Recycled Water Policy Amendment
Response to Peer Review Comments

November 20, 2018

TABLE OF CONTENTS

may exert combined biological activity in, e.g., an additive manner, but are individually present at levels that are below analytical reporting levels, trigger levels, or detection levels.

These receptor bioassays are toxicologically relevant because transactivation of these receptors can initiate a sequence of causally linked events that can lead to an adverse health effect, referred to as an adverse outcome pathway. The ER-α bioassay is capable of detecting estrogenic compounds, including hormones and industrial chemicals e.g., alkylphenols, some of which have been detected in wastewater and are potentially present in monitoring samples. The AhR bioassay is capable of detecting a variety of compounds with planar configurations that are ubiquitous in the environment, including polyaromatic hydrocarbons, dioxins, polychlorinated biphenyls, and polychlorinated dibenzofurans. The ER-α and AhR bioassays are well developed and have adequate sensitivities for detecting bioactive substances in water. When used in conjunction with targeted and nontargeted chemical analysis, the bioassays permit bioactivity- directed identification of previously unknown constituents of toxicological interest.

The ER-α and AhR bioassays have been widely used in water quality assessment, and are among the CEC indicator bioassays that have been advanced for routine water quality screening applications to monitor for the presence of active chemicals. Monitoring recycled water used for groundwater recharge and reservoir augmentation with ER-α and AhR in vitro bioassays should provide robust and reliable information regarding the aggregate agonist and antagonist bioactivity of CECs in the sample affecting the aforementioned receptors. The ER-α and AhR in vitro bioassays could also prove useful for evaluating treatment processes and identifying performance issues in the treatment train. In the absence of complementary targeted and non- targeted chemistry analysis, the in vitro bioassays will not provide qualitative or quantitative information regarding specific known and unknown CECs present in the samples.

In summary, I concur with Conclusion 5 that monitoring recycled water used for groundwater recharge and reservoir augmentation with the ER-α in vitro bioassay and the AhR in vitro bioassay will provide robust and reliable information regarding the aggregate bioactivity of CECs (including unknown CECs) that affect these receptors, and thereby information regarding potential unknown CECs.

CONCLUSION 5, RESPONSE TO COMMENT 1 (JE)

No response is needed. The reviewer is in support of Conclusion 5.

CONCLUSION 5, COMMENT 2 (LC)

I agree that functional bioassay such as estrogen-receptor-alpha (ER-a) and aryl hydrocarbon receptor (AhR) in vitro bioassays in recycled water will be useful to screen out possible unknown CEC that have these properties. It is almost impossible to measure all the possible CECs in the recycled water. The 2018 Science Advisory Panel discussed non-targeted analytical chemistry tools as promising tools that with further development may be useful in analyzing recycled water. When there is detection of bioactivity in an in vitro bioassay, there is some knowledge of what types of chemicals may be causing the bioactivity, which then limits the types of non-targeted analyses that would need to be employed to potentially identify the CECs in a sample. This is an efficient way to monitor the cumulative effects of multiple chemicals that affect human health by these two well documented receptor pathways.

In addition to these two assays, I recommend the State Board to consider adopting other powerful non-specific bioassays such as DNA microarray-based transcriptome analysis to study

more than the two specific biological pathways or effects. For example, antibiotic resistance transmission that has been identified as an important concern can be addressed by such bioanalytical assays. The 2018 Science Advisory Panel also stated that future research on bioanalytical screening tools would include developing a broader suite of in vitro bioassays to be used as screening indicators of a wider array of potential impacts to humans and wildlife. However, before such bioanalytical tools can be routinely used for regulatory purposes, continued validations by comparing results from the chemical analyses are needed. Moreover, before results of such assays can be adopted for regulatory purposes, validation with existing protocols are needed. Consensus on optimized methods needed to be built and competency of laboratories and QA/QC protocols needed to be established. Finally, guidance on data interpretation of these assays for potential effects on both human health and wildlife are also needed.

CONCLUSION 5, RESPONSE TO COMMENT 2 (LC)

When the State Water Board next reconvenes the Science Advisory Panel on CECs in recycled water, the Panel will evaluate the relevant scientific literature and submit a report to the State Water Board that describes the current state of scientific knowledge regarding the risks of CECs to public health and the environment as well as recommendations regarding monitoring for CECs in recycled water. In evaluating monitoring recommendations, the Panel may consider whether it is appropriate to recommend monitoring for additional bioassays beyond ER-α and AhR, such as non-specific bioassays. The Panel favors a more targeted approach than DNA microarrays for monitoring antimicrobial resistance of wastewater. See response to Conclusion 7, Comment 1.

In addition, the State Water Board is funding research to further develop bioanalytical tools, including validation of existing protocols, method development, and QA/QC protocol development. The scope of the research includes build consensus on optimized analytical methods and standard operating procedures, build capacity for more laboratories to conduct the analyses, develop guidance on data interpretation, and develop additional bioanalytical screening endpoints. The research will identify a suite of additional *in vitro* bioassay candidates that could be used to screen for CECs that affect other physiological endpoints (e.g., thyroid, glucocorticoid); further develop standardized methods for multiple *in vitro* bioassays; build consensus on optimized methods, build capacity for more laboratories to conduct these analyses; and develop guidance on data interpretation for potential effects on both human health and wildlife.

Also, see Response to Comment Document I, Response to Comments 1.046, 1.049, and Section 4.14.4 of the Staff Report with SED for additional discussion of method validation and approval and other QA/QC requirements in the Amendment.

CONCLUSION 6

The equivalency agonists for the bioanalytical screening tools will provide a sensitive standard for calculation of bioanalytical equivalent concentrations and the monitoring trigger levels for evaluation of bioanalytical screening tool results are scientifically sound.

CONCLUSION 6, COMMENT 1 (JE)

The equivalency agonist recommended for the ER-α in vitro bioassay is 17β- estradiol (E2), and the equivalency agonist recommended for the AhR in vitro bioassay is 2,3,7,8-

tetrachlorodibenzo-p-dioxin (TCDD). These are potent agonists for the respective receptors, and are commonly used as reference substances for calculation of bioanalytical equivalent concentrations of chemicals affecting the bioassay receptor (Escher et al., 2018).

Monitoring trigger levels were recommended for evaluation of in vitro bioassay results (memorandum on behalf of the Panel dated May 14, 2018). For the ER-α bioassay, a health-based MTL of 3.5 ng E2 (equivalents)/L is proposed, and for the AhR bioassay, an MTL was based on the method reporting limit of 0.5 ng TCDD (equivalents)/L. The Panel recommended that no mandatory actions be required in response to an exceedence of the MTL. Voluntary follow-up actions included resampling and analysis, and targeted and non-targeted chemical analysis to determine the chemicals contributing to the response. Such response actions were included in the Draft Recycled Water Policy Amendment when the measured bioanalytical equivalent concentration in the sample is >10-fold the MTL. As indicated in the Draft Staff Report, this added guidance on evaluation of the results of the bioanalytical screening tool, is intended to provide follow-up investigatory actions when monitoring trigger levels are exceeded.

In explaining the selection of the ER-α MTL, the memo refers to a PNEC of 3.5 ng/L for 17-α-ethinyl estradiol (EE2), from values compiled in Appendix D of the Panel report (Drewes et al. 2018). The MTL of 3.5 ng E2/L is based on AwwaRF's DWEL of 3.5 ng EE2/L (Panel report, Table D.3), and is intended as an initial screening MTL for the first phase of data collection. The use of the criteria for EE2 rather than criteria for E2 is stated to be based on studies showing that the in vitro potency of EE2 is within a factor of 2 relative to E2, and that the lower threshold of 0.9 ng/L for E2 is overly conservative and not a practical screening level.

Given that E2 is an endogenous hormone, and that the listed "PNEC" of 0.9 ng/L is based on a 10E-6 cancer risk level associated with chronic exposure, it is reasonable to set this criteria aside for MTL purposes. What is not clear is why the USEPA HRL for E2 of 350 ng/L (per CCL3 and CCL4), or another drinking water benchmark for E2 from Table D.3 was not selected as the basis for determining the MTL. Similarly, in deciding to use criteria for EE2 rather than criteria for E2, it is unclear why the AwwaRF DWEL of 3.5 ng/L was chosen, rather than the USEPA HRL of 280 ng/L (CCL3) or, more recently, 35 ng/L (CCL4). Thus, the choice of the AwwaRF DWEL of 3.5 ng/L as the MTL for the ER-α assay appears to be arbitrary, and is not consistent with the Panel's stated sequence of selecting first, CA NL and second, USEPA criteria for MTLs, if available, over other benchmarks when multiple values are available.

A greater concern with respect to the choice of MTL for the ER-α assay is that it is based directly on a drinking water benchmark, i.e., human health criteria for acceptable intake via drinking water, with no in vivo-in vitro extrapolation procedure to convert the drinking water criteria to an equivalent in vitro concentration. Such an extrapolation procedure takes into account bioavailability and other pharmacokinetic data, (Brand et al., 2013; Wetmore, 2015) and can be expected to result in bioassay equivalent concentrations that are lower than the original drinking water criteria. Based on the approach used by Brand et al. (2013), and relative endocrine potencies, the bioassay trigger value for E2 is ~80-fold lower than the drinking water criteria on which it is based, and the bioassay trigger value for EE2 would be ~8-fold lower than the corresponding drinking water criteria (due to the higher bioavailability of the latter compound).

The procedure followed to establish bioassay MTLs was stated to be outlined in Textbox 7.1. The contents of textbox 7.1 show the pre-selected human health EE2 "PNEC" of 3.5 ng/L superimposed on a ER-alpha bioassay EE2 concentration-response curve. The PNEC corresponds to the approximate EC50 response. This is a method commonly used for establishing ecotoxicity screening thresholds for use with in vitro bioassays (Jarosova et al.,

2014; Mehinto et al. 2018). However, since the intent is to use the in vitro bioanalytical screening tool to protect human health, this reviewer believes it would be more appropriate to first convert the human health criteria for EE2 to the equivalent in vitro concentration, as discussed in the paragraph above, prior to superimposing the value on the concentration-response curve, in order to determine the bioassay response (percent effect concentration) associated with human health. Ultimately, the ER-alpha bioassay trigger value of 3.5 ng E2 equivalents/L that the Panel identified may be a reasonable and health-protective value, however, the rationale for choosing it is not transparent.

The recommended monitoring trigger level for the AhR bioassay is 0.5 ng TCDD equivalents/L, which is the method reporting level and not a health-based threshold. Due to the wide variety of anthropogenic and naturally occurring chemicals capable of activating AhR, which may interact in a synergistic or antagonistic manner, interpretation of the AhR bioassay data will be challenging. Lacking knowledge of the mixture composition of AhR agonists and antagonists in water samples, it is not practicable to set a health-based threshold at this time. If water samples have bioactivity above the MRL, and undergo further analysis i.e., targeted and non-targeted chemical analysis, to obtain more specific knowledge of the agonist/antagonist mixtures that are prevalent, such information may inform the future selection of a heath-based monitoring trigger level.

In summary, I concur with the conclusion that the equivalency agonists for the bioanalytical screening tools will provide a sensitive standard for calculations bioanalytical equivalent concentrations. The judgement used in setting the MTL equal to the MRL for evaluation of the AhR bioassay results is scientifically sound, recognizing that a health-based MTL is not practicable at the early stages of monitoring and data collection. However, because the underlying scientific basis, including scientific judgment underpinnings, for selection of the ER-alpha MTL was not fully articulated, and appears to deviate from precedent, a determination cannot be made regarding whether the MTL for evaluation of ER-alpha bioassay results is scientifically sound.

CONCLUSION 6, RESPONSE TO COMMENT 1 (JE)

Selection of ER-α MTL. As explained in textbox 7.1, to select the MTL for the ER-α assay the Panel relied on PNECs based on mammalian toxicity presented in Caldwell et al. 2010. A key reason for selecting PNECs presented in Caldwell et al. is that they were based on in-vivo studies of mammalian toxicity and the relative difference of the drinking water PNECs for the different estrogens presented in Caldwell et al. matches the relative response observed in the ER-α assay (Mehinto et al., 2018). The MTLs from different sources shown in Table D.3 (i.e., CCL3, AWWARF, Australia) were derived using a range of approaches and assumptions and do not match the observed ER-α assay response.

In-vitro to in-vivo extrapolation. The Panel identified a concentration of 3.5 ng/L of EE2 in drinking water (the most potent estrogen typically considered) that is assumed to be without adverse effect in humans. The Panel was able to equate that concentration with a level of response in the ER-α assay and proposed to use that associated ER-α assay response level as a MTL to determine whether an effluent requires additional evaluation. In-vitro to in-vivo extrapolation is not necessary to use the 3.5 ng/L drinking water concentration given it was derived directly from the mammalian toxicity data. The Panel is simply recommending use the ER-α assay response to measure the concentration of estrogen-active CECs in an effluent and determine whether that concentration is greater than or less than the MTL and believes the approach to be scientifically sound.

AhR bioassay MTL. The Panel agrees with the reviewer (Dr. English) regarding the lack of information (and "practicality") in establishing a health-based threshold for the AhR assay. That is why in their memo to State Water Board staff (dated 14 May 2018), the Panel declined to recommend a health-based bioscreening action level (or MTL) for the AhR endpoint. Rather, they recommended a practical method reporting level (MRL) of 0.5 ng TCDD/L that would serve to initiate voluntary investigative action to identify bioactive substances. Furthermore, the sensitivity of the AhR bioassay in screening mode allows it to be a conservative sensor for unknown compounds that may not be targeted by chemical analysis. As suggested in the Panel's 2018 report, if a consistent response is noted in a water sample, effects-directed analysis coupled with targeted and, if necessary, non-target chemistry can be used to identify the causative agent(s) and using mass balance methods, synergism, potentiation and antagonism of anthropogenic and naturally occurring ligands can be addressed.

CONCLUSION 6, COMMENT 2 (LC)

The equivalency agonists approach can integrate the combined effects of multiple chemicals that have the same mode of action. Such approach is therefore desirable to evaluate the cumulative effects of multiple chemicals found in recycled water. The proposed standard equivalency agonists of 17-beta-estradiol for the ER-α in vitro bioassay and 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) for the AhR in vitro bioassay are widely used strong agonists for their respective receptors and therefore should provide a sensitive response for calculating bioanalytical equivalent concentrations. The monitoring trigger levels for 17-beta-estradiol and TCDD were recommended to be 3.5 ng/L and 0.5 ng/L, respectively.

The increased from the initial MTL for 17-beta-estradiol at 0.9 ng/L to the recommended 3.5 ng/L is reasonable as the MEC for 17-beta-estradiol in 2018 was 0.5 ng/L. Keeping the 0.9 ng/L will be overly conservative resulting on too many false positive results. Evaluations of the Joint FAO/WHO Expert Committee on Food Additives (JECFA) in 2000 determined that the ADI of 17-beta-estradiol is 0-50 ng/Kg bw based on the NOEL of 0.3 mg/day or 5 ug/kg/day. Therefore, 3.5 ng/L is a conservative cut-off that is appropriate for screening threshold purposes.

The recommended monitoring trigger level for TCDD is 0.5 ng/L. The method reporting level is recommended as the monitoring trigger level instead of a health-based threshold (Attachment 5). The argument is that TCDD is significantly more potent than other chemicals that activate the Ah receptor, and a health-based threshold for TCDD would likely be overly conservative for other known AhR agonists that can be present in recycled water. This argument is not clear to me as the output from AhR bioassay are referenced to TCDD to generate BEQs. A BEQ is generated from the summation of all the chemicals in the recycled water that has TCDD-like activities. Therefore, the BEQ concentrations represent the TCDD equivalent concentrations in ng/L in the recycle water. Sensitivity should not be an issue either as the commonly used chemical activated luciferase gene expression (CALUX) reporter gene assay system can detect TCDD at 0.01 nM.

The MTL is higher than the health risk based thresholds used in the US. The US EPA advises that children should not have more than 1 nanogram 2,3,7,8-TCDD per liter of water (ng/L) in 1 day or more than 0.01 ng/L per day for long-term exposure. For long-term exposure in adults, EPA recommends that there should not be more than 0.04 ng/L in drinking water. Therefore, the USEPA set the maximum contaminant level goal for dioxin at 0.00000003 mg/L or 0.03 ng/L in drinking water. ATSDR uses health guidance value for chronic oral exposure to TCDD, i.e. Minimal Risk Levels of 0.000001 µg/day (1×10-6 µg/kg/day). Therefore, someone drinking 4 L

of recycled water at the recommended monitoring trigger level of 0.5 ng/L will have a daily TCDD dose exceeding these health-based guidelines.

Some clarifications and justifications for the MTL for TCDD are needed.

CONCLUSION 6, RESPONSE TO COMMENT 2 (LC)

While the Panel did not include a monitoring trigger level for AhR, the Amendment includes a monitoring trigger level of 0.5 nanograms/liter. The Panel did not recommend a health-based monitoring trigger level for AhR in part because there was not consensus for an appropriate health-based trigger level since there are numerous constituents that can activate AhR and may have varied effects depending on the composition of the mixture. The State Water Board agrees there is still uncertainty around identifying an appropriate health-based trigger level for AhR. However, the Table 9 of Attachment A includes a monitoring trigger level of 0.5 ng/L as a matter of Policy, which should be used as an interim trigger level to evaluate the AhR bioassay data until a health-based monitoring trigger level can be established.

Some agonists of the AhR receptor, such as TCDD, can elicit an effect on the receptor at concentrations far below the reporting limit for the AhR assay, which is currently 0.5 ng/l. This means low concentrations of AhR agonists such as TCDD may be present, but not be reliably detected by the AhR assay. The AhR assay is not intended to supplant or replace current existing monitoring requirements for TCDD and other chlorinated dioxin and furan constituents. Rather, utility of the AhR assay is to screen for other, unknown substances that would be responsive to this assay.

The use of the monitoring trigger level for AhR is consistent with a conservative and precautionary approach that should be implemented to protect public health, and the monitoring trigger level of 0.5 ng/l should be used until the sensitivity of the AhR assay improves (i.e., a lower reporting limit) or there is more consensus on a health-based monitoring trigger level, at which time the monitoring trigger level in Table 9 could be updated. See p. 81 of the Staff Report with SED for more detail. Also, see Response to Conclusion 6, Comment 1.

CONCLUSION 7

The available scientific information on antibiotic resistant bacteria (ARB) and antibiotic resistance genes (ARGs) in recycled water does not indicate that recycled water applications that comply with existing regulations cause antibiotic resistance transmission and monitoring requirements for ARB/ARGs in recycled water are not proposed because of the lack of standardized methods for investigating the occurrence and removal of, and risks associated with, ARB/ARGs.

CONCLUSION 7, COMMENT 1 (AMY PRUDEN PH.D., HEREAFTER AP)

Overview:

The State Water Resources Control Board (State Water Board) is developing an amendment to the Policy for Water Quality Control for Recycled Water (Recycled Water Policy). I have been requested specifically to review Conclusion 7, as stated in summary form above. The scientific assumption and findings supporting Conclusion 7 is summarized in Attachment 2 of the provided materials as follows:

The available scientific information on antibiotic resistant bacteria (ARB) and antibiotic resistance genes (ARGs) in recycled water does not indicate that recycled water applications that comply with existing regulations cause antibiotic resistance

***transmission and monitoring requirements for ARB/ARGs in recycled water are not proposed because of the lack of standardized methods for investigating the occurrence and removal of, and risks associated with, ARB/ARGs*.**

Further details on the state of the science are found in Section 4.14 of the Draft Staff Report and SED and the 2018 Science Advisory Panel Report on CECs in Recycled Water. In sum, "*uncertainty in this field of research and how to apply the results*" were cited as major factors for not recommending monitoring of this time, as well as lack of "*standard monitoring protocols and guidance on data interpretation for ARBs/ARGs*." The judgement was that methods and interpretation "*need further development before recommending monitoring ARBs/ARGs in recycled water*."

In preparing this review, I reviewed all materials provided, along with other relevant scientific literature, bearing in mind precedent for how policy relating to other CECs has historically developed as part of the existing Recycled Water Policy and that the overall goal of the proposed amendment is "*Support the increased development and use of recycled water in a manner that protects the environment and public health as one piece of a broader strategy to mitigate the effects of long-term drought, climate change, and water supply uncertainty*" (Staff Report, p.1, Executive Summary).

<u>Background- ARBs and ARGs as CECs</u>

Chapter 8 in The 2017 Science Advisory Panel Report on CECs in Recycled Water summarizes concerns related to antibiotic resistance in recycled water (Drewes et al., 2018). Overall, the chapter provides a useful and accurate summary about what is known about removal of ARBs/ARGs during various treatment processes and the fate of ARBs/ARGs when soil is irrigated with recycled water. Specific research needs identified in the chapter include the need for standard monitoring methods, the need to develop risk assessment frameworks, the need for better understanding of effects of disinfection, and need for further study on the fate of ARBs and ARGs during aquifer recharge, reservoir augmentation, and crop irrigation. The overall recommendation was that "*The State Water Board can encourage the collection of data in recycled water and sites within California while waiting for the above scientific advances*" (Research Recommendations, p. 99). I agree with this conclusion and offer advice herein on how to proceed.

First, it is useful to expand some on why ARBs/ARGs are a concern specifically in the case of recycled water and how they compare and contrast with chemical CECs. As is the case with the chemical CECs that have largely been the focus of California Recycled Water Policy and the CEC Science Advisory Panel, there are concerns because ARBs/ARGs occur in higher concentrations in raw sewage and there are uncertainties with respect to the efficiency of various wastewater and recycled water treatment technologies for their removal. Clinical, hospital and certain industrial sewage are particularly suspect as they tend to contain higher levels of ARBs, including pathogens, co-mingled with residual antibiotics resulting from excretion or disposal.

Also, as in the case of chemical CECs, there are uncertainties associated with specific human health effects of ARBs/ARGs, as measured in the environment. Specifically, at one end of the spectrum, presence of a pathogenic ARB, for example, an extended-spectrum beta lactamase (ESBL)-producing pathogenic strain of *E. coli* may be considered to be of greatest concern because such bacteria are known to be able to cause life-threatening infections that are difficult to treat with antibiotics. However, for this to happen, in addition to break-down of multiple water treatment barriers already in place and proven effective to remove *E. coli*, there would need to be opportunity for exposure (e.g., ingestion or swimming) by the individual and potentially vulnerability to infection (e.g., weak immune status). At the other end of the

spectrum, individual ARGs and other genes are beginning to be surveyed in wastewaters and recycled waters in the U.S. and globally, as a means of researching and understanding the potential for new strains of antibiotic resistant pathogenic ARBs to evolve as a result of co-occurrence of ARBs/ARGs and antibiotics in wastewater and if various treatment technologies reduce this potential. An advantage to direct monitoring of ARGs, using molecular methods such as quantitative polymerase chain reaction (qPCR), is that it captures potential for antibiotic resistance to be present in uncultured bacteria (which is important because most bacteria do not readily grow in culture media) and for it to spread among bacteria, given that bacteria have the capability to share genes with each other and spread antibiotic resistance via horizontal gene transfer. The disadvantage is that the DNA detected may be extracellular/from dead organisms, with limited knowledge of likelihood/rates of uptake by live bacteria via transformation. Thus, ARGs are a more conservative marker of antibiotic resistance than ARBs.

ARBs and ARGs also differ from chemical CECs in that they are biological in nature and capable of multiplying under certain conditions. This has been documented in terms of regrowth in irrigation piping systems at the point of application (e.g., Fahrenfeld et al. 2013, Negreanu et al. 2012). Thus, it is fair to judge that there are distinct complexities of ARBs and ARGs compared to some chemical CECs monitored historically, as was concluded in the 2010 Monitoring Strategies for CECs in Recycled Water (Anderson et al. 2010), providing grounds for further investigation leading to the recommendations supporting Conclusion 7 in the proposed Amendment to the Recycled Water Policy.

In my judgement as an expert conducting research in this area, the state of the science in terms of understanding ARBs/ARGs as CECs in wastewater and recycled water systems is developing quite rapidly. Research efforts in the U.S. and internationally have made considerable progress in improving understanding the fate and behavior of ARBs/ARGs in the environment. In particular, since the time of the 2017 Scientific Advisory Panel report (Drewes et al., 2018), a comprehensive chapter summarizing the fate of ARBs/ARGs through wastewater treatment is slated to be published online as part of the Global Water Pathogens Project: http://www.waterpathogens.org/toc. Rapidly developing next-generation DNA sequencing and bioinformatic technologies have also contributed to new knowledge, particularly in the realm of deeper understanding of the factors contributing to the evolution and spread of antibiotic resistance and informing improved monitoring and mitigation strategies. **Thus, it is particularly important that any new policy regarding antibiotic resistance in recycled water is flexible and regularly revisited.** This is consistent with a general recommendation by the 2017 Science Advisory Panel of "*several institutional changes for the Water Boards, including developing a more flexible and responsive program to update CEC monitoring recommendations in response to new information*" (Draft Staff Report, 2018, p. 1).

In summary, the spread of antibiotic resistance is a major public health concern. ARBs, ARGs, antibiotics and other selective agents are known to be present in sewage, but it is likely that ARBs, and to a good extent, ARGs, are reduced significantly during treatments required by Title 22 for water reuse and that they can be further attenuated following soil application. Still, this is important to verify and quantify. The value in monitoring ARBs/ARGs to be sure that this is the case was laid out in particular in Chapter 7 of the 2016 Expert Panel Final Report: Evaluation of the Feasibility of Developing Uniform Water Recycling Criteria for Direct Potable Reuse (Olivieri et al. 2016). The 2016 report offered three specific recommendations with respect to antibiotic resistance in recycled water, which can essentially be summarized as: **1)** Need to develop risk models **2)** Need for standardized ARB/ARG monitoring methods and **3)** Need to assess efficacy of various advanced water treatment technologies for ARB/ARG removal. Note that these recommendations were specific to direct potable reuse (DPR), where

the most stringent criteria are applied due to direct human consumption of the water. In general, I agree with these three recommendations and that they extend to recycled water use in general, in particular for the aquifer recharge and reservoir augmentation scenarios for potable reuse.

I also understand the logic, with respect to Conclusion 7 of the proposed amendments, that because there is a "***lack of standardized methods for investigating the occurrence and removal of, and risks associated with, ARB/ARGs***" that ARB/ARG monitoring is not recommended at this time. However, as described in the next section, **this situation has actually changed and consensus has been gained recently on a standard method for monitoring ARBs in water and wastewater by the World Health Organization**. **In my opinion, it is important to begin some form of monitoring of ARBs/ARGs in order to establish a baseline and to make progress towards the recommendations of Olivieri et al. (2016) and Drewes et al. (2018)**. In the following sections, I provide some logic for why this is the case and make suggestions for what could be targeted for monitoring.

Rationale for Monitoring ARBs/ARGs in Recycled Water

I disagree with the overall judgement that "*Monitoring for antibiotic-resistant bacteria (ARB) and antibiotic-resistant genes (ARGs) are not indicated by current scientific information*." There are two key assumptions to this judgement: 1) lack of standardized methods (which is now no longer the case) and 2) lack of risk assessment framework (which I agree are currently lacking, but are under development). In fact, if some form of antimicrobial resistance monitoring is not adopted, it will be difficult to fill the important knowledge gaps of risk assessment and occurrence/removal of ARBs/ARGs in recycled water systems that are cited in Conclusion 7 as grounds for not initiating monitoring.

Here I emphasize that the Recycled Water Policy can be informed by precedent of how other CECs in recycled water have effectively been addressed historically. Notably, scientific complexity has also been substantial for the various chemical CECs that have been recommended for monitoring in the past, but, as the state of the knowledge of CECs has improved, so have the monitoring recommendations. For example, the proposed amendments have been modified to add new CECs to the list (e.g., 1-4 dioxane) and remove others (e.g., triclosan) due to evolving knowledge. Similarly, a starting place is needed for monitoring of ARBs/ARGs, which can be revisited, improved, and modified with time. Some form of ARB/ARG monitoring is needed to establish a "baseline," much in the way baselines have been established for other CECs.

I do generally agree with the value the "risk-based framework" that has applied to chemical CECs when possible and also agree that effort in the scientific community is needed to develop and test analogous risk-based frameworks for antibiotic resistance in recycled water. It is worth noting that The Water Research Foundation recently released Request for Proposals 4813 "*Critical Evaluation and Assessment of Health and Environmental Risks from Antibiotic Resistance in Reuse and Wastewater Applications*." This effort will likely get underway in early 2019 and will ultimately be of value to guide antibiotic resistance monitoring and treatment in the water reuse community. Also, a basic human health risk assessment framework for environmental sources of antibiotic resistance was published in 2013 and has been widely cited by the scientific community (Ashbolt et al., 2013), providing a useful starting place. However, given the importance of antibiotic resistance, it is not advised to wait until such efforts are 100% complete before beginning some sort of monitoring. By definition, according to the CEC staff report, "*Constituents of emerging concern …. may or may not pose a risk to human health and aquatic species*" (p. 32, section 3.7). Thus, **it should <u>not</u> be expected that the precise human health risks of whichever antibiotic resistance**

markers that are selected for monitoring must be fully quantified before initiating monitoring. In fact, the monitoring itself is needed in order to effectively inform development of risk frameworks, including establishment of baselines and identification potential "trigger" levels, analogous to what has been recommended for other CECs.

ARBs and ARGs: Suggested Monitoring Targets

If monitoring of ARBs/ARGs is to be recommended in the amendments to the Recycled Water Policy, it is understandable that such a campaign would be modest to start. It would also be important to communicate to the public that the health effects and potential trigger levels are not known (as is the case with other CECs), but that the State Water Board, together with having extremely rigorous treatment as part of Title 22, is being proactive in implementing a monitoring program.

Berendonk et al. (2015) (as cited in Drewes et al. 2018) provides a very useful starting place in considering a monitoring program for antibiotic resistance in recycled water. The Berendonk review represents the consensus of 123 European scientists from 20 different countries and a variety of disciplines (including human health, toxicology, epidemiology, risk assessment, and civil & environmental engineering, as specified in the Conclusion 7 synthesis statement in Attachment 2). In sum, several target ARBs and genetic determinants, including ARGs and the class 1 integron, are suggested for monitoring.

In terms of ARBs, the targets suggested by Berendonk et al. (2015) tend towards those which contain pathogenic members, occur in water, and can be problematic for antibiotic resistance. Of these, there are fecal bacteria, such as *E. coli* and *Klebsiella pneumonia*e, which more readily tend to die-off in water systems, and water-based pathogens, such as *Aeromonas* and *Pseudomonas aeruginosa*, which can be native to aquatic systems and thus tend to survive and persist longer. **Based on this list, *E. coli* seems a reasonable starting place to recommend for monitoring in recycled water. A tremendous advantage of *E. coli* is that there ARE in fact standard methods already in place for monitoring regularly used by the water industry in California and across the U.S**. **Further, there are also standard methods for assaying antibiotic resistant *E. coli***, e.g., EUCAST (Berendonk et al. 2015). In fact, given such advantages, the Advisory Group for Integrated Surveillance of Antimicrobial Resistance (AGISAR) of the World Health Organization, recently released a standard method for monitoring ESBL *E. coli* (i.e., cefotaxime-resistant *E. coli*) for the purpose of integrated One Health antimicrobial resistance surveillance (Matheu et al. 2017). Specifically, the method has been developed for monitoring of sewage and surface water, in addition to human clinical and animal (poultry) samples. Several countries have already joined this monitoring program, which will provide useful points of comparison for a recycled water monitoring program in California. Notably, ESBL *E. coli* monitoring has been adopted by the 27 countries forming the Joint Programming Initiative on Antimicrobial Resistance (JPIAMR) for water and sanitation monitoring purposes (Wuijts et al. 2017). **Given that *E. coli* can contain pathogenic members, ESBL *E. coli* could be considered as a "Health-Based CEC" monitoring target.**

Given that most bacteria do not grow readily in culture media and that targeting any one bacterial species will not provide information about the resistance reservoir of the broader microbial community and the potential for antibiotic resistance to evolve and spread, gene-specific monitoring is also advised to complement culture-based monitoring. In terms of potential genetic determinant targets, there are a wide variety ARGs and other genes to choose from.

Berendonk et al. (2015) suggest a cross section of "clinically-relevant" ARGs as well as more commonly detected ARGs, which are proposed candidates for assessing treatment performance. Clinically-relevant ARGs include those encoding resistance to last-resort

antibiotics or extreme multi-drug resistance, such as ESBL, metallo-beta-lactamase, carbapenemase, and quinolone ARGs. Clinically-relevant ARGs are less likely to be detected, which is "good" from an immediate human health standpoint, but not as useful for performance-based monitoring purposes. On the other hand, sulfonamide and tetracycline ARGs (e.g., *sul*1, *sul*2, and *tet*M, suggested by Berendonk et al.) are more widespread and have been applied widely to assess treatment technologies (Ashbolt et al. 2018). **Of the genetic determinant targets recommended by Berendonk et al. (2015), I would recommend monitoring the class 1 integrase gene, *int*I1.**

There are numerous advantages to targeting *int*I1 for antibiotic resistance monitoring, as summarized by Gillings et al (2015). First, class 1 integrons are not technically ARGs, but they are gene capture elements that tend to carry several ARGs and are thus indicative of multi-drug resistance. Second, class 1 integrons have high clinical relevance, as they have been associated with numerous multi-drug resistant infections in humans. Third, class 1 integrons in general, and *int*I1 in particular, are a strong indicator of anthropogenic sources of antibiotic resistance, and thus can aid in distinguishing from ARGs that occur in the background. This is analogous to *E. coli* being a stronger fecal indicator than Total Coliforms. Fourth, *int*I1 is likely to be encountered through various stages of water treatment, decreasing as treatment progresses and thus serving as a sensitive indicator of performance. Finally, *int*I1 is embedded in a transposon and thus is also an indicator of potential for antibiotic resistance to spread via horizontal gene transfer. **For these reasons, *int*I1 could be an ideal "Performance-Based CEC" monitoring target for antimicrobial resistance in recycled water.**

While there are not yet standard methods specifically for *int*I1, it would be quite straightforward for the Water Board to develop and adopt such a standard. *Int*I1 is quantified via qPCR, which is already widely implemented for monitoring of viruses and validating log removal requirements in recycled water. The same principles of quality control in standard preparation, reagent maintenance, positive controls, and negative controls could easily be implemented. In 2004, the

U.S. Environmental Protection Agency published guidelines on best practices for applying PCR to environmental samples, which could be practiced in-house or by commercial labs for quantification of *int*I1 (EPA, 2004). Further, Water Environment & Reuse Foundation Project 14-17 was recently completed, which provides further guidance specifically on the application of molecular methods for monitoring in potable reuse scenarios (Wigginton et al. 2018).

It is also worth noting that **monitoring of sulfamethoxazole**, as recommended for continued monitoring as a Performance-Indicator CEC in the Proposed Amendments to the Recycled Water Policy, **will also provide useful supporting information with respect to potential to influence the prevalence of antibiotic resistant bacteria**. Note that Bengtsson-Palme and Larsson (2016) have proposed discharge limits for antibiotics, including sulfamethoxazole, specifically with the intention for limiting the potential for antibiotic resistance to be selected, transferred, or otherwise spread and these may be a useful reference point for further development of recycled water policy. However, if antibiotic monitoring is eventually to be implemented as an aspect of antibiotic resistance monitoring, it will be critical to consider that there are numerous other antibiotics that are also commonly present in secondary effluents that could also have an influence. Also, heavy metals are known to influence antibiotic resistance as well. Other antibiotics and metals could be considered for monitoring in the future.

<u>Location and Frequency of Monitoring</u>

A reasonable starting place for location of suggested ESBL *E. coli* and *int*I1 monitoring would be only in situations of potable reuse, i.e., groundwater recharge and reservoir augmentation.

Water should be tested before and after treatment (prior to recharge or augmentation) as well as at the point where the water is drawn for use. Examining the water when it is recovered for re- use is important given that ARB and ARG are biological CECs and can be subject to re-growth. Semi-annual frequency is reasonable, following the model of other CECs. In the future, monitoring of non-potable recycled water should be re-evaluated, given ongoing national and international research on fate of ARBs/ARGs on edible crops.

Conclusions

As reviewers of the proposed amendments, we were asked to "*consider the Big Picture… relying on professional judgment where available scientific data are not as extensive as desired to support the statutory requirement for absolute scientific rigor*" (Review Request, Attachment 2, p. A8). **Based on my review of the materials provided and my knowledge of the state of the science to date, my judgement is that ESBL *E. coli* and *int*I1 represent a reasonable and likely informative starting place for monitoring AMR in California's recycled water.** Specifically, ESBL *E. coli* could be considered a "Health-Based CEC" and *int*I1 a more conservative "Performance-Based CEC." At the same time, it is important that any monitoring plan be flexible and regularly revisited. The science is rapidly developing in this field and promises to offer improved targets in terms of cost, accessibility, relevance to recycled water, and significance to human health. Such an approach would be analogous to the "*Phased Monitoring Requirement*" currently implemented for other CECs, to "*allow monitoring to be refined based on results and findings*" (Recycled Water Policy Proposed Amendment, Attachment A, Section 4, p. A10). While a "risk-based" approach is not yet available, beginning some form of monitoring can help inform development of risk frameworks. Efforts are in motion to develop frameworks for assessing risk due to antimicrobial resistance in recycled water (e.g., Water Research Foundation RFP 4813) and can inform future refinement of monitoring targets. Beginning some sort of monitoring for antimicrobial resistance at this time is also advised by the Science Advisory Panel's 2017 Report on CECs in Recycled Water (Drewes et al. 2018) as well as the 2016 Expert Panel Report for the specific case of direct potable reuse (Olivieri et al. 2016).

It is important to note that *E. coli* is a fecal organism and therefore does not survive well in water. While it is a very logical starting place to track resistance in an organism relevant to recycled water and human health, the State Water Board may want to consider expanding monitoring in the future to include other resistant bacteria, such as *Pseudomonas* and *Aeromonas*. *Pseudomonas* and *Aeromonas* also occur in multi-drug resistant forms and are important to human health, but also survive much better in water environments and thus have more chance to acquire antibiotic resistance. In addition, the State Water Board may wish to consider more expansive monitoring of genetic determinants via shot-gun metagenomic sequencing, for a more in-depth profile of all detectable ARGs. Such a metagenomic approach is rapidly developing and gaining popularity. For example, in part through support from the World Health Organization, a standardized metagenomic approach to profiling ARGs in sewage is now being implemented as part of the Global Sewage Surveillance Project (https://www.compare- europe.eu/Library/Global-Sewage-Surveillance-Project). If not now, the State Water Board will surely want to re-visit the possibility of using metagenomics as a powerful tool for monitoring ARGs and other genetic elements of interest in recycled water in the future.

While presently an antimicrobial resistance monitoring program aimed at potable reuse scenarios, i.e., groundwater recharge and reservoir augmentation, makes sense, monitoring non- potable systems should be re-visited in the future. This is justifiable because ingestion is not the only relevant exposure route for antimicrobial resistance, e.g., aerosols and skin contact can also be important (Garner et al. 2015). It is agreed that there is good evidence

that soil is a natural buffer against build-up of antibiotic resistance, as reported by Negreanu et al. (2012) and elaborated upon by Pepper et al. (2018). However, soil build-up can be observed (Fahrenfeld et al. 2013, Wang et al., 2014), indicating that soil type and other factors could be important.

CONCLUSION 7, RESPONSE TO COMMENT 1 (AP)

While Dr. Pruden has made a number of valid points and recommendations, the State Water Board is funding a grant (with the Water Research Foundation) for research on this subject[1] and has thus initiated addressing the Panel's recommendation. The State Water Board can consider Dr. Pruden's comments and recommendations as part of the Water Research Foundation's development of the request for proposals and scope of work for the project. The State Water Board can include a review and update of AMR as part of a future CEC Panel effort (or separate ARG/ARB expert panel, if needed). The Water Research Foundation research results as well as the results from the international community (which we understand that Professor Mark Sobsey is engaged with) will most likely be available for future CEC Panel review and allow for a more robust analysis of Dr. Pruden's monitoring recommendations and any potential policy modifications to the Policy.

At this time, we are not requiring monitoring for antimicrobial resistance in Attachment A due to the uncertainty in this field of research and how to apply the results. The recommended targeted chemistry and bioanalytical screening tools in Attachment A are further along and were recommended back in 2012. This field of science for ARB/ARGs is progressing, and the World Health Organization has an analytical method for monitoring ARB/ARGs in water and wastewater, but there is not a risk assessment framework for ARB/ARGs. Consequently, the Panel did not make recommendations for monitoring ARB or ARGs in recycled water at this time, but did recommend the State Water Board to continue to fund and track research in this field and investigate monitoring protocols and constituents to analyze. Standard monitoring protocols and guidance on data interpretation would need further development and stakeholder input before being implemented in routing monitoring at recycled water treatment facilities.

See section 4.14.5 of the Staff Report with SED for additional information. See 4.14.6 of the Staff Report with SED for institutional changes the State Water Board plans to make to better respond to address CECs, including antimicrobial resistance, on an ongoing basis. The State Water Board has authority through Water Code sections 13267 and 13383 to require monitoring for antimicrobial resistance, meaning that as this field of science progresses, monitoring could be implemented outside a future process to amend the Recycled Water Policy.

[1] WRF solicited research RFP entitled *Critical Evaluation and Assessment of Health and Environmental Risks from Antibiotic Resistance in Reuse and Wastewater Applications (RFP No. WRF 4813 LINK 17-10).* The goal of the research project is "to examine approaches to quantify the public health and environmental risks related to antibiotic resistance in reuse and wastewater applications."

GENERAL COMMENTS

The Big Picture

(a) Are there any scientific issues not mentioned in this document that are part of the scientific basis of the Proposed Amendment?

(b) Taken as a whole, is the scientific portion of the Proposed Amendment based upon sound scientific knowledge, methods, and practices?

GENERAL COMMENT, COMMENT 1 (LC)

In my view, the conclusions were based on the recommendations made by a panel of experts using the latest results and the state of knowledge in the literature. There is no major outstanding scientific issue that have been missed or needed to be highlighted. The conclusions are based upon sound scientific knowledge, industry standard methods, and the practices. However, I have made some recommendations and suggested measures for future improvements on the monitoring program. Also, the State Water Board should consider and evaluate the ecological risk of the recycled water for non-portable use.

GENERAL COMMENT, RESPONSE TO COMMENT 1 (LC)

See response to Conclusion 4, Comment 1.

GENERAL COMMENT, COMMENT 2 (PG)

The Draft Amendment includes section "10. Constituents of emerging concern" and the Science Advisory Panel purports to handle "constituents of emerging concern (CECs)". BUT, the Science Advisory Panel Recommendations Report consistently mixes CECs AND Priority Pollutants without distinction (e.g., their Table 4.3). In the following, I am distinguishing "contaminants of emerging concern" from "priority pollutants". I am assuming priority pollutants will all be measured in the waters before recycling. If that is not true, then one needs MECs and MTLs (many, but not all, included in the Advisory Panel report) on all the priority pollutants too. And then one needs the risk assessments on these.

The proposed policy change states: "The purpose of the Policy for Water Quality Control for Recycled Water … is to encourage the safe use of recycled water from municipal wastewater sources … in a manner that implements state and federal water quality laws and protects public health and the environment." Later, the Policy amendment states "10.2.4. Each report shall recommend actions that the State of California should take to improve our understanding of CECs and, as may be appropriate, to protect human health and the environment." But the Science Advisory Panel was directed (Preface of their report) "… to limit their deliberations to impacts on human (and not ecological) health." This lack of ecological risk assessment is an incredibly large omission (e.g., comparable to ignoring the ability of freons to damage "the environment"). Such potential impacts must be addressed by scientific experts before the Recycled Water efforts can safely go forward.

The Draft Amendment states (8.1.4): "Nothing in this Policy shall be construed to prevent a regional water board from imposing additional requirements for a proposed recharge project that has a substantial adverse effect on the fate and transport of a contaminant plume or changes the geochemistry of an aquifer thereby causing the dissolution of constituents, such as arsenic, from the geologic formation into groundwater." This is incredibly important issue (i.e., one that has caused severe problems elsewhere in the world (see Bangladesh). But I do not see

where/how/by whom key water quality properties (e.g., pH, BOD) will be used to assess the prospects for such aquifer/reservoir changes.

The Policy Amendment appears vague on the matter of "8.2.4.1. If a groundwater recharge project proposes to utilize less than 10 percent of the available assimilative capacity in a basin or subbasin…" And still further: "For compliance with this subparagraph, the available assimilative capacity shall be calculated by comparing the mineral water quality objective with the average concentration of the basin or subbasin,…" what does "comparing" mean? This is too vague and may not even be protective. How does one quantify the "average concentration" of something like nitrate in a basin? What depth of groundwater does one include? Also, in the matter of "salt and nutrient assimilative capacities of groundwaters", what metrics besides maximum nutrient and salt concentrations belowground will be assessed: corrosiveness? scaling impacts? Na+ heart effects? F- effects? others? And section 6.2.4.1. states: "Salts, nutrients, and the constituents identified in 6.2.1.1 shall be monitored." No salts, nutrients, or constituents are listed in 6.2.1.1.

The following is confusing: "6.2.6. Data assessment. The regional water boards, in consultation with stakeholders, shall assess and review monitoring data generated from these plans approximately every 5 years but no more than every 10 years,…" To me, "no more than every 10 years" means one cannot assess conditions more frequently than once every 10 years. Continuing in the same section, modeling is noted: "groundwater quality impacts predicted in the salt and nutrient management plan based on most recent trends and any relied-upon models, including an evaluation of the ability of the model to simulate groundwater quality;" But the metrics used to quantify "the ability to simulate" are vague. What spatial and temporal scales of measurement-modeling correspondence will be required?

Other comments:

In section 3.1 of the Attachment A, it is stated: "(2) At monitoring well locations designated in consultation with the State Water Board within the distance groundwater travels downgradient from the application site in 30 days." This may not prove to be very far from the injection well. Further, sampling of an upgradient well should also be done. A conservative tracer should also be used to understand the degree of mixing of injected water with pre-existing groundwater to enable the extent of CEC loss 30 days after injection.

GENERAL COMMENT, RESPONSE TO COMMENT 2 (PG)

Priority pollutants. The Panel was tasked to investigate and make recommendations on emerging contaminants, which did not include a thorough assessment in priority pollutants as there has already been significant work on the priority pollutants. However, the Panel includes some information on priority pollutant in their report, but these were not considered to be emerging contaminants. See response to Conclusion 1, Comment 3 regarding the analysis of priority pollutants in this risk assessment. Priority pollutants are required to be monitored in NPDES permits, but may not be required monitoring for all WDRs or WRRs.

Ecological risk assessment. See response to Conclusion 4, Comment 1 regarding the ecological assessment of CECs.

Additional monitoring requirements for groundwater recharge projects. The regional water board has discretion through Water Code section 13267 to require monitoring for constituents that may indicate water quality changes in groundwater and surface waters.

Basin assimilative capacity. Section 8.2.4.1. of the proposed amendment was modified to make explicit the regional water board's discretion in determining a concentration representative of the

basin or subbasin, rather than constraining the analysis to be a basin-wide average. In addition, the State Water Board is contracting with UC Davis to conduct additional research to enumerate methods for determining basin-wide concentrations of salts and nutrients for the purpose of achieving water quality objectives.

Additional constituents for monitoring. Section 6.2.1.1 of the Amendment states that the salt and nutrient management plan may address constituents other than salts and nutrients that adversely address groundwater quality. These constituents would be specific to each salt and nutrient management plan depending on the groundwater quality of the basin or subbasin for which a salt and nutrient management plan is in development. It is therefore not appropriate to constrain the constituents that may be considered by including specific constituents in the Amendment.

Frequency of monitoring data review. Section 6.2.6. of the proposed amendment was modified to require a review of monitoring data every 5 years, rather than allowing the regional water board discretion in conducting the review. Regarding the metrics for review of salt and nutrient management plans, section 6.2.6. was left as-is to allow the regional water boards to determine metrics for evaluation of the ability of any relied-upon groundwater models to simulate groundwater quality.

Monitoring well locations. When recycled water producers apply for a permit, monitoring locations will be reviewed by Water Board staff consistent with section 3 of Attachment A. Groundwater recharge projects and reservoir water augmentation projects require monitoring either (1) prior to application to the surface spreading area, (2) prior to treatment by RO and following RO treatment, prior to release into the aquifer, or (3) prior to treatment by RO and following RO treatment, prior to release into the reservoir. These monitoring events should therefore occur before the recycled water mixes with ambient groundwater or reservoir water. The regional groundwater background levels would thus not impact the recycled water quality. The State Water Board advocates the release of high-quality recycled water so that it does not contribute to the degradation of existing water resources.

In addition, the Staff Report with SED section 4.14.1 and Science Advisory Panel (SAP) report section 5.5 explain that the Panel built in a level of conservatism into their risk-based framework. According to the SAP report, "The process the Panel used to screen CECs considered concentrations measured in secondary or tertiary treated wastewater effluent, not the point of exposure. Attenuation of CECs during advanced water treatment was not given any credit but these processes (including SA, integrated membrane systems or advanced oxidation processes) represent very effective barriers against a wide range of CECs. As a result of numerous physical, chemical and biological processes (e.g. dilution, dispersion, volatilization, sorption and biotransformation), CEC concentrations will be further reduced in an environmental buffer. Post-treatment after abstraction either at the well-head (for GWR) or at a regular surface water treatment plant (for SWA) provide additional barriers to some CECs. Finally, blending with other drinking water sources might occur either prior to or in the drinking water distribution system before this water reaches the point of exposure." While 30 days may not be that far from the injection site, it will take into consideration soil aquifer treatment and the State Water Board could require monitoring at an upgradient well if needed.

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