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Re: **Evaluation of the feasibility of developing uniform water recycling criteria for direct potable reuse**

In 2010, the California State Water Resources Control Board ("Water Board") convened a Science Advisory Panel (SAP)to develop recommendations regarding monitoring of constituents of emerging concern (or CECs) in recycled water applications across the State. We, members of that SAP, are submitting these formal comments about the recent report entitled "**Evaluation of the feasibility of developing uniform water recycling criteria for direct potable reuse**" because we are concerned about both the factual basis and the conclusions reached in Chapter 5, titled Application of Bioanalytical Tools to Water Analyses.

Bioassays will improve, not replace current monitoring methods. While the report as a whole is well done, we believe Chapter 5 fails to recognize the necessity of incorporating cell-line assays into the routine testing protocols for recycled water. There is simply no way that chemical-by-chemical monitoring can keep pace with the discovery of new chemicals, either manufactured intentionally or produced unintentionally as by-products of e.g., recycled water treatment practices. We agree with the report's assertion that work remains to be done before these assays are ready for routine regulatory application, and further that the best use of the tools is to complement analytical chemistry, particularly in a non-targeted approach to help identify known and unknown agents. However, our vision is that as our knowledge of Adverse Outcome Pathways broadens, and more tools become available that allow comparison with guidelines already in place, the bioanalytical measurements will become an essential tool health protection and the State should focus on their development as rapidly as possible.

SAP recommendations for bioanalytical tools were misrepresented.
Further, we feel the report significantly misinterpreted recommendations made by the SAP in reaching their conclusions. We interpret Chapter 5 as suggesting that the recommendations of the 2010 SAP were to utilize bioanalytical tools through the Adverse Outcome Pathway paradigm to set guidelines for drinking water safety. While the members of the SAP were (and remain) staunch supporters of the Adverse Outcome Pathway and Toxicology in the 21st Century recommendations for chemical safety testing, we did not believe this process could be used to set *in vivo* water safety guidelines. Rather, we adhere to the paradigm of using Adverse Outcome Pathways to identify specific molecular responses that can be used as tools to evaluate recycled water for mixtures of known and unknown compounds. Adverse endpoints of cancer or reproductive dysfunction can be inferred by measuring activation of one or more molecular initiating events, and it is this data linkage of events that warrants the use of bioanalytical tools. In contrast to what was proposed in Chapter 5, we propose use of the Pathway to move "backwards" to evaluate exposure rather than "forward" to set a guideline. The benefits of this strategy to water assessment is the identification of linkages between in vivo responses and receptor-driven molecular initiating events that can be used in conjunction with preset guidelines for screening water.

The Expert Panel is highly critical of three publications from published literature; however, a wealth of additional literature is readily available. In 1975, the World Health Organization published a report entitled, "Health effects relating to direct and indirect re-use of waste water for human consumption" (WHO, 1975). This report by the WHO advocated the use of bioassays, including in vitro techniques, for the monitoring of recycled water. In fact, a review published in 2015 provides numerous examples of the application of bioassays specifically applied to recycled water for over 50 years (Leusch and Snyder, 2015). In addition, while the Expert Panel does provide a citation for WateReuse Research Foundation Project 10-07, it seems the Expert Panel may not have connected that the manuscripts in peer-reviewed literature are highly limited by word count restrictions. Some of the criticisms raised by the Expert Panel are well explained with the WRRF 10-07 report. Regardless, the Expert Panel report could have benefited by a more comprehensive review of widely available literature on this topic (Escher and Leusch, 2012). The Expert Panel focuses primarily on the use of in vitro bioassays to detect estrogens in UK studies from the 1990s, but could have benefited by considering more recent success stories such as identification of highly potent glucocorticoid steroids in recycled water (Jia et al., 2016). In addition, the Expert Panel did not consider that the US EPA already uses in vitro bioassay data. For instance, US EPA method 4435 "Screening for Dioxin-Like Activity in Soils and Sediments Using the CALUX Bioassay and TEQ Determinations" is an approved method already (http://www3.epa.gov/epawaste/hazard/testmethods/sw846/pdfs/4435.pdf).

The case for specific, receptor-based screening bioassays. As was stated in the SAP report (Anderson et al. 2010), identification of ligands that are specific for a receptor-mediated response can be quantified via biological equivalence values (i.e. BEQs), i.e. concentrations that can be interpreted in the same way one interprets individual chemical concentrations, or more appropriately summed concentrations of chemicals that

collectively activate a specific receptor. In this capacity, a guideline for the ligand is already present. For example, the SAP report led to selection of receptor-based bioanalytical assays that targeted CECs for which risk-based estimates of compounds indicated a potential hazard (Mehinto et al. 2015). The risk-based assessments already had guidelines for that ligand in water. It was our recommendation that if the BEQ of that molecular initiating event exceeded that guideline (a risk/hazard based process), then additional testing in a tiered approach could be initiated either to confirm the response or to potentially identify the causative agent. In no way did our report (Anderson et al. 2010) suggest that the bioanalytical response could be used in a refined risk assessment strategy to set a guideline for water quality, whether it be for a potable water supply or for a receiving water application. Moreover, since the proposed tools were selected contingent on their ability to be quantified via a BEQ response, and with a documented, credible linkage to an adverse outcome based on an existing standard or guideline, "reverse toxicokinetics" to characterize exposure is not necessary. If the goal of managers is to assess the potential hazards of recycled water, then use of these tools under "worst-case" scenario exposure (assuming 100% exposure) represents the most conservative exposure assessment approach. If molecular event bioactivation is not detected under the most conservative approach, then no further testing is needed (see associated figure 1).

Non target analyses. The Expert Panel seems to condone the use of non-targeted analyses (NTAs), yet barely mentions that many of the same limitations of bioassays also apply to NTA. For instance, the Expert Panel specifically addresses the issues of false positives/negatives, extraction efficiency, and limitations of mass spectrometric techniques. In fact, most laboratories would advocate for the use of matrix spikes of cellular bioassay positive controls within the waters to be evaluated. This allows for some certainty that the a well-known agonist is actually recovered from the sample preparation methods used. NTA is also generally limited to those substances that can be extracted or purged from water samples. For instance, it is extremely unlikely that NDMA, perchlorate, or 1,4-dioxane would have been detected using the most widely applied NTA procedures. While the SAP also agreed that NTA is a valuable and necessary tool, we believe it is highly complementary to bioassay analyses. As a recent case in point, medium pressure UV advanced oxidation has been shown to result in genotoxic byproducts, yet NTA has not yet been successful to identify those substances causing the reproducibly observed mutagenicity (Martijn and Kruithof, 2012; Kolkman et al., 2015; Martijn et al., 2016). Thus, we maintain that bioassays as part of routine monitoring programs provide valuable information regarding mixture toxicity that is otherwise not possible using analytical methods currently employed for water quality monitoring.

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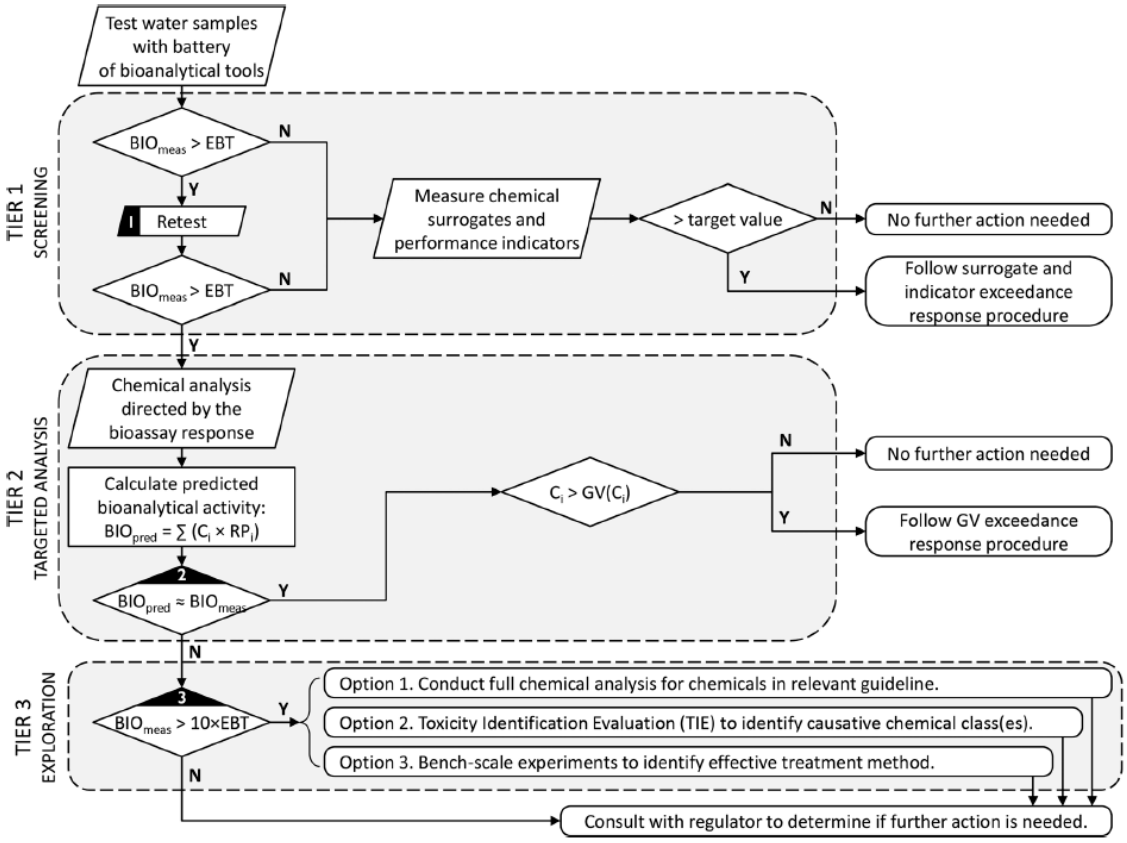

Figure 1. Proposed framework for using bioanalytical tools in water screening.
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