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COMMENT

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Response to commentary article on environmental quality standards for diclofenac derived under the European water framework directive: 1. Aquatic organisms, by Maack et al. 2022

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Abstract

In this short article, we respond to a Commentary by Maack et al. (Environ Sci Eur 34:24, 2022) in which they challenge recommendations in Leverett et al. (Environ Sci Eur 33:133, 2021) for setting an aquatic Environmental Quality Standard (EQS) for the pharmaceutical diclofenac. Maack et al. recommend the use of results from a stream mesocosm study as the main point of departure for setting the EQS and dismiss the use of a Species Sensitivity Distribution (SSD) containing relevant and reliable single species data because of bimodality in this distribution. We present the key mesocosm data used by Maack et al. and note that these are highly variable, include control mortalities of up to 60%, and, as reported by the original authors, show a significant effect only at the highest test concentration and not at the estimated value proposed by Maack et al. We also show that there are neither regulatory nor technical grounds for dismissing the use of an SSD and respond to minor criticisms of our compliance assessment (comparison of different EQS values with reported concentrations in European surface waters). Finally, we provide comment on the EQS derivation process and subsequent opinion of the diclofenac EQS dossier by the European Commission's Scientific Committee on Health, Environmental and Emerging Risks (SCHEER).

Keywords: Diclofenac, Environmental quality standard, European water framework directive

Introduction

Maack et al. [8] published a Commentary in Environmental Sciences Europe on a paper by Leverett et al. [6], published previously in the same journal. We are grateful for the right of reply to Maack et al. [8], whose Commentary comprises technical criticisms of our recommended approach to EQS derivation, particularly our suggestion that a Species Sensitivity Distribution (SSD) of single species diclofenac aquatic toxicity data should not be replaced by highly uncertain data from a single stream mesocosm study. In this short article, we respond to that technical critique, and also to the subsequent opinion on the EQS derivation by the EC's own Scientific Committee on Health, Environmental and Emerging Risks [11].

Environmental quality standard derivation for diclofenac

When setting an EQS for diclofenac, Maack et al. [8] state their preference for stream mesocosm data reported by Joachim et al. [5] instead of an SSD of single species data and question whether a "hypothesis testing" or regression approach should be used to analyse highly variable data from this mesocosm study.

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They advocate use of a regression approach, including data on fish (stickleback, *Gasterosteus aculeatus*) which they consider critical to the EQS derivation. In contrast, Leverett et al. [6] point out that after five months of exposure to diclofenac in these mesocosms stickleback displayed both very high mortality in control replicates (up to 60% died) and significant variability between replicates in controls and treatments. The responses in controls and treatments overlapped, except at the highest exposure concentration. Joachim et al. [5] did not estimate an EC10 for these endpoints in their paper. Instead, they reported that '*…at the end of the experiment, the total number of founder fish were lower in the high treatment compared to the control (ANOVA, $F(3,8)=18.6$, $p<0.0005$ for F_o females, ANOVA, $F(3,8)=5.4$, $p=0.024$ for F_o males…. No effects were found for the other treatments.*' This, therefore, established an NOEC (no observed effect concentration) and LOEC (lowest observed effect concentration) for these endpoints of 0.44 and 3.82 µg L^{-1}, although the high degree of control mortality could reasonably be argued to render these thresholds unreliable for direct use in deriving an EQS. The European Commission [3] assessment used the Joachim et al. [5] data to estimate an EC10 for female stickleback mortality of 0.22 µg L^{-1} diclofenac with a 95% confidence interval ranging over two orders of magnitude (0.0385–1.30 µg L^{-1}) and then used this value "deterministically" to derive an EQS of 0.040 µg L^{-1} by dividing the EC10 by an assessment factor (AF) of 5 and rounding down.

Figure 1 and Table 1 support the Leverett et al. [6] interpretation of the mesocosm data (and, indeed, that of the original study authors) and raise the following reasonable technical questions:

- What model(s) should be fitted to these data to allow reliable estimation of an ECx value with acceptable precision?
- Are these data of sufficient quality to use as the main point of departure in defining an aquatic EQS to be applied across the entire European Union?

Maack et al. [8] state that, in studies with high variability, "traditional" hypothesis testing approaches used to identify toxicological summaries, such as an NOEC, are inferior to calculation of an ECx value such as an EC10, because the power of hypothesis testing is low when variability is high. However, Green et al. [4] explain why this is a simplistic response to the statistical evaluation of toxicity tests, a topic that has been intensively discussed for over 20 years (e.g., [1]). In the case of survival analysis, such as the EC10 estimates recommended by Maack et al. for stickleback, Green et al. [4] point out that step responses are common, *"but estimation of an EC10 is highly problematic because no information is available to derive its location."* This is the situation illustrated in Table 1 and Fig. 1 in which mortality (characterised in this study as "missing" fish at the study end) is high and variable in the controls and the two lowest test concentrations, but then increases significantly only at the highest test concentration. This distribution of data renders estimation of an EC10 highly uncertain for stickleback mortality and is one reason why Leverett et al. [6] recommend that the mesocosm study is used only to support, but not to be the primary driver of, a diclofenac EQS.

Instead of this reliance on uncertain estimates from a highly variable study, Leverett et al. [6] recommend use of the full range of single species data in an SSD to derive a diclofenac EQS of 0.126 µg L^{-1} (hazardous concentration for 5% of species (HC5) divided by an AF

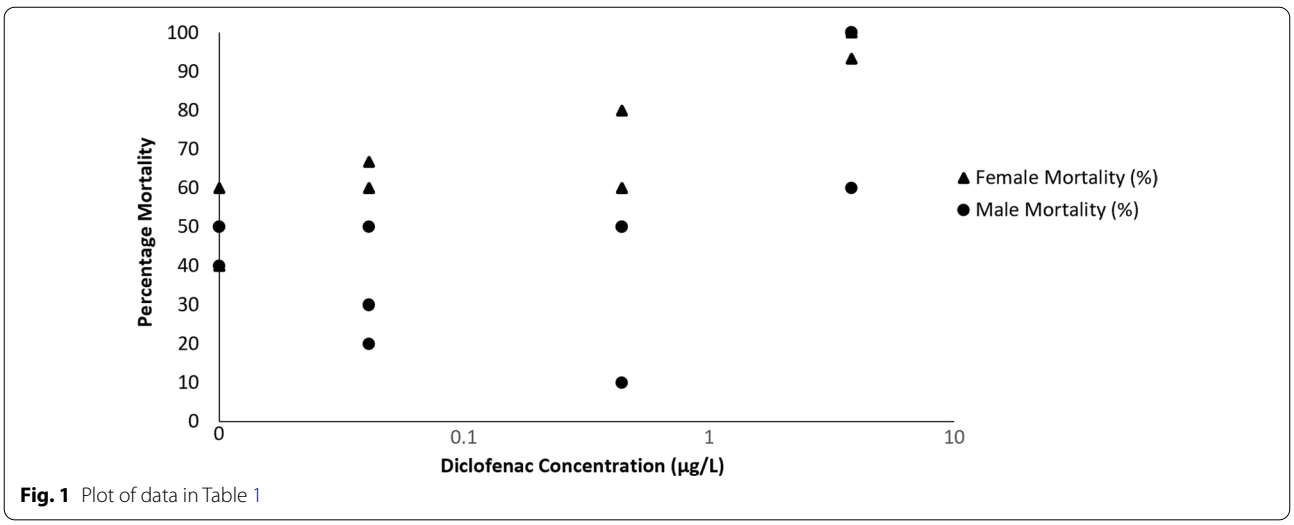

Fig. 1 Plot of data in Table 1

Table 1 Mortality of male and female stickleback after 5-month exposure to measured diclofenac concentrations reported as control, 0.041, 0.44, and 3.82 µg L^{-1} (nominal concentrations of 0.1, 1, and 10 µg L^{-1})

Mesocosm channel	Exposure concentration (µg L^{-1})	Total number of females at study start	Total number of females at study end	Female mortality (%)	Total number of males at study start	Total number of males at study end	Male mortality (%)
3	0	15	6	60	10	5	50
8	0	15	9	40	10	6	40
12	0	15	9	40	10	5	50
6	0.041	15	6	60	10	7	30
7	0.041	15	5	66.67	10	8	20
9	0.041	15	6	60	10	5	50
2	0.44	15	6	60	10	5	50
5	0.44	15	3	80	10	9	10
11	0.44	15	6	60	10	5	50
1	3.82	15	1	93.33	10	4	60
4	3.82	15	0	100	10	0	100
10	3.82	15	0	100	10	0	100

of 5), with mesocosm data used only as an additional line of evidence to support this value by demonstrating "real world" applicability. Maack et al. [8] dismiss the SSD presented by Leverett et al. [6], because it is bimodal, with no obvious mechanistic reason for this bimodality. They base their dismissal of the diclofenac SSD on the following quote from the European Commission's Technical Guidance Document for EQS derivation (Chapter 3.3.1.2, page 44, EC [2]): '*If the data do not fit any distribution, the left tail of the distribution (the lowest effect concentrations) should be analysed more carefully. If a subgroup of species is particularly sensitive and, if there are sufficient data, an SSD may be constructed using only this subgroup. However, this should be underpinned* ***if possible*** *by some mechanistic explanation, e.g., high sensitivity of certain species to this particular chemical. The SSD method should not be used in cases where* ***there is a poor data fit to all available distributions****.*' (our bolding). However, contrary to Maack et al.'s assertion, this text very clearly states that although a mechanistic explanation is desirable, it is not a necessary prerequisite before deriving an SSD with the most sensitive species. In Leverett et al. [6], we do this, but also show that insensitive data in the SSD do not influence the HC5 to any significant extent and that use of a sensitive subgroup provides a good data fit and, therefore, allows use of an SSD. We remain perplexed at the reluctance of Maack et al. [8] to use estimates based on an SSD from multiple high-quality studies with a wide range of species when deriving a diclofenac EQS.

Measured diclofenac concentrations in European freshwaters

The compliance assessment presented in Leverett et al. [6] was included at the suggestion of peer reviewers, primarily because it provides useful context, but Maack et al. [8] suggest that scientifically incorrect simplifications were made in this section. We reported both weighted and unweighted mean 90th percentiles values in the original manuscript, of 0.090 and 0.141 µg L^{-1}, respectively, though Maack et al. are correct that the weighted mean 95th percentile was reported when discussing the worst-case approach. All values were to be included in the submitted manuscript, but this section had to be shortened during revision, and this introduced a minor error. Re-analysing the original data set (see Additional file 1) shows an unweighted mean of 90th percentiles from individual countries of 0.162 µg L^{-1}, compared with 0.144 µg L^{-1} from Maack et al. (and 0.141 µg L^{-1} as stated in the original manuscript). It is unclear what data Maack et al. have used in their assessment, because no request for the data used in Leverett et al. was ever received. We agree with Maack et al. [8] that the compliance assessment confirms a degree of environmental risk from diclofenac in EU waterbodies, and better understanding the magnitude and extent of these risks on a European scale would be desirable. We have not made any claims to the contrary and state in Leverett et al. [6] that '*data on concentrations of diclofenac in European surface waters suggest that there are potential risks to aquatic receptors.*'

Maack et al. [8] criticise use of ½ Limit of Detection (LoD) for values below the LoD in Leverett et al. [6]. We

used this approach for consistency with the approach taken by the European Commission's Joint Research Centre (JRC, Loos et al. [7]. However, we agree with Merrington et al. [9] that more appropriate methods exist for processing left-censored data and combining large data sets from different sources. That is why one of us (Merrington) co-chaired the SETAC Technical Workshop to Develop Criteria for Reporting and Evaluation of Exposure Data (CREED) (https://cdn.ymaws.com/www.setac.org/resource/resmgr/workshops/CREED_Prospectus.pdf), the face-to-face part of which was held in Copenhagen, Denmark in May 2022 (several of the authors of [8] were invited to participate in this Technical Workshop, but declined). The results from this workshop will provide a more robust, transparent, and evidence-based way to assess and use such data. However, it is already clear that all data, not just metadata, used in an exposure assessment must be publicly accessible. This is so that these data can be independently verified and, if necessary, challenged. We note that such an examination of exposure data currently used in EQS dossiers, including for diclofenac, is not currently possible, because only the JRC has full access to these data.

EQS derivation process and European commission opinion

Maack et al. [8] highlight that four of the authors of the original article [6] were participants in the "expert" group which developed the diclofenac EQS. While this is true, Maack et al.'s paper disappointingly omits that our requests for further analyses with respect to the specific issues highlighted in the original article (Leverett et al. [6]) were ignored, and we were excluded from some discussions. We were, therefore, unable to support the conclusions in the final dossier submitted for review by the European Commission's Scientific Committee on Health, Environmental and Emerging Risks (SCHEER), because we do not believe that they were based on a full assessment of all the available evidence. Indeed, if the issues of concern were merely a matter of "disagreement" as claimed by Maack et al., it would not have been necessary to publish our original article (Leverett et al. [6]), but we had serious concerns that our appeals for additional evaluation of the available data were being disregarded precisely *because* we represented industry stakeholders.

Maack et al. [8] also state that the EQS derivation process for diclofenac was ongoing when Leverett et al. [6] was published and, therefore, suggest that any critical analysis was "very premature". This is disingenuous. The draft final dossier for the EQS assessment of diclofenac was submitted for an opinion to the SCHEER in July 2021. Our previous experience of European EQS derivation for other substances has shown that it is very rare for an assessment to change after an SCHEER Opinion. If stakeholder input is not adequately considered during "expert" discussions to derive an EQS, it becomes vital to ensure that there is open and informed debate before the SCHEER Opinion of this derivation is finalised.

The SCHEER Opinion is now complete [11] and, as our previous experiences suggested, supports the EQS value proposed in the draft final dossier for diclofenac. However, SCHEER do highlight the high degree of control mortality in the mesocosm study [5]. Unfortunately, the SCHEER Opinion also suggests that the population and community NOEC proposed in the conclusion of Joachim et al. [5] may be used as a "line of evidence" to support an EQS derived using other means. This overlooks the fact that this NOEC is based on the specific stickleback endpoints (including mortality) that are compromised by the high control mortality observed in the study. In addition, and as highlighted in Leverett et al. [6], the concentrations of diclofenac to which the stickleback (and other organisms) were exposed were inconsistently maintained in the mesocosm.

During the development of their Opinion, SCHEER apparently asked the EC to generate SSD curves for the chronic diclofenac dataset, although the details of these analyses are not presented in the Opinion [11]. It is assumed that several SSD options were assessed by the EC, possibly using different statistical models and/or different sub-sets of the chronic data set for diclofenac, although this is not clearly reported. The range of HC5 values given in the SCHEER Opinion are 1.78 to 5.6 µg L^{-1}, and the stated range of EQS values from 0.076 to 0.23 µg L^{-1}, using these HC5s. This suggests that the use of assessment factors (AF) of 23.3 or 23.4 has been applied to the HC5s, which is certainly not in accordance with the EC's own EQS Technical Guidance (EC 2018) on AFs to apply to an HC5 in a probabilistic EQS assessment, which is a maximum value of 5. No details are provided in the SCHEER review to justify these unique AFs.

The two highly questionable EQS values given in the SCHEER opinion; that is, one derived from the stickleback "population NOEC" of 0.4 µg L^{-1} from the mesocosm divided by an AF of 10 to give 0.04 µg L^{-1}, and the other representing the lowest HC5 from the EC's unreported SSD analysis (1.78 µg L^{-1}) divided by an AF of 23.3 to give 0.076 µg L^{-1}; are then compared in an apparent "weight of evidence" assessment. This assessment then concludes that '*the lower* [EQS] *of 0.076 µg* L^{-1} *from an SSD is "not far" from the tentative* [EQS] *of 0.04 µg* L^{-1} *from the mesocosm study and so could be justified', and that 'therefore, an* [EQS] *of 0.04 µg* L^{-1}*.....could now be supported by the SCHEER'.* Based on the reliable and relevant scientific data and following the accepted EC Technical Guidance (EC [2]), this outcome is extraordinarily opaque. The apparent "agreement" of the two

highly questionable and uncertain EQS values generated in the SCHEER Opinion are subsequently suggested to be mutually supportive as a weight of evidence. What is less opaque is that an appropriate data-driven weight of evidence assessment, according to SCHEER's own "Memorandum on weight of evidence and uncertainties" revised in 2018 [10], is not presented in the opinion.

Supplementary Information

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Additional file 1. Measured diclofenac concentration in European freshwaters.

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Author contributions
Each author made contributions to the conception, analysis, and interpretation of data and assisted in drafting the work. Each author approved the submitted version (and any substantially modified version that involves the author's contribution to the study) and agrees both to be personally accountable for the author's own contributions and to ensure that questions related to the accuracy or integrity of any part of the work, even ones in which the author was not personally involved, are appropriately investigated, resolved, and the resolution documented in the literature. All authors read and approved the final manuscript.

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Consent for publication
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Competing interests
Dean Leverett, Graham Merrington and Iain Wilson are employees of a consultancy (WCA Environment Ltd) which works for companies on projects focusing on the environmental risk assessment of chemicals. Mark Crane is a partner in a consultancy (AG-HERA) which undertakes similar work.

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