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## State Water Resources Control Board

July 2, 2025

**TO:** Potable Recycled Water Producers and Laboratories Conducting Bioanalytical Monitoring Pursuant to the Water Quality Control Policy for Recycled Water

**SUBJECT:** RECYCLED WATER POLICY CONSTITUENTS OF EMERGING CONCERN MONITORING PROGRAM – PROPOSAL FOR AN ALTERNATIVE QUALITY ASSURANCE QUALITY CONTROL MATRIX SPIKE FOR THE ARLY HYDROCARBON BIOASSAY

The purpose of this letter is to inform potable recycled water producers (producers) and laboratories conducting in vitro bioassays that they may select the most suitable chemical as the matrix spike specifically for assessing matrix spike recovery in the Aryl Hydrocarbon Receptor (AhR) bioassay. This must be done as part of the quality assurance and quality control (QA/QC) measures for the AhR assay, provided the results are reported in 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) equivalents and meet the established recovery acceptance criteria. The AhR bioassay is utilized as a monitoring tool within the constituents of emerging concern (CEC) monitoring requirements of the [Water Quality Control Policy for Recycled Water](#) (Recycled Water Policy).

### Background

On December 11, 2018, the State Water Board adopted an amendment to the Recycled Water Policy that included updated CEC monitoring requirements. Attachment A of the Recycled Water Policy requires producers to monitor for bioactivity indicative of the presence of CECs using two bioanalytical screening tools, estrogen receptor alpha (ER- $\alpha$ ) and AhR bioassays. Producers must also develop and maintain a Quality Assurance Project Plan (QAPP) to ensure data quality and compliance with the Recycled Water Policy. The QAPP requires laboratories to use validated analytical methods and demonstrate competency through established performance criteria.

Laboratories must submit a method validation package and obtain approval from the State Water Resources Control Board (State Water Board) before producers may use that laboratory to conduct CEC monitoring using bioanalytical tools. Approval requires a standard operating procedure (SOP) and supporting data that demonstrate that the laboratory procedures meet the specified performance criteria. The [National Water Research Institute \(NWRI\) guidance document](#) for developing an SOP provides useful guidance to help laboratories and facilities comply with the Recycled Water Policy. It includes QA/QC criteria, such as criteria for matrix spikes, which are used as a

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performance indicator for the extraction efficiency and can also clarify issues associated with matrix interference.

In addition to the matrix spike, reference standard chemicals, or agonists, are used in bioassays to determine the potency of the sample. The activity induced by the sample is compared to that of these agonists, providing a benchmark for assessing how strongly the chemicals in the sample activate specific receptors (like ER- $\alpha$  or AhR). The results are expressed as bioanalytical equivalents (BEQs), indicating the sample's potency relative to the reference agonist. The compounds 17 $\beta$ -Estradiol (E2) and 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) are used in ER- $\alpha$  and AhR bioassays, respectively, as both matrix spike compounds and reference agonists due to their well-established roles in receptor activation.

In 2019 and 2020, State Water Board staff worked with regional water boards staff to update potable recycled water producer monitoring and reporting requirements to include the updated Recycled Water Policy requirements, including the requirement to monitor using the two bioanalytical tools and to develop and maintain a QAPP for CEC monitoring. Since 2021, four laboratories have been approved for the ER- $\alpha$  bioassay and two laboratories have been able to demonstrate competency and have been approved for the AhR assay.

Since the implementation of the bioassay monitoring requirements in the Recycled Water Policy, laboratories contacted the State Water Board to indicate they were having challenges meeting the NWRI guidance document's matrix spike acceptance criteria of 70-130% for the AhR bioassay. Specifically, laboratories expressed challenges with the solid-phase extraction step in the AhR bioassay due to low matrix spike recovery, which is likely attributed to the low solubility of the matrix spike compound TCDD in polar solvents. As a result, laboratories were not able to consistently meet the acceptance criteria for matrix spike recovery. To address this issue, in June 2022, the State Water Board emailed laboratories to expand the acceptance range of the matrix spike recovery criteria from the original range of 70-130% to 40-160%. The laboratories attempted various modifications to the solid-phase extraction method to improve TCDD recovery, including testing different solvents, solvent exchange methods, and cartridges, among other adjustments. However, the laboratories continued to report difficulties in consistently meeting the revised criteria.

#### [Proposal to Change Matrix Spike for AhR Bioassay](#)

Discussions and evaluations conducted by State Water Board staff and laboratories through workshops, email exchanges, and virtual meetings to address ongoing issues with AhR Bioassay matrix spike recovery, resulted in this flexibility to allow laboratories to change the matrix spike compound from TCDD to an alternative chemical, provided the results are reported in TCDD equivalents and the established recovery acceptance criteria (40-160%) is met. The selected chemical must also be a strong AhR agonist with a mode of action similar to that of TCDD.

Laboratories and facilities may select an alternative chemical as the matrix spike compound and/or the reference agonist via the following two options:

**Option 1: Use an alternative chemical as both the reference agonist and the matrix spike compound**

In this option, laboratories would use the alternative chemical for all spike applications, including both blank and matrix spikes, when calculating spike recoveries. In addition, laboratories would use the alternative chemical instead of TCDD as the reference agonist but would still need to report results in TCDD equivalents. They would generate a dose-response curve for the alternative chemical to measure the sample's potency in the alternative chemical's equivalents. The results would then be converted to TCDD equivalents by applying the relative potency factor (RPF) of the alternative chemical, ensuring standardized reporting.

**Option 2: Use the alternative chemical as the matrix spike compound while retaining TCDD as the reference agonist**

In this case, laboratories would use the alternative chemical for all spike applications, including both blank and matrix spikes, when calculating spike recoveries. However, TCDD would continue to be used as the reference agonist for the calibration curve, with results reported in TCDD equivalents to maintain consistency in data interpretation.

Please note that if laboratories and facilities choose either of these options to start using an alternative chemical, they must submit a method validation package. Approval from the State Water Board must be obtained before implementing the revised method for bioanalytical screening under the CEC monitoring program.

Sincerely,



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Senior Environmental Scientist  
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cc:

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