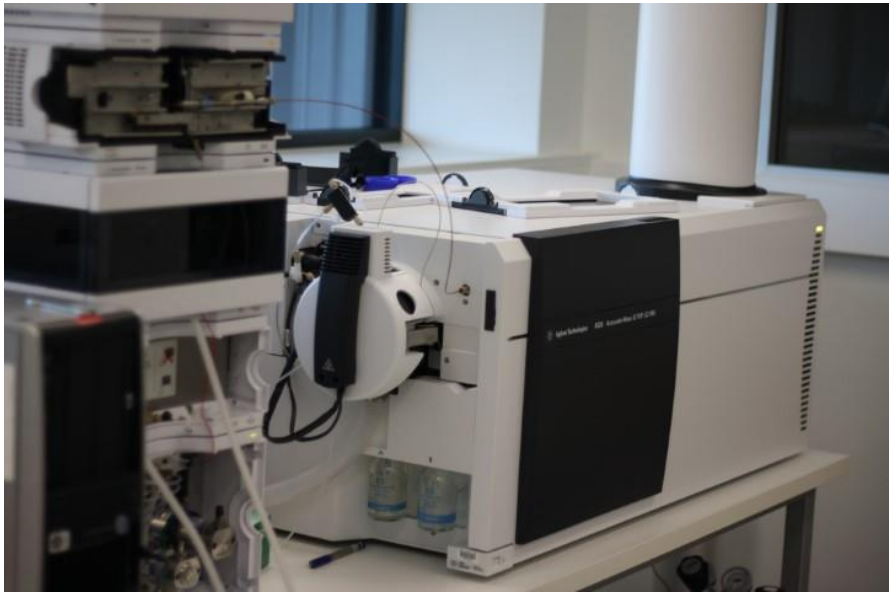
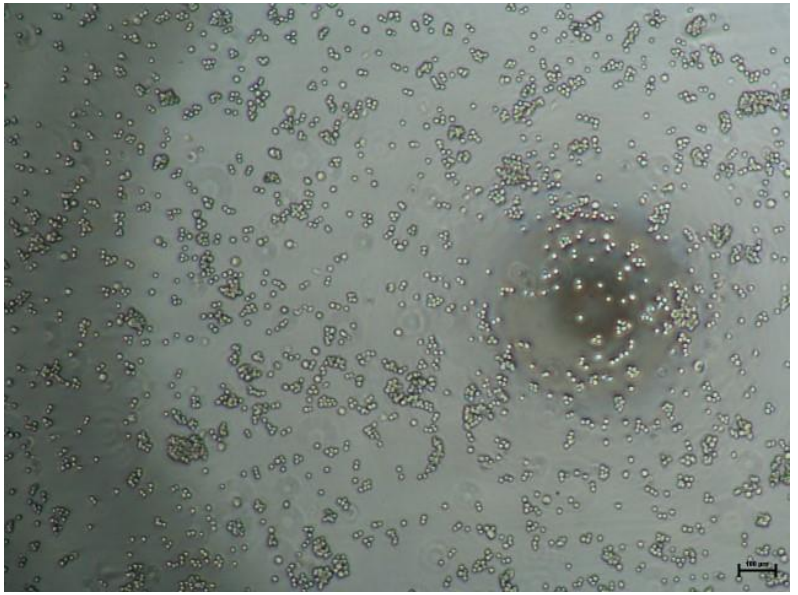


Figure 2. Proposed adverse outcome pathway (AOP) for acetylcholinesterase (AChE) inhibition leading to acute lethality. Key events (KEs) are in bold letters, starting with the inhibition of AChE, the molecular initiating event (MIE), and proceeding to the accumulation of acetylcholine in cholinergic synapses (KE2), excitatory response in tissues (KE3), and lethality, the adverse outcome (AO) of regulatory significance. Dashed arrow indicates a plausible connection for which sufficient direct empirical evidence was not available.

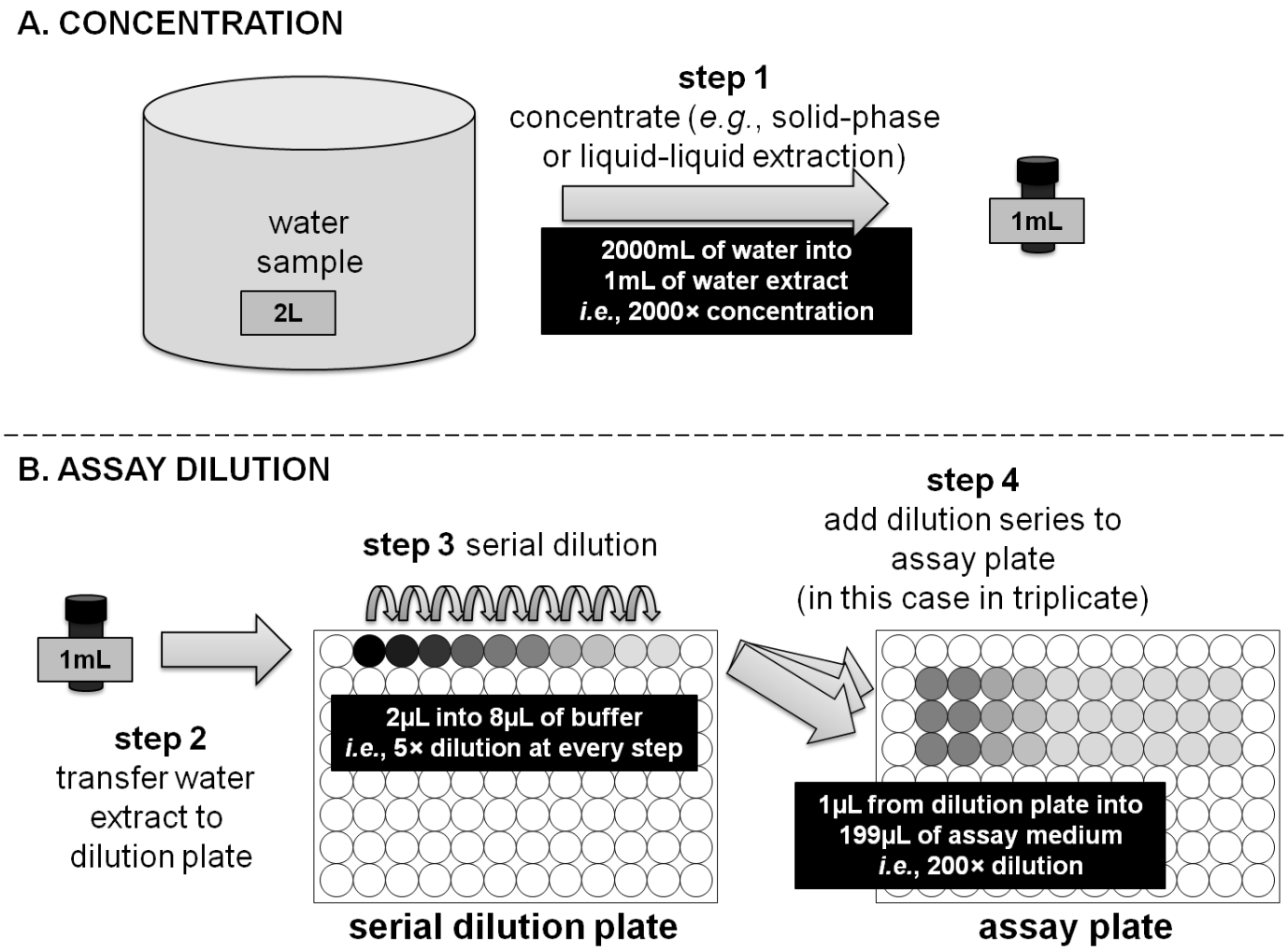
Bioanalytical tools

- Bioanalytical tools = *in vitro* bioassays
- Effects testing at the cellular level to predict effects at the organism level (*in vivo*)





Applying Bioanalytical Tools to a water sample



Escher and Leusch (2012) Bioanalytical tools in water quality assessment. IWA Publ

Application of Bioanalytical Tools to recycled water

- mutagenicity and genotoxicity

Scheme	Endpoints (assays)
1960-present: Dan Region Sewage Reclamation Project, Israel	Mutagenicity (Ames test)
1962-present: Montebello Forebay Groundwater Recharge Project, California, USA	Mutagenicity (Ames test) Carcinogenicity (Mammalian cell transformation assay)
1975-present: Orange County Water Factory 21 and Groundwater Replenishment System, California, USA	Mutagenicity (Ames test)
1980-1982: Potomac Estuary Experimental Water Treatment Plant, Virgina, USA	Mutagenicity (Ames test) Carcinogenicity (Mammalian cell transformation assay)
1987-1989: Tampa Water Resource Recovery Project, Florida, USA	Mutagenicity (Ames test) Genotoxicity (Sister chromatid exchange test)
1981-1999: San Diego Total Resources Recovery Project, California, USA	Mutagenicity (Ames test) Genotoxicity (Micronucleus test, 6-thioguanine resistance assay) Carcinogenicity (Mammalian cell transformation assay)
1989-present: Tucson Reclaimed Water System, Arizona, USA	Mutagenicity (Ames test)

Leusch and Snyder (2015) *Environ Sci: Wat Res Technol* 1: 606-621

Bioanalytical Assay Workshops

- Aleura, Australia February, 2015
- Huntington Beach, Ca February, 2016
- Singapore July, 2016

Examples of Bioassays Research: Near-Term Projects

Example #1: Selection of Endpoints to Monitor CECs in Recycled Water (SWB Project #12)

- Identify and determining the usefulness of bioassays in detecting known and unexpected CECs in waters produced from various treatment technologies.

Example #2: Assess the Universe of Bioassays and Universe of Chemicals of Interest (SWB Project #16)

- Assess possible bioassays (including health endpoints of concern). Develop a list of specific endpoints that is meaningful.

Example #3: Develop Standard Operation Procedures (SOPs) for Sample Preparation and QA/QC for Bioassays (SWB Project #17)

- Address standardization with appropriate quality assurance/quality control (QA/QC) to ensure that cell assays are run correctly and results are accurate.

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Contents lists available at ScienceDirect

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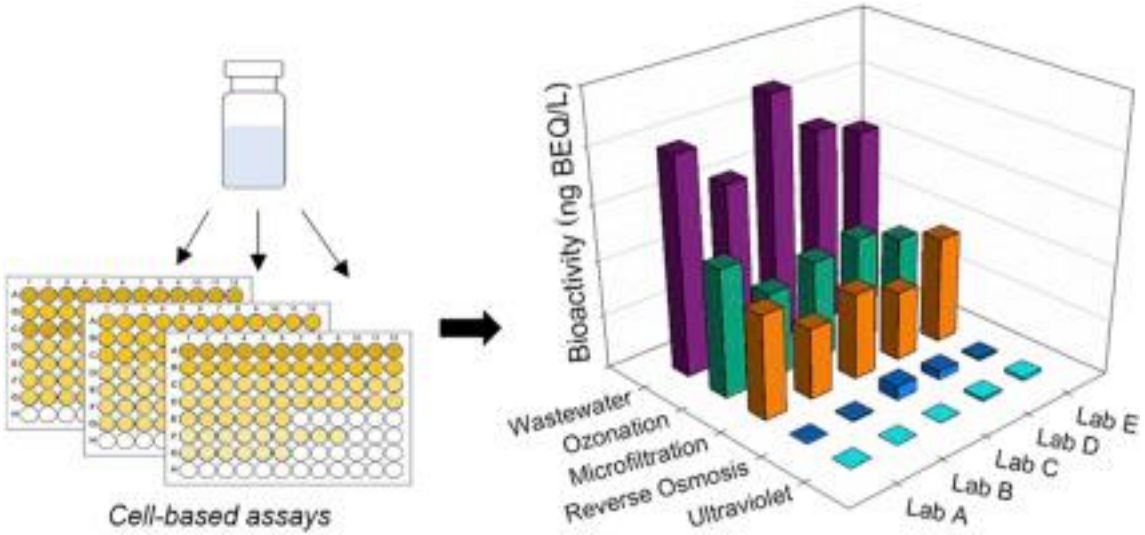
journal homepage: www.elsevier.com/locate/watres

Interlaboratory comparison of *in vitro* bioassays for screening of endocrine active chemicals in recycled water

Alvine C. Mehinto [a, *], Ai Jia [b], Shane A. Snyder [b], B. Sumith Jayasinghe [c], Nancy D. Denslow [c], Jordan Crago [d], Daniel Schlenk [d], Christopher Menzie [e], Sandy D. Westerheide [e], Frederic D.L. Leusch [f], Keith A. Maruya [a]



STUDY OBJECTIVES & GOALS

- Identify most promising bioassays
  - Which endpoints are most relevant?
- Optimize & evaluate selected bioassays
  - Can they be standardized for robust, cost-effective monitoring?
- Provide interpretive guidance & framework for results
  - Do bioassays get us closer to effects or simply screen for exposure?
- Transfer technology to stakeholders
  - Deliver protocols for robust measurement and tips on best use of information

PRIORITY ENDPOINTS

ENDPOINT	INDICATOR CEC	REF TOXICANT	CANDIDATE BIOASSAY(S)
estrogenicity	17β-estradiol	17β-estradiol	estrogen receptor (ER)
androgenicity	dihydrotestosterone	testosterone & derivatives	androgen receptor (AR)
progesterone activity	levonorgestrel	trenbolone levonorgestrel	progesterone receptor (PR)
thyroid hormone activity	triclosan	T3	Thyroid receptor (TR)
genotoxicity	NDMA	benzo[a]pyrene	Ames II, UMU, p53 reporter
aryl hydrocarbon reactivity	caffeine	TCDD; PCB126; benzo[a]pyrene	Aryl hydrocarbon receptor (AhR)
CEC-specific response	gemfibrozil	gemfibrozil	PPAR gamma

Anderson et al. 2010

Round Robin and Water Treatment Assessments

Table 2
Interlaboratory comparison of calibration performance for GeneBLAzer® estrogen receptor-alpha (ER), glucocorticoid receptor (GR) and progesterone receptor (PR) bioassays. Results deemed not acceptable were excluded. The calibration performance of the GeneBLAzer® androgen receptor (AR) bioassay was not evaluated as no bioactivity was observed in any of the samples analyzed.

	Lab A	Lab B	Lab C	Lab D	Lab E
ER (referenced to 17β-estradiol)					
Hill slope	1.6	1.6	1.4	1.5	1.0
R^2	0.99	0.99	0.99	0.97	0.99
Log EC_{50} (M)	−9.8	−9.9	−9.6	−10.2	−9.6
LOD	11	7	10	4	7
Data accepted	yes	yes	yes	no	yes
GR (referenced to dexamethasone)					
Hill slope	2.3	1.9	2.5	2.4	2.2
R^2	0.99	0.99	0.99	0.98	1.00
Log EC_{50} (M)	−8.6	−8.5	−8.4	−8.7	−8.5
LOD	5	4	2	9	4
Data accepted	yes	yes	yes	yes	yes
PR (referenced to levonorgestrel)					
Hill slope	2.1	1.8	1.4	1.5	1.3
R^2	0.99	1.00	0.96	0.98	0.99
Log EC_{50} (M)	−9.1	−9.9	−9.5	−9.9	−9.5
LOD	3	2	3	2	8
Data accepted	no	yes	yes	yes	yes

LOD – limit of detection, expressed as a percent effect concentration (EC) relative to the referenced chemical.

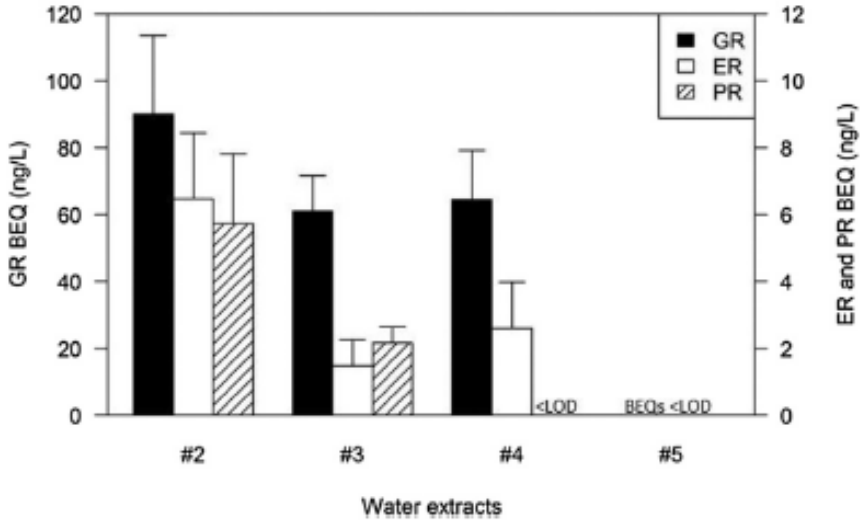


Fig. 1. Mean bioassay equivalent concentrations (BEQ) for ER (expressed as ng 17β-estradiol/L ± SD), GR (expressed as ng dexamethasone/L ± SD) and PR (expressed as ng levonorgestrel/L ± SD) for samples from an operational recycled water facility (Plant 1). Samples 2, 3, 4 and 5 correspond to Plant 1 influent, OZ – ozonation, MF – microfiltration and RO – reverse osmosis, respectively. The median limit of detection (LOD) was <1.7 ng/L for ER-BEQ, <52 ng/L for GR-BEQ, and <1.4 ng/L for PR-BEQ.

Examples of Bioassays Research: Near-Term Projects

Example #4: Benchmark Bioassay Techniques across and within Treatment Systems, and Compare to Other Water Sources (SWB Project #18)

- Develop an appropriate approach and test plan to (1) assess biological activity within potable reuse treatment systems, and (2) benchmark advanced treatment recycled water with other water sources.

Example #5: Interpretation Framework for Cell Bioassay Results (SWB Project #13)

- Interpret and extrapolate cell bioassay results to inform the public regarding the quality of recycled water.
- Linkage studies (i.e., analysis of the relationship between in vitro cell bioassay responses and biological/health effects) is needed to establish thresholds of concern.

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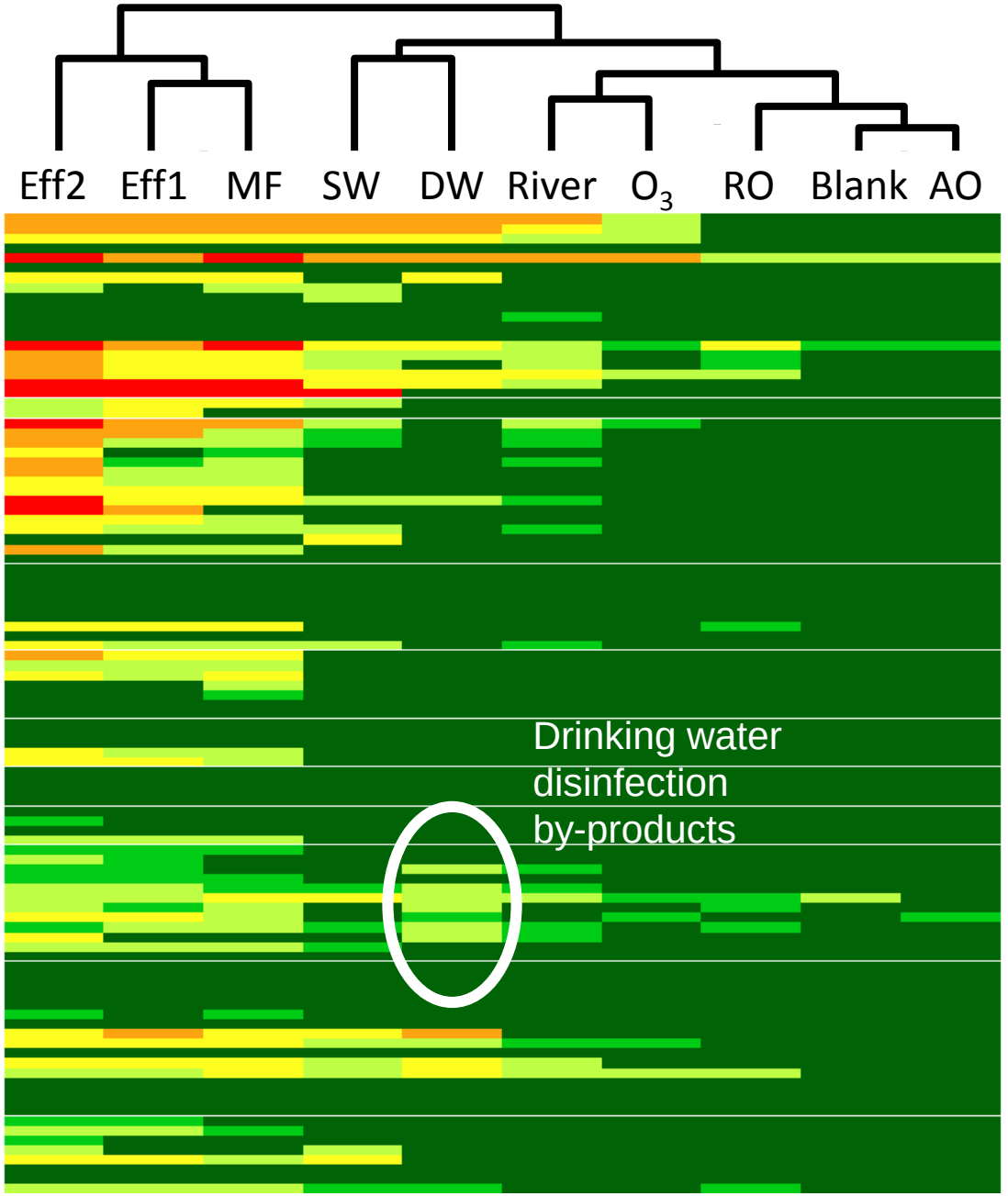
Benchmarking Organic Micropollutants in Wastewater, Recycled Water and Drinking Water with In Vitro Bioassays

Beate I. Escher,*[,†] Mayumi Allinson,[‡,§] Rolf Altenburger,[∥] Peter A. Bain,[⊥] Patrick Balaguer,[#] Wibke Busch,[∥] Jordan Crago,[▽] Nancy D. Denslow,[○] Elke Dopp,[◆] Klara Hilscherova,[¶] Andrew R. Humpage,[☆] Anu Kumar,[⊥] Marina Grimaldi,[#] B. Sumith Jayasinghe,[○] Barbora Jarosova,[¶] Ai Jia,[▬] Sergei Makarov,[⊡] Keith A. Maruya,[Φ] Alex Medvedev,[⊡] Alvine C. Mehinto,[Φ] Jamie E. Mendez,[✲] Anita Poulsen,[†,●] Erik Prochazka,[⋈] Jessica Richard,[◆] Andrea Schifferli,[⊗] Daniel Schlenk,[▽] Stefan Scholz,[∥] Fujio Shiraishi,[§] Shane Snyder,[▬] Guanyong Su,[∞] Janet Y. M. Tang,[†] Bart van der Burg,[◓] Sander C. van der Linden,[◓] Inge Werner,[⊗] Sandy D. Westerheide,[✲] Chris K. C. Wong,[■] Min Yang,[@] Bonnie H. Y. Yeung,[■] Xiaowei Zhang,[∞] and Frederic D. L. Leusch[⋈]

- 103 bioassays
- 10 water types with varied treatment
- 21 laboratories from 9 countries

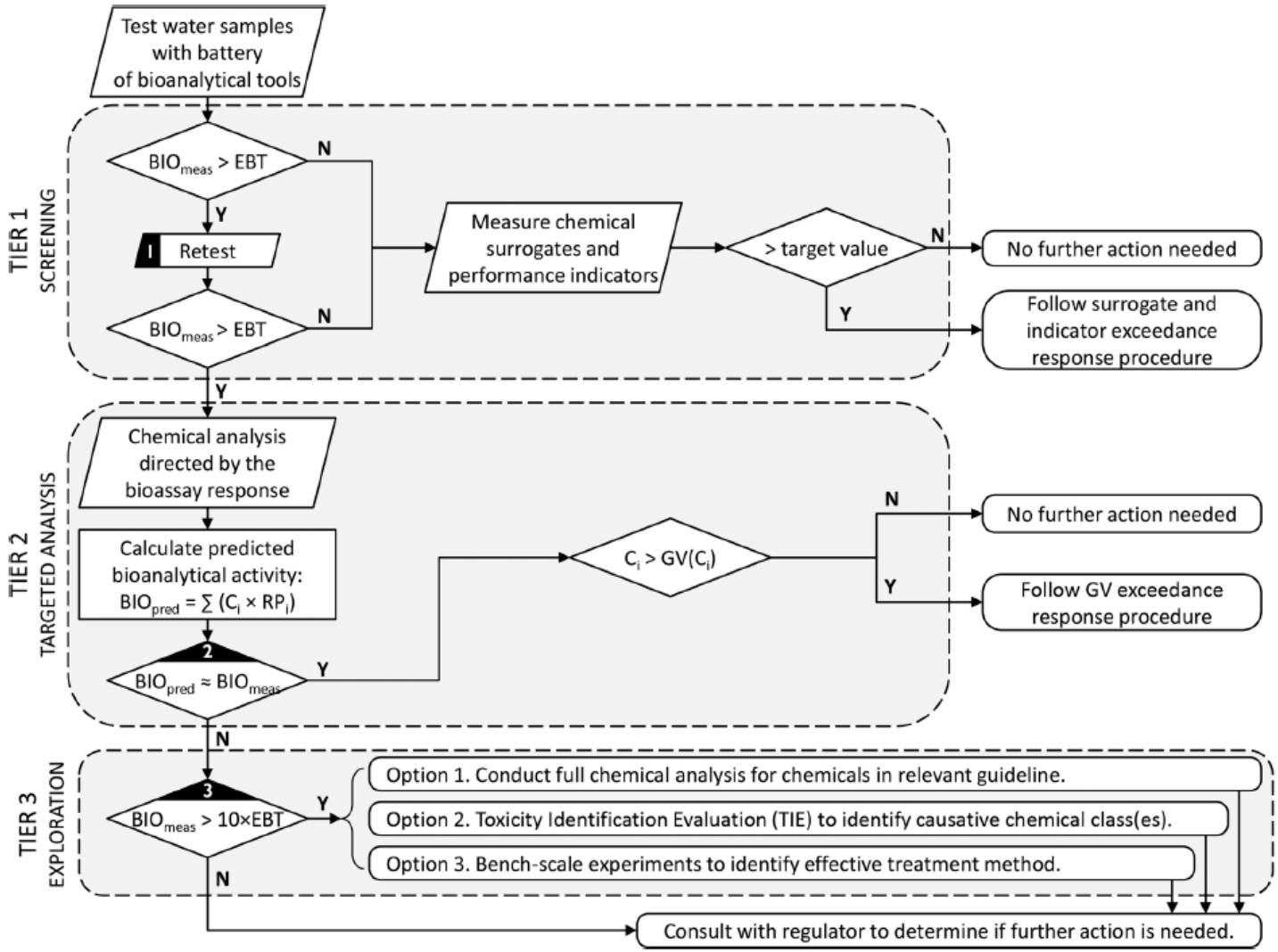
Bioassay responses in assorted water types with varied treatments



Cell viability:
(cells representative of system response)

Escher et al (2014) *ES&T* 48(3):1940-1956

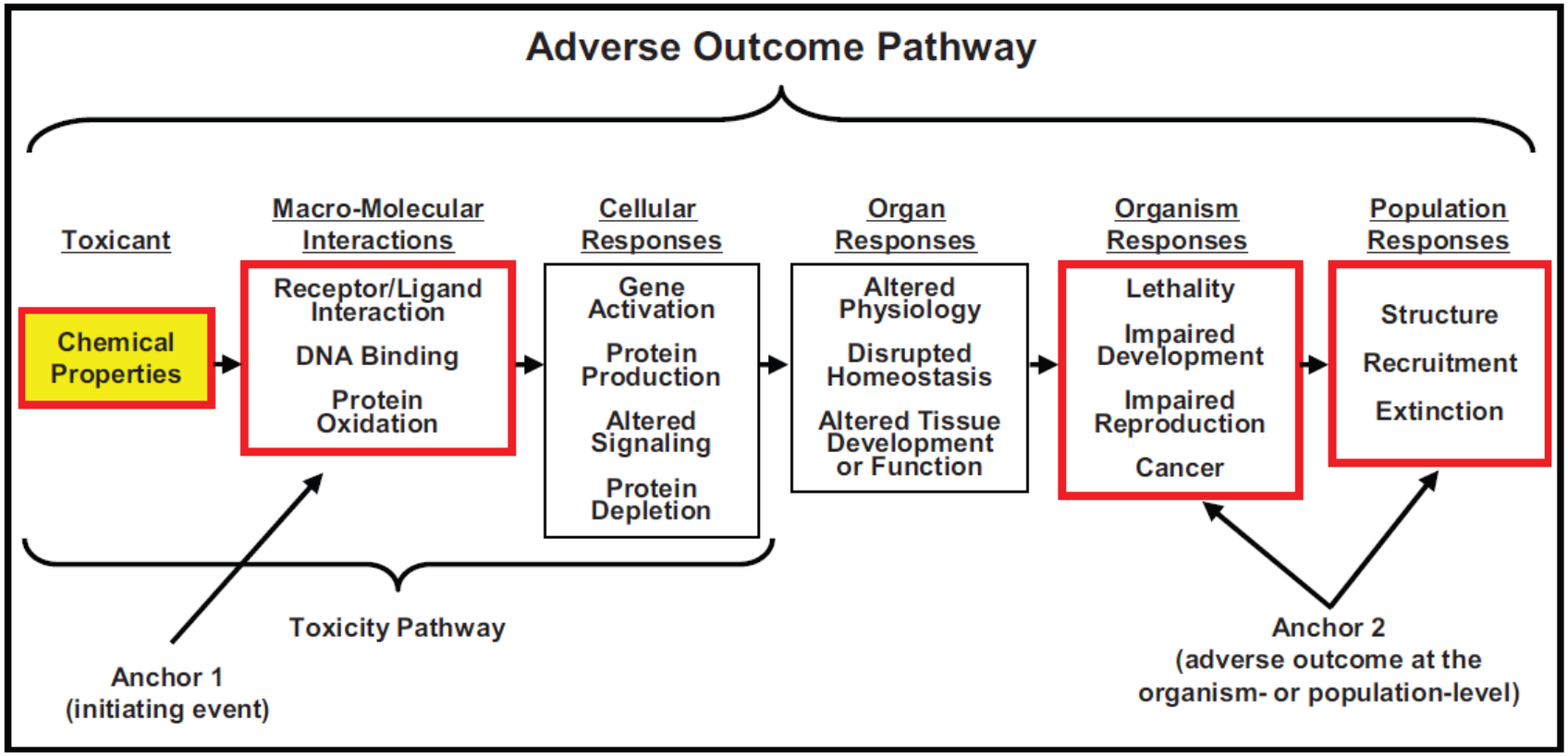
How do we use bioassay results?



Leusch and Snyder 2015 *Environ Sci: Wat Res Technol* 1: 606-621

Adverse outcome pathway

- Link between initiating event at molecular/cellular level and whole organism response



Ankley *et al* (2008) *Environ Toxicol Chem* 29(3): 730-741

Environ. Sci. Technol. **2001**, *35*, 3620−3625

Identification and Quantification of Estrogen Receptor Agonists in Wastewater Effluents

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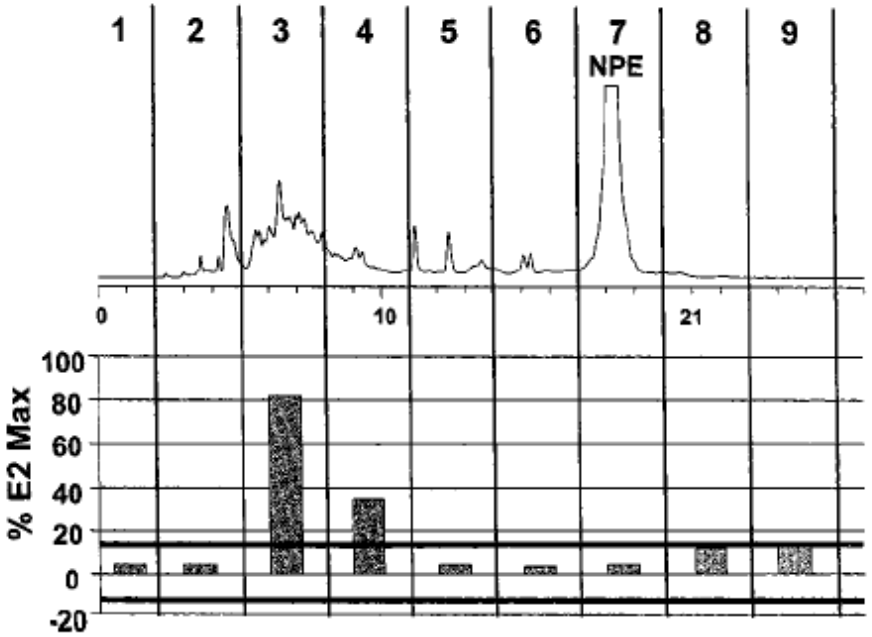

FIGURE 2. Fine fractionation of LV Wash, Lake Mead (April), F3 extract using RP-HPLC with fluorescence detection followed by luciferase induction in the MVLN cell bioassay (estrogen responsive) by the corresponding fractions. Response magnitude presented as percentage of the average maximum response observed for a 1000 pM 17β-estradiol standard (%-E2-max). Horizontal lines represent $\pm$ 3 SD from the mean solvent control response (set to 0%-E2-max).

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Balancing the Budget: Accounting for Glucocorticoid Bioactivity and Fate during Water Treatment

Ai Jia, Shimin Wu, Kevin D. Daniels, and Shane A. Snyder*

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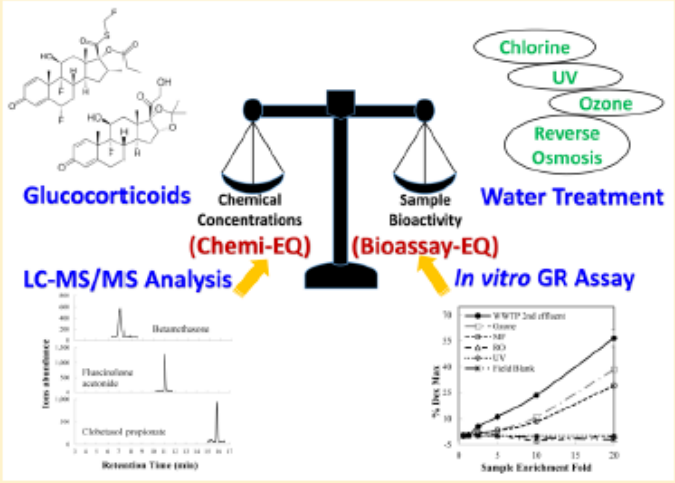
Supporting Information

ABSTRACT: Numerous studies have identified the presence and bioactivity of glucocorticoid receptor (GR) active substances in water; however, the identification and activity-balance of GR compounds remained elusive. This study determined the occurrence and attenuation of GR bioactivity and closed the balance by determining those substances responsible. The observed in vitro GR activity ranged from 39 to 155 ng dexamethasone-equivalent/L (ng Dex-EQ/L) in the secondary effluents of four wastewater treatment plants. Monochromatic ultraviolet light of 80 mJ/cm^2 disinfection dose was efficient for GR activity photolysis, whereas chlorination could not appreciably attenuate the observed GR activity. Ozonation was effective only at relatively high dose (ozone/TOC 1:1). Microfiltration membranes were not efficient for GR activity attenuation; however, reverse osmosis removed GR activity to levels below the limits of detection. A high-sensitivity liquid chromatography with tandem mass spectrometry (LC-MS/MS) method was



Conclusions

- Too many chemicals to measure
- Bioanalytical tools offer short term benefits for water screening (not threshold derivation for risk)
- Bioanalytical tools with discrete functional relevance to adverse outcomes offer the ability to identify potential unknowns

Thank you

- California State Water Resources Control Board
- Southern California Coastal Water Research Project
  - Keith Maruya, Alvina Mehinto, Steve Weisberg
  - Nancy Denslow
  - Shane Snyder
  - Sandy Westerheide
  - Fred Leusch
  - Beate Escher
  - Jeff Mosher