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Tebuconazole bioconcentration in European Eels (*Anguilla Anguilla*): the role of pollutant cocktails

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Abstract. European eel (*Anguilla anguilla*) populations are in decline across Europe, with organic pollutants (OPs) identified as one of the contributing factors. In the Albufera of Valencia, a critical feeding and maturation habitat for this species, a significant number of OPs have been identified, with tebuconazole exhibiting the highest concentrations among the compounds detected. This study aimed to assess the bioconcentration of tebuconazole in European eels following waterborne exposure, both alone and in combination with other commonly detected OPs. Eels were exposed under controlled laboratory conditions to environmentally relevant concentrations of tebuconazole and other OPs, and tissue samples were analysed to determine their bioconcentration. Statistically significant differences in tebuconazole accumulation were observed during the exposure phase, with notably higher concentrations in eel tissues when the compound was administered alone, compared to co-exposure with other OPs. These results suggest that the presence of pollutant cocktails may alter the bioconcentration of individual substances, potentially through interactions affecting absorption, distribution, or metabolism. This study highlights the importance of considering the complex nature of contaminants mixtures in natural habitats. Understanding how these interactions affect species like the European eel is crucial for protecting vulnerable populations, and ensuring ecosystem and food safety.

Keywords: European eel, tebuconazole, Albufera de Valencia, liver, muscle.

1. Introduction

The European eel, a critically endangered species in Europe, faces major conservation challenges due to its complex life cycle, long migratory route, and susceptibility to pollution. Pollutant exposure may impair its physiological condition, contributing to population decline alongside other stressors such as habitat degradation and overfishing (Vitale et al., 2021). OPs —such as pesticides, perfluoroalkyl substances (PFASs), and pharmaceuticals—are increasingly detected in aquatic ecosystems, raising significant environmental concerns. Although their occurrence in water and sediments is well documented, there remain substantial knowledge gaps regarding their accumulation in aquatic organisms (Álvarez-Ruiz et al., 2021). In the Albufera of Valencia, a key habitat for European eels, the fungicide tebuconazole has been identified as one of the most prevalent OPs. Since environmental pollutants are typically present as mixtures rather than in isolation, assessing their combined bioconcentration is essential for understanding their ecological risk (Soriano et al., 2023). Therefore, this study investigates the tissue-specific accumulation of tebuconazole in the liver, muscle, and plasma of European eels under waterborne exposure, both individually and in combination with eight other commonly reported OPs: diclofenac, venlafaxine, carbamazepine, caffeine, difenoconazole, acetamiprid, perfluorooctanoic acid (PFOA) and perfluorooctane sulfonate (PFOS). The results provide valuable insights into the different accumulation trends of OPs, interactions among co-occurring compounds, and their broader implications for aquatic ecotoxicity and potential human exposure.

2. Methods

The bioconcentration study was conducted over 55 days, following a 3-week acclimation period. Upon completion of acclimation, European eels were assigned to one of three experimental groups: control group, group exposed to tebuconazole (1 µg/L), and group exposed to tebuconazole (1 µg/L) in combination with other OPs (0.125 µg/L each). All contaminants were administered via water during the exposure phase (days 0–34). The exposure phase was followed by a depuration phase (days 35–55), during which the polluted water was replaced with fresh, clean water. Eels were collected on days 0, 2, 8, 15, 28, 34, 36, 44, and 55, and samples of liver, muscle, and plasma tissues were taken. Water from the tanks was also sampled to ensure stable concentrations of pollutants. Eels' tissues were extracted using ultrasound-assisted solvent extraction and purification kits (Supel™ QuE Z-Sep+). The resulting extracts were analysed using high-

pressure liquid chromatography coupled with high-resolution mass spectrometry (HPLC-HRMS/MS).

3. Results

The results demonstrated significant bioconcentration of tebuconazole in liver, followed by muscle and plasma of European eels during both the exposure and depuration phases. Peak concentrations were observed on days 28 and 34 (end of the exposure period). In the group exposed to the cocktail of OPs, difenoconazole was also detected in liver and muscle tissues, whereas PFOS, PFOA, carbamazepine, and caffeine were detected exclusively in liver tissue. Furthermore, tebuconazole bioconcentration was significantly higher when administered alone than when co-exposed with other OPs. This effect was evident in both liver and muscle tissues, as shown in Figures 1A and 1B, respectively. These differences were statistically significant on days 28 and 34 of the exposure phase ($p < 0.01$). Depuration of these pollutants was similar in both groups.

4. Conclusions and further research

This study highlights the bioconcentration of several OPs in European eels, with two pesticides, two PFASs, and two pharmaceutical compounds detected in the liver and muscle. It also demonstrates how co-exposure to a mixture of pollutants influences the bioconcentration of tebuconazole, which tended to decrease in the presence of other OPs—suggesting that such interactions may alter its pharmacokinetics. These findings underscore the complexity of pollutants interactions and the need to consider mixture effects in ecotoxicological assessments. While this study focused on ten OPs at environmentally relevant concentrations, wild eels are likely exposed to far more complex and variable pollutant profiles, particularly during extreme environmental events such as floods (as the one of October 2024). To better understand the mechanisms driving these changes in bioconcentration, further research is required on the metabolic processing of pesticides, PFASs, and pharmaceuticals under co-exposure conditions. Such insights will be essential for improving ecological risk assessments and ensuring the protection of both eel health and food safety.

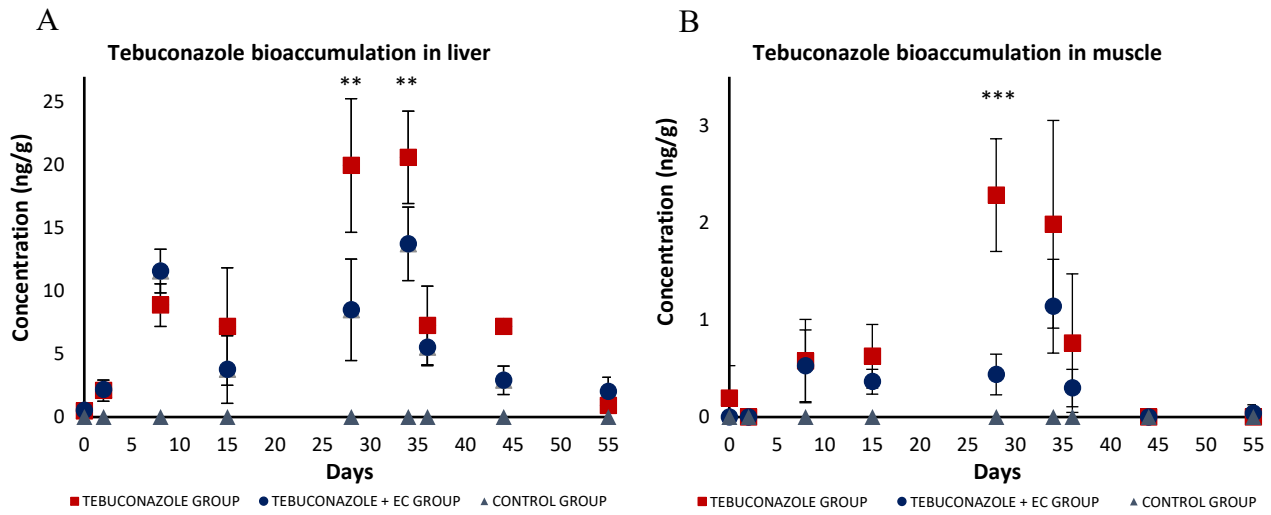

Figure 1. Tebuconazole concentrations in the liver (1.A) and muscle (1.B) of European eels from the control, tebuconazole, and tebuconazole + OPs groups throughout the exposure phase (days 0–34) and depuration phase (days 35–55). Values are expressed as mean ± standard deviation (n = 5). ** = $p < 0.01$, *** = $p < 0.001$.

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