SOUTHERN CALIFORNIA COASTAL WATER RESEARCH PROJECT
A Public Agency for Environmental Research

May 14, 2018

Tessa Fojut
Division of Water Quality
State Water Resources Control Board
Sacramento, CA

Re: Information request for bioscreening endpoints (email dated 2/2/18); revised 5/14/18

Dear Tessa:

On behalf of the Panel, and in response to your questions regarding the ER-α and AhR bioscreening endpoints recommended for the next round of data collection associated with potable reuse applications as described in the draft final report, please see below for the Panel's responses.

The Panel appreciates the importance of providing clear and actionable levels for bioscreening, and thus appreciates the nature of your request. With that in mind, the Panel reiterates that the purpose of bioscreening is not to replace or compete with current analytical methods, e.g. those that target regulated dioxin-like chemicals like TCDD, but rather to complement existing monitoring by providing a screening level safeguard for unmonitored, unknown or new agonists that may pose human health risks. The collection of bioscreening data as part of the next data collection phase of the State Water Board Recycled Water Policy (RWP) is meant to accomplish this objective, while also paving a path for identifying substances that are potentially problematic using the framework for addressing unknowns in Chapter 7 of the draft report. At this time, the Panel also stresses that their recommendations for bioscreening should not be misconstrued as suitable for incorporation into the RWP as a regulatory limit for compliance but rather, as noted above, for screening level analysis only. For example, the term "action level" in the current draft report could lead to misinterpretation of the Panel's intent, even though the steps for interpreting bioscreening results are clearly delineated in the draft report (see Section 7.5.3) as adaptive, flexible, non-binding and non-regulatory. Should objections be raised regarding this terminology, the Panel is happy to consider alternatives, such as "bioscreening level", which is employed in the Panel's responses that follow.

Q1. *What is the reference agonist of choice for AhR?*

Response: **The Panel recommends 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) as the reference agonist of choice for AhR**. The Panel's selection of TCDD provides the desired sensitivity for screening of unknown AhR agonists as recommended by the Panel (see also response to Q2). The Panel recognizes that TCDD is already regulated and does not fit within their definition of a CEC. However, this point is irrelevant when applying the AhR assay in bioscreening mode, as the selection of TCDD is simply a reference point for quantitation.

Q2. *What are the appropriate action levels (ALs) for ER-α and AhR?*

Response: **The Panel recommends a bioscreening level of 3.5 ng E2/L for the ER-α endpoint (E2 = 17β-estradiol).** As outlined in Textbox 7.1 of the draft report, the Panel identified a PNEC of 3.5 ng/L for 17α-

ethinylestradiol (EE2), another potent estrogenic compound and a synthetic analog of E2 that can occur at trace levels in treated municipal wastewater effluent, from the threshold values compiled in the updated report (listed in Table D.3, Appendix D). Recent studies have shown that E2 has been used far more frequently and successfully as a reference agonist in ER bioscreening studies, and is the preferred reference agonist for the ER-α screening bioassay. In these studies, the *in vitro* potency of EE2 has been shown to be within a factor of 2 to that of E2, thus, the Panel believes an initial bioscreening level of 3.5 ng E2/L is appropriate for Phase I data collection. Although a lower threshold of 0.9 ng E2/L is also listed Table D.3, the Panel unanimously feels that this latter value is overly conservative and, also noting the 0.5 ng E2/L method detection limit, is not appropriate at this time as a practical screening level for the ER-α assay.

For AhR, the Panel does not recommend a health-based bioscreening level at this time. Instead, **the Panel recommends using the method reporting level (MRL) of 0.5 ng TCDD/L for the AhR assay, as currently recommended in the draft report and which the Panel has confirmed can be readily achieved.** Existing studies suggest residual AhR activity that cannot be explained by current monitoring of known AhR agonists can be present in recycled water. The Panel believes water samples with activity above the MRL represent good candidates for additional evaluation to identify the agonists causing such residual activity. As more information becomes available, a health-based bioscreening *trigger* level that parallels the approach outlined for ER-α in Textbox 7.1 can be developed for AhR.

Q3. Should labs be able to use different reference agonists, or stick to specified ones?

Response: To maximize comparability of results during this initial round of bioscreening data collection, the Panel recommends that participating labs select E2 and TCDD as their reference toxicants.

I trust the above responses will prove helpful in updating the Recycled Water Policy amendment for monitoring of CECs. Please contact me if you have any further questions.

Sincerely yours,

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