California Regional Water Quality Control Board

Central Coast Region

External Scientific Peer Review and Staff Responses to Comments

for

Proposed Resolution No. R3-2024-0002 Amendment to the Water Quality Control Plan for the Central Coastal Basin to Establish Total Maximum Daily Loads for Organophosphate Pesticides and Toxicity in the Lower Salinas River Watershed, Monterey County, California

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1 INTRODUCTION

This document provides the comments submitted by external scientific peer reviewers along with Central Coast Regional Water Board Quality Control Board (Central Coast Water Board) staff responses to the peer review comments.

Health and Safety Code section 57004 requires external scientific peer review for certain water quality control policies. On February 15, 2023, staff requested that the CalEPA Scientific Peer Review Program (CalEPA) initiate the process with the University of California, Berkeley (University) to identify and select external scientific peer reviewers for the scientific portions of the TMDLs. The peer reviewer's responsibility is to determine whether the scientific findings, conclusions, and assumptions are based upon sound scientific knowledge, methods, and practices. The University confidentially identified two qualified reviewers and initiated reviews. The detailed step-by-step guidance for setting up and obtaining reviews appears in an Interagency Agreement between CalEPA and the University of California (see Exhibit F of the guidance document). A January 7, 2009, Supplement to the Guidelines, in part, provides guidance to ensure confidentiality of the process. No person may serve as an external scientific peer reviewer if that person participated in the development of the scientific basis or scientific portion of the proposed rule, regulation, or policy. CalEPA provided the final reviews to the Central Coast Water Board on May 11, 2021.

Approved reviewers:

David C. Volz, Ph.D.
Professor of Environmental Toxicity
Department of Environmental Sciences
University of California, Riverside

Daniel Schlenk, Ph.D.
Professor of Aquatic Toxicology & Environmental Toxicity
Department of Environmental Sciences
University of California, Riverside

Central Coast Water Board Staff requested the reviewer's comment on whether the scientific portions of the TMDL Technical Report, Section 7.2 Additive Toxicity Numeric Target Chlorpyrifos, Diazinon, and Malathion are based upon sound scientific knowledge, methods, and practices. Specifically, the reviewers were asked to comment on two specific conclusions related to the Section 7.2, Additive Toxicity Numeric Target Chlorpyrifos, Diazinon, and Malathion as stated below:

Conclusion #1: "Applying the concept of concentration additivity is an appropriate assumption and a technically valid approach for the derivation of additive toxicity numeric targets."

Conclusion #2: "The proposed additive toxicity numeric targets are a technically valid numeric interpretation of narrative water quality objectives."

Staff reproduced the peer review comments in the chronological order they were received.

California Health and Safety Code section 57004 states that if the external scientific peer reviewers find that a State agency failed to demonstrate that the scientific portion of the proposed rule is based upon sound scientific knowledge, methods, and practices, the reviewer's report shall state that finding, and the reasons explaining the finding. After receiving the reports from the reviewers and considering their comments, staff concludes that the reviewers found the two conclusions are based on sound scientific principles. The reviewers have extensive knowledge and experience in the areas addressed in the reports and their reviews provide valuable feedback that staff has addressed in this response document.

The Central Coast Water Board appreciates the thoughtful reviews provided by these two scientific peer reviewers.

Format used for staff response to comments:
In the following sections of this document, staff reproduce direct and unmodified transcriptions of the comments from each reviewer using *italic* text and insert staff responses using **bold text**.

2 PEER REVIEW COMMENTS OF DAVID C. VOLZ, PH.D.

Review Date: April 16, 2023

2.1 Conclusion #1: "Applying the concept of concentration additivity is an appropriate assumption and a technically valid approach for the derivation of additive toxicity numeric targets."

2.1.1 Overall Comments:

I agree that applying the concept of concentration additivity is an appropriate assumption and a technically valid approach for the derivation of additive toxicity numeric targets for chlorpyrifos, diazinon, and malathion – all three of which are OP insecticides that inhibit acetylcholinesterase (AChE). However, the authors should address the specific comment below to help clarify whether specific protection goals will be met for the Lower Salinas River Watershed.

Staff RTC 2.1.1

Staff acknowledges and appreciates the reviewer's finding that the "concept of concentration additivity is an appropriate assumption and a technically valid approach for the derivation of additive toxicity numeric targets for chlorpyrifos, diazinon, and malathion" and provides responses to the specific comments below.

2.1.2 Specific Comment:

Page 71, Section 7.2, 1st paragraph: Why is there an exclusive emphasis on "additive toxicity to aquatic invertebrates"? Is this because the data presented in Section 6.5 were based on aquatic invertebrate toxicity bioassays? Aquatic vertebrates (e.g., fish) are also very sensitive to the effects of OP pesticides (in some cases more sensitive than invertebrates), so it's unclear why additive toxicity is only being assessed for invertebrates. As a result, an exclusive emphasis on "additive toxicity to aquatic invertebrates" may have significant implications about whether specific protection goals will be met for the Lower Salinas River Watershed, especially if model invertebrates used for toxicity testing are less sensitive/vulnerable to chlorpyrifos, diazinon, and malathion compared to aquatic vertebrates.

Staff RTC 2.1.2

Aquatic invertebrates are the focus because multiple studies indicate that invertebrate species are more sensitive than vertebrate species for chlorpyrifos and diazinon (CDFW, 2000: Appendices A-C), and for malathion (Faria, 2010: see Section 12, Sensitive Species, pg. 11; for acute see Table 3, pg. 34; and for chronic see Table 5, pg. 41). Staff agree that vertebrates can be very sensitive and have accordingly proposed the use of criterion continuous concentration (CCC) values in the additive toxicity numeric targets because CCC values include toxicity evaluations for both vertebrates and invertebrates (CDFG, 2000, pgs. 42 and 57; Faria, 2010, pg. 41).

The reviewer questions whether the emphasis on aquatic invertebrates is due to the water quality data analysis which uses invertebrate toxicity bioassays (TMDL Project Technical Report Section 6.5). Although a large body of vertebrate (fish) toxicity data[1] for this watershed is available, staff did not include an analysis because there are very few instances of toxic effects. Based on available studies and the references cited above, staff has concluded that invertebrates are the most sensitive species and suitable for evaluating aquatic toxicity conditions.

[1] Fish toxicity data. Several sites with more than 30 fish toxicity samples each and very few significant effects to lethal or sublethal (growth) endpoints.

2.2 Conclusion #2: "The proposed additive toxicity numeric targets are a technically valid numeric interpretation of narrative water quality objectives."

2.2.1 Overall Comments:

I agree that the proposed additive toxicity numeric target (S ≤ 1) is a technically valid numeric interpretation of the narrative water quality objective. However, the authors should address the specific comments below to help clarify whether specific protection goals will be met for the Lower Salinas River Watershed.

Staff RTC 2.2.1

Staff acknowledges that the proposed additive toxicity numeric target is a technically valid numeric interpretation of the narrative water quality objective and has addressed the specific comments below.

2.2.2 Specific Comments:

Page 71, Section 7.2, bottom paragraph: Although an EC50 is used as an example, it's unclear what effect concentration – EC50, EC25, EC10, EC5, etc. – will ultimately be used as the numeric target (NT) to calculate additive toxicity. The text should clarify what ECx will be used to calculate concentration additivity since the ECx selection will drive whether the narrative water quality objective has been met or not. If the goal is to prevent a significant toxic effect on survival, growth, and/or reproduction, then this goal will not be achieved by relying on EC50s (concentrations that result in effects on 50% of the population) for chlorpyrifos, diazinon, and malathion. Alternatively, if an EC5 (a concentration that results in effects on 5% of the population) is used to calculate additivity toxicity, then selection of an EC5 plus the use of safety (uncertainty) factors (see comment below) will provide greater confidence that specific protection goals will be met, especially considering that the model invertebrates used for toxicity testing may not be representative of more susceptible aquatic invertebrates and vertebrates inhabiting the Lower Salinas River Watershed.

Page 72, Section 7.2, bottom paragraph: Importantly, the authors acknowledge that combined exposure to chlorpyrifos, diazinon, and malathion has the potential to result in synergistic toxicity based on previously published studies, suggesting that additive toxicity numeric targets may underestimate toxicity in the Lower Salinas River Watershed. The authors also state that "current research has not identified a coefficient of interaction or any other means in which to derive numeric targets that would accurately characterize the synergistic effect and therefore be more protective." Therefore, in the absence of approaches to estimate synergistic toxicity for chlorpyrifos, diazinon, and malathion, the authors should consider applying a safety (uncertainty) factor (e.g., 10X) to all three effect concentrations (e.g., EC5) prior to calculating concentration additivity. For example, the numeric target (NT) for each pesticide would be calculated by dividing the EC5 by 10; the adjusted NT would then be used additive toxicity according to the equation presented in Figure 7.1. This will ensure that specific

protection goals will more likely be met for the Lower Salinas River Watershed given uncertainties associated with potential synergistic toxicity.

Staff RTC 2.2.2

To clarify, the effective concentration (EC) represented in the formula to calculate additive toxicity is the acute and/or chronic numeric target concentrations presented in Section 7.1 of the TMDL Project Technical Report (see pg. 70), with the chronic numeric target for each OP pesticide being the most stringent. The purpose of having both acute and chronic numeric targets in the additive toxicity numeric target is to accommodate both acute and chronic water quality toxicity testing results, should only one test result be available. Use of the term EC50 in the TMDL Project Report is only for descriptive purposes of the concentration additivity concept and corresponding formula and should not be construed to indicate that compliance with water quality standards is being defined using a 50% effect level concentration. Note that EC50 values are incorporated into the derivation of water quality criterion (e.g., numeric targets used for this TMDL Project Technical Report) and are presented in the references contained as CDFW 2000 and Faria 2010.

In the absence approaches to estimate synergistic toxicity, the reviewer suggests applying a safety (uncertainty) factor (e.g., 10X) to all three effect concentrations. As clarified above, effect concentrations (EC) have already been incorporated into the derivation of water quality criterion which represent the numeric targets for each compound and an additional safety factor is unnecessary and not proposed in this TMDL.

3 PEER REVIEW COMMENTS OF DANIEL SCHLENK, PH.D.

Review Date: April 23, 2023

3.1 Conclusion #1: "Applying the concept of concentration additivity is an appropriate assumption and a technically valid approach for the derivation of additive toxicity numeric targets."

As provided in Section 7.2 of the TMDL Project Technical Report, I have evaluated the derivation of the Additive Toxicity Numeric Target for chlorpyrifos, diazinon and malathion. The values utilized for the individual compounds have been well-characterized and consist of acute and chronic values derived from multiple documents (CDFW, 2000, CDFW, 2004, and Faria et al., 2010). The acute values identified as the Criterion Maximum Concentration (CMC), and the chronic value, identified as Criterion Continuous Concentration (CCC) are primarily based upon toxicity measurements in aquatic invertebrates, which tend to be the most sensitive species for acute toxicity of

these compounds. These are generally conservative values and are appropriate for use as target values.

The acute toxicity of each of these compounds is caused by inhibition of the enzyme acetylcholinerase (Achase) (and other cholinesterases as well as serine esterases) that inhibits the metabolism of the neurotransmitter, acetylcholine. When acetylcholine is not metabolized, persistent neuronal activation occurs in the central and peripheral nervous systems. Death is usually caused by lack of respiration in the organism. Chronic toxicity may also be caused by Achase inhibition, but other modes of action may be present for the impairment of reproduction or growth (see below).

Staff RTC 3.1
Staff acknowledges these comments and the reviewer's finding that the proposed numeric targets are "generally conservative values and are appropriate for use."

3.2 Conclusion #2: "The proposed additive toxicity numeric targets are a technically valid numeric interpretation of narrative water quality objectives."

Since each of these compounds has the same mode of action for acute lethality, then a concentration additivity model can be used for the acute lethality endpoints in a Toxic Unit or Concentration Additivity model (Deneer 2000). The numeric target for each compound can be used in a ratio of the measured concentrations for the compound. These values can be summed for an overall mixture toxicity threshold.

As mentioned in the technical report (and above), additional sublethal endpoints may also be affected through other modes of action, particularly in fish. Thus, synergistic activity has been observed. This may be lead to altered behavioral responses which may limit predation or cause predation (Scholz et al, 2006; Maryoung et al. 2015a). While it is hypothesized that Achase may be involved in these behavioral modifications, there is other evidence to suggest that other neuronal signaling pathways may also be affected including those modulated by intracellular molecules such as Calcium and lipids/Fatty Acids (Maryoung et al, 2015b; Greer et al, 2019). This may explain the greater than additive responses (synergism) noted in other studies. Differences in metabolism of the pesticides may also be an issue that may cause less than or greater than additive responses in fish (Schlenk et al, 2008). As aquatic invertebrates generally have more limited metabolism of these compounds than fish, they tend to be more susceptible to the acute responses of Achase inhibition and the resulting lethality. This is not to say that metabolism is absent in invertebrates. Clearly the interactions of other pesticides and OPs appear to have a metabolism component (Cedergreen 2014; Pape-Lindstrom and Lydy, 1997). Overall, given the conservative values for acute toxicity using invertebrates, CA models for OP mixtures are valid, but uncertainty exists when this is extended to chronic or sublethal responses (e.g. behavior/reproduction/growth) and responses in all species (e.g. Fish). The reason for this uncertainty is based upon varied modes of action for growth, reproduction and behavior that may not occur as a

result of Achase inhibition, particularly in fish. That said, the inclusion of an uncertainty value of ~10 for the Acute-Chronic ratio may provide some degree of safety. However, the derivation of these values which often include the incorporation of growth with lethality in species sensitivity distributions adds some degree of uncertainty as well. Thus, CA should use the common mode of action and its result (acute lethality) to parameterize the model.

Staff RTC 3.2
Staff acknowledges the reviewer's comment that "a concentration additivity model can be used for the acute lethality endpoints in a Toxic Unit or Concentration Additivity model (Deneer 2000)" and that "the numeric target for each compound can be used in a ratio of the measured concentrations for the compound" with the values "summed for an overall mixture toxicity threshold."

Staff appreciates the reviewer's comments regarding other modes of action, particularly behavioral modifications in fish, that may explain the greater than additive responses (synergism).

Staff acknowledges the reviewer's comment that "Overall, given the conservative values for acute toxicity using invertebrates, CA models for OP mixtures are valid, but uncertainty exists when this is extended to chronic or sublethal responses (e.g., behavior/reproduction/growth) and responses in all species."

The reviewer suggests that incorporating an uncertainty value of around 10 for the Acute-Chronic ratio may provide some degree of safety, but this may introduce some uncertainty as well. As such, staff does not propose to modify the proposed additivity toxicity numeric targets using an uncertainty value.

REFERENCES CITED BY REVIEWER SCHLENK AND STAFF

CDFW, 2000. Siepmann, S, and B.J. Finlayson. Water quality criteria for diazinon and chlorpyrifos. California Department of Fish and Game. Office of Spill Prevention and Response Administrative Report 00-3. Sacramento, CA.

Cedergreen N. 2014. Quantifying synergy: A systematic review of mixture toxicity studies within environmental toxicology. PLoS ONE 9(5) e96580.

Deneer J. W. 2000. Toxicity of mixtures of pesticides in aquatic systems. Pest Manage Sci 56: 520.

Faria, I.R., A.J. Palumbo, T.L. Fojut, R.S. Tjeerdema. 2010. Water Quality Criteria Report for Malathion. Phase III: Application of Pesticide Water Quality Criteria Methodology. Department of Environmental Toxicology. University of California, Davis. Report prepared for the Central Valley Regional Water Quality Control Board,

Rancho Cordova, CA. March 2010.

Greer, JB, Magnuson, JT, Hester, K, Giroux, M,.Pope, K.. Anderson, B. Liu, J. Dang, V, Denslow,ND and D. Schlenk, D (2019) Effects of Chlorpyrifos on Cholinesterase and Serine Lipase Activities and Lipid Metabolism in Brains of Rainbow Trout (*Oncorhynchus mykiss*). Toxicological Sciences 172: 146–154.

Maryoung,LA, Blunt, B, Tierney, KB, and Schlenk, D (2015a) Sublethal toxicity of chlorpyrifos to salmonid olfaction after hypersaline acclimation. Aquatic Toxicology 161:94-101

Maryoung, LA, Lavado, R, Bammler, TK, Gallagher, EP, Stapleton, PL, Beyer, RP, Farin, FM. Hardiman, G, Schlenk, D(2015b) Differential Gene Expression in Liver, Gill, and Olfactory Rosettes of Coho Salmon (Oncorhynchus kisutch) After Acclimation to Salinity. Marine Biotechnology17:703-717.

Pape-Lindstrom, P.A. and M.J. Lydy. 1997. Synergistic toxicity of atrazine and organophosphate insecticides contravenes the response addition mixture model. Environ. Toxicol. Chem. 16: 2415-2420.

Schlenk, D (2005) Biotransformation of Pesticides IN: Biochemical and Molecular Biology of Fishes Vol. 6 - Environmental Toxicology - volume (editors T.W. Moon and T.P. Mommsen) Elseiver, New York pp171-190.

Schlenk, D, Celander, M, Gallagher, E, George, S, James, M, Kullman, S, van den Hurk, P, Willett, K (2008) Biotransformation in Fishes In: *The Toxicology of Fishes* (R. Di Giulio and D. Hinton editors). Taylor and Francis Publishers, Washington DC pp 153-234.

Scholz N.L., Truelove N.K., Labenia J.S., Baldwin D.H., Collier T.K. 2006. Dose-additive inhibition of chinook salmon acetylcholinesterase activity by mixtures of organophosphate and carbamate insecticides. Environ Toxicol Chem 25(5):1200–1207.