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Aquatic thresholds for ionisable substances, such as diclofenac, should consider pH-specific differences in uptake and toxicity

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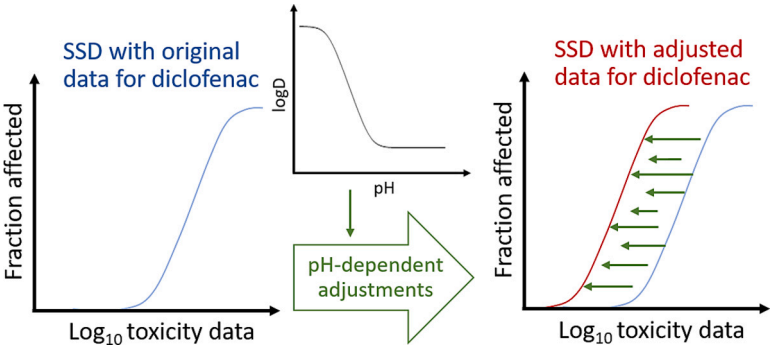
HIGHLIGHTS

- Toxicity of ionisable substances to aquatic organisms is pH-dependent, which influences the respective hazard assessment.
- We adjusted the effect values for diclofenac based on their pH-dependent uptake potential as a function of logD.
- We observed decreased heterogeneity of acute data and an improved species sensitivity distribution of chronic data.
- The normalization resulted in a lower chronic HC5 value for environmental quality standard (EQS) derivation.
- We recommend the adjustment of effect data for ionisable substances based on a worst-case pH in hazard assessments.

GRAPHICAL ABSTRACT



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ABSTRACT

Diclofenac, a widely used nonsteroidal anti-inflammatory drug (NSAID), enters the aquatic environment worldwide. The effect values available for the derivation of an environmental quality standard (EQS) are markedly heterogeneous, even within the same species. This heterogeneity could partially be attributed to inter-laboratory variation, but is also observed in repeated tests within the same facility. Diclofenac is ionisable; its speciation and potential for uptake and thus toxicity is influenced by pH. A high correlation has previously been observed between effects in zebrafish embryos and the pH-specific partitioning coefficient logD for diclofenac. We hypothesized that the observed heterogeneity could also be attributed to differences in study pH. To test this hypothesis, we reviewed physicochemical data and selected ecotoxicity data that were considered to be reliable and relevant in the latest EU EQS Dossier for which a study pH was reported for further analysis and EQS derivation. We adjusted the reported effect concentrations for differences in uptake using the delta logD value for the worst case pH value of 6.5. pH adjustment of effect values resulted in decreased heterogeneity of the acute effect data and a better fit of the chronic species sensitivity distribution. Both, the MAC-EQS and the AA-EQS were derived using the deterministic approach as data requirements for deriving EQS based on the SSD were not fulfilled. Many studies had to be discarded because test pH was not reported or exposure concentrations had not been analytically verified. Physico-chemical data had to be discarded due to non-relevant experimental conditions or missing information. We strongly encourage scientists publishing ecotoxicity data for ionisable

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substances to report the test pH together with the effect values and encourage measurement of physico-chemical parameters at environmentally relevant conditions. We recommend to consider adjusting the effect data for ionisable substances according to a worst-case pH in future hazard assessments.

1. Introduction

Around 80 % of prescribed drugs are ionisable (Manallack, 2007). Ionisable substances tend to be increasingly toxic at pH levels that result in an increasing fraction of the neutral species. Weak acids are therefore more toxic at lower pH, while the opposite is true for weak bases (Rendal et al., 2011; Simon and Beevers, 1952). Especially the latter classes of ionisable substances change their speciation in the environmentally relevant pH range of 5–9, which is most relevant for aquatic risk assessment, and freshwater aquatic risk assessment in particular. A well-known example for a weak acid is diclofenac (2-[2,6-dichloranilino] phenylacetic acid), a nonsteroidal anti-inflammatory drug (NSAID) that has analgesic, anti-inflammatory, and antipyretic properties making it effective in treating acute and chronic pain and various inflammations. Diclofenac is the most frequently used NSAID for humans and domestic livestock worldwide and is available in over the counter products in most countries (McGettigan and Henry, 2013). In the EU, diclofenac is still authorised for veterinary use on union level and in most member states (e.g. in 25 MS for systemic application in 2019 (EMA, 2019)), none are authorised in Switzerland. Globally, diclofenac was reported to have been consumed in amounts of 1443 ± 58 tons per year (from 2010 to 2013, Acuña et al. (2015)). The total consumption in Europe was estimated to be around 180 tons per year, including 86 tons per year for Germany alone - the largest European user (Lonappan et al., 2016). Diclofenac is excreted via human urine and faeces, if administered orally or intravenously. If applied topical as ointment, it may also be washed off e.g. with showering. Diclofenac and its two main metabolites (4′-hydroxydiclofenac and 5-hydroxydiclofenac) usually enter wastewater treatment plants (WWTPs) via the domestic grey-water, where the removal efficiency mostly depends upon the treatment methods (Alessandretti et al., 2021). Conventional treatment systems exhibit a degradation efficiency of 40–75 %, while ozonation can increase removal to >90 % (Altmann et al., 2014). If sewage sludge or manure from treated diclofenac treated livestock is used as fertilizer in agriculture diclofenac may enter the soil and subsequently leach to surface waters and groundwater. However, diclofenac can also be degraded readily and rapidly by sunlight. Photolysis (with DT_{50} below 4 days) was identified as the main removal process in lakes and it was estimated that up to 90 % of the drug can be eliminated by this process (Buser et al., 1998). Nevertheless, diclofenac has been widely detected in the aquatic environment (surface water, groundwater, drinking water, seawater and wastewater) and can be considered as pseudo-persistent contaminant. This term refers to chemicals that are continuously released via treated wastewater, resulting in a steady-state exposure concentration, which can be higher than their degradation properties suggest (e.g. Barceló and Petrovic, 2007). Measured concentrations span from the ng to µg per litre range worldwide (Sathishkumar et al., 2020). Maximum concentrations of >1 µg/L often occur downstream of WWTPs in densely populated areas. High concentrations of diclofenac in small watercourses in rural areas can also be linked to (Carvalho et al., 2015) livestock where these uses are allowed. Diclofenac was listed on the first Watch List (Carvalho et al., 2015) of the EU Water Framework Directive (2000/60/EC; WFD) for a continuous monitoring for up to four years.

Several attempts to derive an Environmental Quality Standard (EQS) for diclofenac have been performed in recent years (e.g., Maack and Schudoma (2014)). In 2019, the European Commission tasked an expert group with the derivation of a new EQS for diclofenac, based on the EU Technical Guidance (i.e. EU TGD 27) for Deriving Environmental Quality Standards (EC, 2018). The resulting EQS dossier has been reviewed by the EC SCHEER committee in 2022 and diclofenac has been added to the draft updated EQS Directive (2013/39/EU (EC, 2022)). The expert group noted a substantial heterogeneity of toxicity test results among organism groups and even within single species which they failed to explain.

For lipophilic substances and metals, the EU TGD 27 describes specific considerations as to compartmentalization and bioavailability in the environment for derivation of respective thresholds. For ionisable organic substances, such as diclofenac, such specific procedures are not yet prescribed. Diclofenac has an acidic dissociation constant (pKa) around 4, which means that small changes in pH above the pKa can significantly alter its speciation. Below the pKa, almost 100 % of the compound remains non-dissociated, i.e. in its neutral state. However, the pH-dependent distribution pattern of the two diclofenac species, according to the logD (pH-dependent partitioning coefficient) concept, shows that small variations in pH between 5 and 8 can greatly affect partitioning, and thus bioavailability and toxicity (Bittner et al., 2018; Köhler et al., 2023; Schweizer et al., 2021). A recent study by Köhler et al. (2023) reported a strong correlation between the severity of effects in zebrafish embryos at different pH and the corresponding pH-dependent partitioning coefficient logD for >20 ionisable drugs. For diclofenac, a correlation close to 1:1 was observed.

We hypothesize that the observed heterogeneity in published effect data for diclofenac is thus at least partially caused by differences in the respective potential for uptake of diclofenac due to the particular test pH. Hence, an adjustment of the effect data for pH-dependent log D should facilitate the EQS derivation for diclofenac. Further, we aimed to analyse the proportion of the assessment factors (AF) required by the EU TGD 27 to differences in EQS with and without adjustment of effect data. For this purpose, we performed a literature review of data on physico-chemical properties (water solubility, dissociation constants (pK_a) and octanol-water partition coefficients ($logP/K_{ow}$)), and selected the acute and chronic ecotoxicity studies assessed as reliable and relevant in the JRC dossier for diclofenac for which a pH adjustment was possible. Using logD as descriptor of uptake and hence bioavailability, we adjusted the reliable and relevant effect concentrations of diclofenac for the logD at the worst-case pH of 6.5 as well as pH of 7 and used these values to derive a proposal for an adjusted EQS.

2. Methods

2.1. Physico-chemical properties of diclofenac

An open literature search was performed in November 2021 using PubMed, Google Scholar and Scopus with the search terms: diclofenac, diclofenac sodium, solubility, dissociation constant, partition coefficient, lipophilicity, pKa, logP, $logK_{ow}$, and physicochemical, resulting in >500 publications. References within the retrieved publications were checked for relevance and verified were possible.

Physico-chemical properties data for diclofenac sodium were considered as well, as it was mostly used as test item in the aquatic ecotoxicity studies due to its higher solubility.

2.2. Acute and chronic aquatic ecotoxicity data

Acute and chronic aquatic toxicity studies were retrieved from the draft EU JRC EQS dossier (JRC, 2022a). Moreover, the two recent studies reporting the results of diclofenac in the Fish Embryo Acute Toxicity (FET) test with *Danio rerio* at different pH conditions were added to the acute toxicity dataset (Bittner et al., 2019; Schweizer et al., 2021).

The following experimental parameters were retrieved from the original publications listed in the EU JRC EQS dossier and the two additional publications: type of substance (source, purity), guideline applied, temperature, pH, medium, solvent, additional endpoints and time points. All studies had been evaluated for their relevance and reliability according to Moermond et al. (2016) and in accordance with recommendations in the EU TGD 27 for EQS derivation (EC, 2018) already for the EU JRC EQS dossier. We are aware that the study validation made for the EU JRC EQS dossier has been criticised (Leverett et al., 2021). We would not like to further this debate. For simplicity reasons we rely on the validity assessment in the EU JRC EQS dossier for the specific purpose of adjustment of effect concentrations based on delta logD (see section below). Specifically, as the solubility and partitioning of diclofenac in water are highly dependent on pH, the following criteria were verified: (1) exposure concentrations of diclofenac or diclofenac sodium below their respective solubility limits, (2) analytical verification of exposure concentrations, (3) solvents $\leq$0.01 %. Of the reliable and relevant studies, only those with reported pH values were used to facilitate adjustment of the effect values.

The aquatic toxicity dataset did not contain enough data to perform a comparison between the sensitivity of marine and freshwater species. Hence, following the recommendation of EU TGD 27 (EC, 2018), the datasets retrieved for freshwater and marine biota were combined.

2.3. Adjusting effect concentrations (EC_x) based on delta logD

Adjusting effect concentrations to a fixed "worst-case" pH allows the direct comparison of effect concentrations across taxonomic groups and among species that were obtained at different experimental pH values (Köhler et al., 2023). Recently, a large-scale study with 24 ionisable substances published by Köhler et al. (2023) showed that the differences in the observed embryotoxicity to *Danio rerio* at different pH values correlated very well with the respective differences in the estimated $logD_{o/w}$ of the chemicals at the respective pH values. Based on these findings, the authors propose to use this relationship when deriving regulatory thresholds. We use the proposed methodology in the present study as described below. Accordingly, logD of diclofenac is modelled as a function of pH, log p (lipophilicity of the neutral and ionic species) and the pKa, and a linear relationship can be observed for pH values ranging from 5 to 8 (Fig. S1).

$$logD_X = log10\left(f_{neutral} \times 10^{log\ Px(neutral)} + f_{ionic} \times 10^{log\ Px(ionic)}\right) \quad (1)$$

with

$$f_{neutral} = \frac{1}{\left(1 + 10^{(pH - pK_a)}\right)}$$

and

$$f_{ionic} = 1 - f_{neutral}$$

For $logP_{neutral}$, the value of 4.34 was used (Table 4), for $logP_{ionic}$, a value of 0.7 was applied according to the software Chemicalize developed by ChemAxon (retrieved September 2022). For pKa, the value of 3.99 (Avdeef et al., 1998) was used.

On the basis of experimentally generated EC_x data ($EC_{x,\ test}$) for a given pH (pH_{test}), the effect concentration was then corrected or normalized according to Eq. (2) to the toxicity at the worst-case pH ($pH_{worst\ case}$) of 6.5 for weak acids as well as pH 7, using Delta $logD_{(o/w)}$ as correction factor, resulting in an adjusted EC_x ($EC_{x,\ worst\text{-}case}$) value:

$$log\ EC_{x,adjusted} = log\ EC_{x,test} + \left(logD_{(o/w)}\ [for\ pH_{test}] - logD_{(o/w)}\ [for\ pH_{worst\ case}]\right) \quad (2)$$

To test the suitability of this approach for diclofenac, we used the LC_{50} data experimentally generated in the *Danio rerio* embryotoxicity test (= $LC_{50,\ test}$), available for six pH values (pH 5, 5.5, 6, 7, 8 and 9) (Bittner et al., 2019; Köhler et al., 2023), for the pH of each test (referred to here as pH_{test}) as the basis for calculating the adjusted LC_{50} for all other pH values (referred to here as $pH_{adjusted}$). Subsequently, we correlated the $LC_{50,\ adjusted}$ with the 'real', experimentally generated $LC_{50,\ test}$ for the respective test pH values. We performed this procedure several times, each time using a different test pH value serving as the basis for the re-calculations of the $LC_{50,\ adjusted}$ values.

2.4. EQS derivation

EQS derivation was performed according to the EU TGD 27 for EQS derivation (EC, 2018) and as described in (Korkaric et al., 2019). The chronic EQS. (AA-EQS) was selected from a list of quality standards (QS) as prescribed by the EU TGD 27. Here, we focused on the QS based on direct toxicity to aquatic organisms and derived the respective $QS_{fw,eco}$. The EU TGD 27 suggests that individual toxicity data may be aggregated using the same principles as those in Chapter R.10 of the REACH Guidance where multiple data are available for the same species/endpoint (p. 161). The only two effect concentrations this would apply to are No. 11 and 12 in the chronic dataset (Table S7). As information on the medium is missing in one case, we did not calculate geometric mean. The acute and chronic QS can be derived based on the deterministic method, using laboratory or field/mesocosm data, or based on the probabilistic method, using species sensitivity distribution (SSD) models. In all cases, the resulting threshold concentration is divided by an additional assessment factor (AF) to account for the residual uncertainty associated with a given data set and laboratory to field extrapolations. For the deterministic method, the lowest effect concentrations per group of organisms is identified and compiled and then, the lowest reliable effect value is determined. The AF to be applied to this critical effect concentration ranges from 10 to 100 for the MAC-EQS and from 10 to 1000 for the $QS_{fw,eco}$. If data requirements were met to produce an SSD, i.e. a sufficient number of diverse taxa representative for whole communities, these were modelled using the ETX software (ver. 2.3) (Vlaardingen et al., 2004). The SSD is a statistical approach in which toxicity data are ranked and plotted according to a normal distribution, and a model is subsequently fitted to the data. The model can be used to calculate a hazardous concentration (HCx) of a chemical that is hazardous to no more than x % of all species included. According to the EU TGD 27, SSDs based on a lognormal distribution of the effect data should be constructed, because its mathematical properties are well described. For each species considered in the SSD, only a single endpoint with a reported pH was available. Hence, this original value or the adjusted value were used in the SSDs.

Provided the resulting SSD fulfilled defined criteria regarding lognormality and goodness of fit, EQS were derived by applying appropriate AFs to the resulting HC_5 (concentration at which 95 % of all included species should theoretically be protected). In this case, the relevant AF may vary from 5 to 1 and from 10 to 1 for the derivation of the $QS_{fw,eco}$ and the MAC-EQS, respectively. Acute mesocosm data were not available. Assessment of secondary poisoning to derive a QS_{biota} was not performed. Likewise, QS for saltwater, $QS_{sediment}$, and QS to protect human health were not considered here.

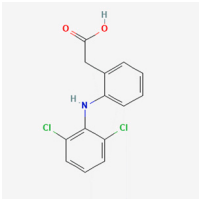
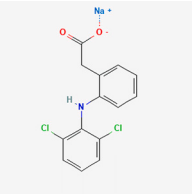
3. Results

3.1. Assessment of physicochemical data

Due to the poor water solubility of diclofenac in standard ecotoxicity tests, its sodium salt is often used as alternative, thus, data for both substances were considered (Table 1). Most of the retrieved physicochemical property data for diclofenac and diclofenac sodium had to be discarded, as experimental conditions were either not relevant for ionisable substances or not relevant for aquatic environments (Tables S1-S4).

Water solubility is an important factor when assessing the reliability

Table 1
Molecular structure, molecular weight and CAS number of diclofenac and diclofenac sodium.

Common name	Diclofenac	Diclofenac sodium
Molecular structure		
MW (g/mol)	296.1	318.1
CAS number	15307-86-5	15307-79-6

of ecotoxicity studies. The solubility of diclofenac, a weak acid, depends on pH, temperature and solution chemistry. We sifted published water solubility data based on the data being experimental, measured at relevant pH (pH $\geq$ 6 and pH $<$ 9), and at temperatures relevant for ecotoxicity tests for temperate species.

With respect to the methodology, the EU TGD 27 (EC, 2018) does not recommend the shake-flask method for compounds with a solubility lower than 10 mg/L. However, it can be used for solubility determination for ionisable substances with low water solubility when essential parameters are well controlled and reported to allow data comparison (Avdeef et al., 2016).

Out of 20 verified and reliable solubility data (Table S1), three values were identified as relevant for ecotoxicity studies (Table 2) yielding a geometric mean of 0.345 mg/L.

For diclofenac sodium, 20 verified and reliable experimental solubility data were retrieved (Table S2), including eight values that were relevant according to the criteria defined in the methods section (Table 3), yielding a geometric mean of 3088 mg/L. This value was used as threshold for accepting ecotoxicity studies as reliable. Please note that increasing pH increases water solubility while solubility correlates negatively with ionic strength (Kincl et al., 2004).

The determination of the pKa of the weak acid diclofenac is important to understand the possible relationships between pH and bioavailability and thus, toxicity.

The pKa data were considered relevant and reliable when they are experimental values measured under ecological relevant conditions and a concentration of solvent which does not exceed 0.01 %. The only experiment which was found valid determined the dissociation constant by potentiometry in 0.15 M KCl at 25 °C to the value of 3.99 $\pm$ 0.01 (Avdeef et al., 1998). All other values are listed in Table S3.

$LogP^0$ or $logK_{ow}$ is the decadic logarithm of the n-octanol/water partition for the neutral form. Based on the pKa value of 3.99, diclofenac is mostly ($\geq$99 %) in its neutral form if the pH of the aqueous phase is 2 units below the pKa. Therefore, $logP^0$ values were considered relevant and reliable when the pH of the aqueous phase was 2 or less. According to EU TGD 27 (EC, 2018), the log P data are reliable if the experimental values are determined by the shake-flask method which works well for log*K*ow values in the range between −2 and 4 (occasionally up to 5). Table 4 summarizes the valid logP data for diclofenac and displays their geometric mean of 4.34. All other values are listed in Table S4.

3.2. Selection of a worst-case pH value for logD-based adjustments

To account for differences in the potential for uptake into an organism due to differences in ambient pH, using logD at a given test pH value and adjust the toxicity to a worst-case pH value, the selection of such a worst-case pH value is necessary. We suggest that this value reflects (1) the pH ranges tolerated by the test species, (2) pH ranges relevant to the ecosystems to be protected, and (3) the worst-case bioavailability of the substance (following to "Guidance Document on Aquatic Toxicity Testing of Difficult Substances and Mixtures" (OECD, 2019b)).

OECD guidelines for testing the toxicity of chemicals vary with respect to information on the allowed pH ranges of the test media and even allow variability of pH during the test (Table S5). When specified, pH values in the range of 6–9 are typical, as well as a tolerated variability of 1.5 pH units during the test. Of the guidelines applicable here, only OECD 203 refers specifically to the guidance document (GD) 23 on testing of difficult chemicals with respect to pH of test media (OECD, 2019b). The OECD guideline on difficult substances (OECD, 2019b) requires ionisable substances to be tested in the most toxic form. The relationship of pH and logD of diclofenac as a measure of uptake potential is depicted in Fig. S1. A linear relationship exists in the pH range between around 4 and 8. Below pH 4, diclofenac is mostly neutral and considered to be most available for uptake, which is, however, rare for European surface waters. Global stream water chemistry data made available in the GLORICH database (Hartmann et al., 2019) comprises pH values from 2.2 to 13. Values reported by European countries range from 3.6 to 10.3. An analysis of data reported under the WFD suggested that 95 % (i.e., between the 2.5th and 97.5th percentile) of all mean values were between 7.0 and 8.5, when replicate measurements per sampling site were excluded (Bundschuh et al., 2016). An analysis with a broader European dataset resulted in 90 % of pH values being between 6.2 and 8.6 (De Vivo et al., 2006). According to (Peters et al., 2022), the 5th and 10th percentile of pH values in European freshwaters are 6.8 and 7.1, respectively.

In summary, it is not straightforward to select a worst-case pH for the weak acid diclofenac, based on the available information. A pH value of 6.5 has been proposed in the EU JRC EQS Dossier for the NSAID ibuprofen (JRC, 2022b) for the critical fish endpoint and for BCF and BAF values. The resulting adjusted endpoints have been accepted by the Scientific Committee on Health, Environmental and Emerging Risks (SCHEER) of the EU (SCHEER, 2023) for the risk assessment. SCHEER endorsed the final EQS on the basis of the pH-adjusted NOEC, which resulted in a threshold eight times lower than without pH-adjustment. Here, we have selected pH 6.5 following the worst-case scenario for ibuprofen, as well as pH 7 considering the distributions of pH values reported for European surface waters, to fulfil the aforementioned requirements (1)–(3). The predicted logD at pH 6.5 and pH 7 are 1.8 and 1.4, respectively.

3.3. Verifying the adjustment of effect endpoints based on pH of test media

We hypothesized that differences in study pH values influences the heterogeneity of the measured effect values due to differences in the

Table 2
Summary of reliable and relevant solubility data for diclofenac. GM: geometric mean.

Entry	Solubility mg/L	Temp. °C	Solution	pH	Experimental method	Reference
1	0.323	20	Phosphate buffer	7.4	Isothermal saturation spectrophotometry	(Perlovich et al., 2007)
2	0.355	25				
3	0.358	30				
GM	0.345					

Table 3
Summary of reliable and relevant solubility data of diclofenac sodium salt. GM: geometric mean.

Entry	Solubility mg/L	Temp. °C	Solution	pH	Ionic strength M	Experimental method	Reference
1	150		Phosphate buffer	6.0	0.06		
2	670		Phosphate buffer	6.8	0.08		
3	1360		Phosphate buffer	7.0	0.09		
4	5150	23 ± 2	Phosphate buffer	7.4	0.12	Shake flask. 72 h spectrophotometry	(Kincl et al., 2004)
5	12,000		Phosphate buffer	7.8	0.13		
6	12,140		Phosphate buffer	8.0	0.14		
7	17,170		Borate buffer	8.0	0.05		
8	4700	25	Phosphate buffer	7.4			(Panchal et al., 2020)
GM	3088						

potential uptake. Hence, we applied the recently proposed concept of a delta logD-based correction of effect endpoints (Köhler et al., 2023) for the weak acid diclofenac. To verify the approach, a total of seven LC50 values at different pH obtained for embryotoxicity in *Danio rerio* (Bittner et al., 2019; Köhler et al., 2023) were assessed for correlation with $LC_{50,\ adjusted}$ being derived by a Delta logD-based adjustment. For all pH values between 5 and 9 that were used as basis for the adjustment of LC_{50} values, we observed a highly significant correlation ($p < 0.0001$) between adjusted and experimentally determined LC_{50} data, with a high degree of agreement (slope of the regression curve close to 1 and y-axis intercept of 0.12; Figs. 1, S2).

3.4. Effect values adjusted for logD at pH 6.5 and pH 7

Twelve of the 36 acute effect concentrations and 22 of the 40 chronic effect concentrations were reported with test pH and analytical verification of exposure concentrations (Tables S6, S7). The most sensitive group of organisms were amphibians in the dataset for acute toxicity and bivalves in the dataset for chronic toxicity, with both groups lacking effect data in the respective other dataset. We could neither identify acute nor chronic data for insects. Non-adjusted acute effect concentrations ranged from 670 µg/L to 185,070 µg/L, covering about three orders of magnitude, while non-adjusted chronic effect concentrations were spread between 0.37 µg/L and 15,540 µg/L, covering about five orders of magnitude (Fig. S3). Adjustment of effect values for the worst-case pH 6.5 resulted in a reduced and slightly shifted spread of 437–62,802 µg/L (acute) and 0.05–1682 µg/L (chronic). Thus, the overall variability decreased in the acute dataset upon logD-based correction.

3.5. EQS derivation

3.5.1. Acute EQS (MAC-EQS)

The final dataset for acute toxicity comprises 12 endpoints from eight species (Table S8), belonging to four different taxonomic groups. The two lowest effect concentrations are both mortality in embryos of the two amphibian species *Trachycephalus typhonius* and *Physalaemus albonotatus*.

The minimum data requirements recommended for the application of the SSD approach for EQS water derivation is preferably >15, but at least 10 effect concentrations from different species, covering at least eight taxonomic groups. The acute toxicity dataset does not fulfil these requirements for both, number of species (8) and number of taxonomic groups (4) and hence, an EQS was only suggested based on the deterministic or AF-approach (EC, 2018).

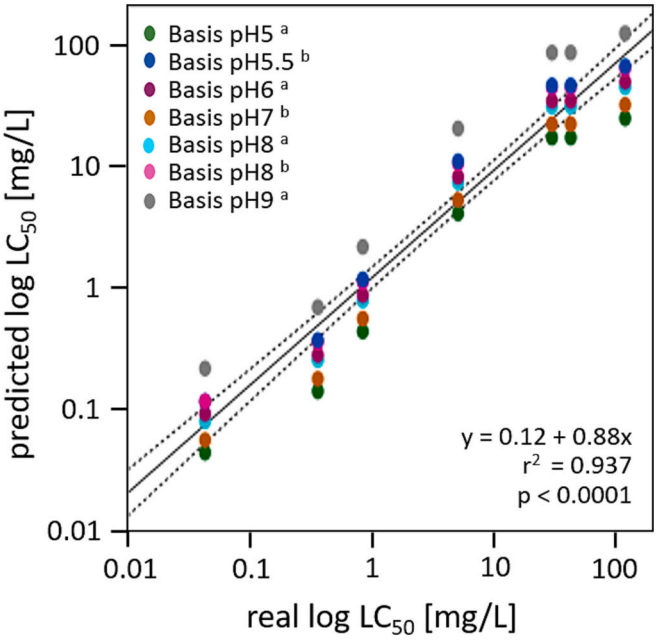


Fig. 1. Experimentally determined log LC_{50} data (real log LC_{50}) from [a]Köhler et al. (2023) and [b]Bittner et al. (2019), versus log LC_{50} data adjusted according to the method introduced by Köhler et al. (2023) and applied here for diclofenac. The respective pH value for which the experimental log LC_{50} was used as a basis from which predicted data were calculated can be deduced from the colouring of the data points shown in the list at the top left. Experimental log LC_{50} values increase in the order of pH 5, 6, 8 and 9 (taken from Köhler et al. (2023)), pH 5.5, 7, and 8 (taken from Bittner et al. (2019)). High reliability of predictions is symbolized by linear regression (and 95 % confidence interval) resulting in a close to 1:1 relationship, with a slope of 0.88 and an intercept of 0.12 (r^2 of 0.937). ANOVA revealed a significant correlation at $p < 0.0001$.

The lowest acute effect concentrations per taxonomic group are listed in Table 5 along with values corrected for logD at pH 6.5, with the lowest reported value being the 96 h LC_{50} of 2462 µg/L for acute mortality of *Physalaemus albonotatus* embryos at pH 7.5. This effect concentration corresponds to an adjusted (i.e. worst-case) LC50 of 437 µg/L

Table 4
Summary of reliable and relevant log P data for diclofenac. GM: geometric mean.

Entry	log P	Temp °C	pH	Experimental method	Reference
1	4.0	25	2.0	Stirring. filtration. spectrophotometry	(Fini et al., 1986)
2	4.40	RT	2.0	Shake flask. spectrophotometry	(La Rotonda et al., 1983)
3	4.43	RT	1.0	Shake flask. spectrophotometry	(Barbato et al., 1986)
4	4.4	RT	2.0	Shake flask. spectrophotometry	(Barbato et al., 1986)
5	4.51	25		pH-metric titration	(Avdeef et al., 1998)
GM	4.34				

Table 5
Lowest relevant and reliable acute and chronic effect concentrations per group of organisms selected from Tables S4 and S5. The lowest acute and chronic values are underlined. Please see Tables S4 and S5 for further details on the selected studies.

Group	Species	Duration	Study pH	Effect concentration	Value [µg/L]	Value [µg/L] Adjusted for logD at pH 6.5	Reference
Basic acute data							
Algae	*Desmodesmus subspicatus*	72 h	8.0–8.2	EC50	60,440	6542	Doležalová Weissmannová et al. (2018)
Crustaceans	*Tisbe battagliai*	48 h	8.0 ± 0.2	EC50	9500	1085	Trombini et al. (2016)
Fish	*Danio rerio*	72 h	8.07	LC50	7800	825	van den Brandhof and Montforts (2010)
Additional acute data							
Amphibian	*Physalaemus albonotatus*	96 h	7.5 ± 0.1	LC50	2462	437	Peltzer et al. (2019)
Basic chronic data							
Algae	*Desmodesmus subspicatus*	96 h	8.0–8.2	NOEC	15,540	1682	Doležalová Weissmannová et al. (2018)
Crustaceans	*Daphnia magna*	21 d	7.61 ± 0.35	NOEC	120	19	Du et al. (2016)
Fish	*Salmo trutta* (*juvenile*)	25 d	8.5 ± 0.04	NOEC	3.5	0.33	Schwarz et al. (2017)
Additional chronic data							
Gastropod	*Lymnaea stagnalis*	28 d	7.29–8.24	NOEC	1540	202	
Bivalve	*Dreissena polymorpha*	171 d	7.5–8.0	NOEC	0.37	0.05	Joachim et al. (2021)

at pH 6.5. As the standard deviation of all log10 effect values listed in Table S6 is >0.5, the AF cannot be lowered from 100 to 10 (EC, 2018). The resulting MAC-EQS$_{AF}$ is thus 24.62 µg/L for non-adjusted data, and 4.37 µg/L for pH 6.5-adjusted data, corresponding to an adjustment ratio of 5.6. For comparison, the MAC-EQS$_{AF}$ based on the same data adjusted to pH 7 would be 11.23 µg/L.

3.5.2. Chronic EQS (QS$_{fw,eco}$)

The final dataset for chronic aquatic toxicity consists of 12 endpoints from 12 species. The two lowest effect concentrations are for mortality in bivalves and fish. The species represent six taxonomic groups. As four orders of fish (Beloniformes, Salmoniformes, Perciformes, Cypriniformes; Table S9) are present, another order can be included as "additional vertebrate data" resulting in seven taxonomic groups. Nevertheless, the dataset does not meet the criteria for SSD modelling according to the EU TGD 27 (i.e., at least eight taxonomic groups). Data were normally distributed (p = 0.01, Anderson-Darling, Kolmogorov-Smirnov, Cramer von Mises tests), irrespective of the correction applied. The resulting SSDs for original and adjusted effect data are shown as overlay in Fig. 2 and separately in Fig. S4. The comparatively large ratio of the upper limit to the lower limit of derived the HC5 (sprHC5) is due to the wide distribution of chronic toxicity data (see above) and the comparatively low number of relevant and reliable data. The resulting HC5 spread (sprHC5) between the upper and the lower limits was decreased from 234 to 174 by adjusting the effect data (Table 6).

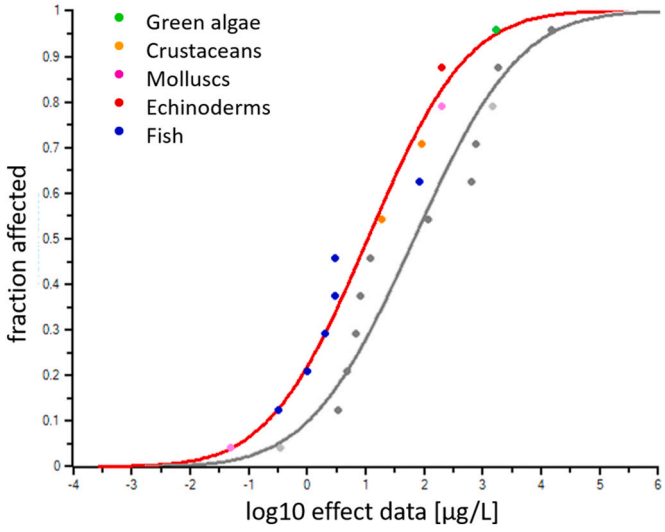


Fig. 2. SSD models for original chronic toxicity data (grey symbols) and logD-adjusted chronic toxicity data (in colour). The resulting HC5 values are 0.2845 µg/L (0.0094–2.2040 µg/L) for the original data and 0.06 µg/L (0.0024–0.4130 µg/L) for the adjusted data.

The application of an additional AF of 5 to the respective HC5 resulted in an QS$_{fw,eco,SSD}$ of 0.057 µg/L for the non-adjusted values, and of 0.012 µg/L based on the pH 6.5-adjusted values, corresponding to an adjustment ratio of 4.6.

For the EQS derivation using the deterministic approach, the lowest chronic effect concentrations per group of organisms are listed in Table 5, along with values corrected for logD at pH 6.5 (for pH 7 see Table S10). The lowest effect value is the NOEC of 0.37 µg/L for mortality in *Dreissena polymorpha*. This effect concentration corresponds to 0.05 µg/L diclofenac at pH 6.5. With regard to the lowest effect value in fish, it is the NOEC of 3.5 µg/L for mortality in *Salmo trutta*. This effect concentration corresponds to 0.33 µg/L diclofenac at pH 6.5. In case of chronic effect concentrations being available for three species representing different living and feeding conditions (or trophic levels), the EU TGD for EQS recommends the application of an assessment factor of 10 to the lowest credible datum (EC, 2018). The application of an AF of 10 to the lowest credible chronic datum results in an QS$_{fw,eco,AF}$ = 0.037 µg/L for non-corrected values, or 0.005 µg/L for the pH 6.5-corrected values, corresponding to an adjustment ratio of 7.4. In case the pH-adjustment would be limited to fish, a QS$_{fw,eco,AF}$ = 0.35 µg/L would

Table 6
HC5 obtained for SSDs modelled for original effect data as well as effect data adjusted for logD at pH 6.5. Lower limits (LL), upper limits (UL) and the ratio of UL to LL (spread; sprHC5) are also listed. The unit of all values is µg/L. Plots of the corresponding SSDs are shown in Fig. S4.

Dataset	LL HC5	HC5	UL HC5	sprHC5
Original data	0.0094	0.2845	2.2040	234
pH 6.5-adjusted	0.0024	0.0600	0.4130	174

be derived for the non-adjusted NOEC, while the pH 6.5-corrected value would result in a value of 0.033 μg/L, corresponding to a 10.6-times lower threshold. For comparison, the $QS_{fw,eco,AF}$ based on the same data adjusted to pH 7 would be 0.012 μg/L (based on lowest NOEC) and 0.085 μg/L (based on lowest NOEC in fish), respectively.

4. Discussion

By adjusting the measured toxicity of diclofenac at a given pH to the toxicity at other pH values retrieved from empirical *Danio rerio* embryotoxicity tests, we were able to demonstrate usefulness of the Delta logD-based methodology recently proposed by Köhler et al. (2023). While this method has not been verified with external data or been applied to other species, mostly due to the lack of such data (i.e. toxicity at 4 different pH values per compound), we assume a general applicability throughout the domain of eukaryotes. Due to the universal blueprint of the eukaryotic cell, it cannot be assumed that these principles apply exclusively to *D. rerio* or "fish", but rather to all eukaryotes, which justifies the transfer of the results across taxon boundaries. Hence, following the precautionary principle, we conclude that the adjustment of effect data based on logD at the pH value of a given study and the logD at another pH, within the range of the linear relationship between pH 5–9, results in a reasonably robust prediction that could be used for the derivation of EQS. Furthermore, it allows for the extrapolation to a worst-case pH-scenario. Nevertheless, future applications might benefit from a broader validation of this approach.

While the revised OECD Guidance Document 23 (OECD, 2019a) on difficult substances requires ionisable chemicals to be tested at the pH at which the most toxic form of the molecule occurs, aquatic toxicity tests are often based on standardized procedures (i.e. OECD or U.S. EPA testing guidelines) that allow test conditions to range roughly between pH 6 and 9, as well as tolerating pH variations of more than one unit during the tests (e.g., 1.5 pH units in OECD201 on growth inhibition in freshwater algae (OECD, 2011)). The actual test pH in a toxicity study is often related to the test conditions in the testing laboratory (e.g. defined by the well water used) or even may be arbitrarily selected by the experimenter. As a consequence, the potential for uptake of ionisable substances can vary substantially between toxicity studies performed at different pH values or in cases where pH is not kept constant during the test. Within the diclofenac toxicity dataset, reported pH values ranged from 7.0 to 8.1 for the acute toxicity data (except for the study by Schweizer et al. (2021) that was particularly designed to study pH effects on the toxicity of diclofenac to fish embryos) and from 6.85 to 8.5 for the chronic toxicity data. Apart from differences in study pH introducing artificial variability, pH values ≫7 can be considered as "best case", resulting in a lower uptake, an underestimation of the actual effects expected in the environment and in setting of EQS values that are too high to achieve the desired conservation target of 95 % of species and environmental conditions. The ratios of original to pH-corrected effect values ranged from 0.33 to 11.4 (on average 6.7) and hence were in the range of or even exceeded the assessment factors prescribed for EQS derivation (i.e., AF 10 for acute and chronic data (EC, 2018)). This means that when an EQS is based on non-adjusted effect concentrations, the standard assessment factor is barely sufficient to account for the higher bioavailability at lower pH values (in case of the weak acid Diclofenac). Consequently, any other uncertainty that assessment factors are meant to account for (e.g. inter-laboratory variability, extrapolation from the laboratory to the field, inter-species variability in sensitivity, etc.) would not be covered, adding to the lack of protectiveness of EQS determined based on non-adjusted data. The ratios of the EQS values derived from original to those derived from pH-adjusted data were 4.6 for $QS_{fw,eco,SSD}$, 7.4 for $QS_{fw,eco,AF}$, and 5.6 for $MAC\text{-}EQS_{AF}$, respectively. Please note that these ratios are actually lower than for most original and adjusted effect values used for SSD modelling. Nevertheless, the derived chronic $QS_{fw,eco}$ (i.e. SSD and AF) are in the same order of magnitude, and differed only by a factor of 1.5 (non-adjusted) and 2.4 (adjusted).

Despite the relatively large number of tests that had to be excluded from our analysis due to lack of information on study pH, our approach resulted into an even lower chronic $QS_{fw,eco,AF}$ (0.005 μg/L) for the adjusted data as compared to the AA-EQS for freshwater in the Dossier of JRC (0.04 μg/L). Hence, we recommend that the value proposed in the current JRC EQS dossier should not be increased. Instead, we suggest to take the adjustment of the effect data for the study pH into account before deriving an EQS, considering the findings of this study.

We noticed that the test pH values used in aquatic toxicity studies are often much higher than the worst-case allowed in the respective test guideline, possibly related to the locally available water or laboratory routines. We recommend that for commissioned tests, laboratory facilities should be selected according to the pH commonly used in their tests and that test species are being adapted to the required worst-case pH conditions. Based on our experience, we believe that there is a general need for test laboratories offering standard tests at low pH values (<7).

The selection of pH 6.5 as an environmentally relevant worst-case default value was based on measured pH values in surface waters in temperate climates and pH values recommended by standard test guidelines, i.e. those tolerated by most test species. Of course, the (necessarily arbitrary) setting of this default pH value has substantial influence on the resulting QS. In the context of EQS derivation for national purposes or for the WFD, standard worst-case pH values for freshwaters should be determined, for both acids and bases (e.g., pH 6.5 and pH 8.5, respectively). Applying a worst-case pH would also allow for protecting most water bodies within the range of reported pH values, and treat ionisable substances consistently independent of the respective pH-logD relationship. An example for the application of this procedure is the non-steroidal analgesic drug ibuprofen, for which the delta logD adjustment was applied recently, resulting in an EQS eight times lower than without a pH adjustment being made (SCHEER, 2023). Such defined standard pH values would allow to take the differences in uptake potential of ionisable substances at study pH compared to worst-case conditions into account.

The EU TGD 27 describes specific considerations as to compartmentalization and bioavailability of lipophilic substances and metals in the environment. For lipophilic substances with a logKow or Koc exceeding a certain threshold, dissolved organic carbon concentrations need to be used for normalization of effect values. For metals, a correction for bioavailability is performed based on modelled speciation at test conditions. In the case of nickel, for example, corrected EQS are used in a site-specific risk assessment under the WFD to account for bioavailability under local conditions, like total organic carbon and hardness (EC, 2011). For ionisable organic substances such as diclofenac, specific procedures are not described. In view of the impact of pH on the uptake of ionisable organic substances and thus potential adverse effects in aquatic organisms, it seems reasonable to define procedures under the WFD to take this into account in future EQS derivations.

Around 50 % and 70 % of the available effect data had to be excluded from the datasets for chronic and acute toxicity, respectively, due to the lack of either analytical verification of exposure concentrations (~30 %) or reported pH values (33 %), or both (~37 %). This resulted in the exclusion of rarely tested species and entire groups of organisms, making the final dataset used less robust. Based on our experience with EQS derivation (Ecotox Centre, 2023), this is a typical case, especially for substances not authorised as plant protection products or biocides. We thus strongly encourage the determination and full reporting of these parameters, especially in scientific publications. Based on a literature search performed for the recent draft EQS dossier for diclofenac, other general data gaps were noted, too (JRC, 2022a). For example, insect toxicity data were not available. Amphibians are the most sensitive group in the logD corrected acute dataset, except for a single effect concentration in *D. rerio* at a worst-case of pH 6. However, amphibians are not represented in the chronic dataset. Consequently, to account for the remaining uncertainty, the assessment factor cannot be lowered

from 100 to 10. In contrast, bivalves are the most sensitive group in the original and logD corrected chronic toxicity dataset, but are not represented in the acute toxicity dataset. This data gap has been considered less relevant in the past, due to the expected chronic type of exposure to diclofenac and other drug substances in surface waters. However, time-resolved measurements have shown that also peak concentrations of drugs occur, e.g. linked to production processes (GalPro, 2023), making a robust acute EQS likewise necessary. Whether other non-published effect data are available for these groups of organisms is unknown to the authors. A common public database (e.g., NORMAN (2023)) of toxicity and bioaccumulation data, as well as physicochemical properties, irrespective of the use of a substance, would likewise facilitate identifying data gaps and to derive a harmonized assessment, following the idea of one substance – one assessment.

5. Conclusions

In our meta-study, more than half of the available effect data (including rarely tested species and certain groups of organisms) had to be excluded from our analysis due to lack of analytical verification and especially the lack of reported pH values, considering the ionisable nature of diclofenac. We therefore recommend that pH values and exposure concentrations should always be determined and fully reported in toxicity studies. We likewise encourage measurement of physicochemical parameters at environmentally relevant conditions with methods adapted to ionisable substances as the majority of reported values did not meet these criteria. The generally wide range of pH values allowed in toxicity test guidelines that is often utilized in toxicity tests, potentially resulted in an increased heterogeneity of the effect data. Data variability decreased upon pH-dependent Delta logD adjustments, resulting in a substantially lower worst-case EQS. We recommend that Delta logD-based adjustments that represent a worst-case pH scenario should be considered to be mandatory for the hazard assessment and EQS derivation of ionisable substances. To this end, we recommend that two environmentally relevant worst-case pH values for freshwaters should be defined for acids and bases (e.g. 6.5 and 8.5), based on the ecological tolerance of the organisms of interest and pH of water bodies, that are mandatory to comply with in aquatic toxicity tests submitted for authorization and registration of ionisable chemicals. Whenever pH values deviate from such conditions, Delta logD-based corrections should be used to derive save thresholds or foster the provision of appropriate effect data.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Peter von der Ohe, Alexandra Kroll and Marion Junghans are regularly involved with EQS derivation for the Working Group Chemicals of the EC. Marion Junghans was involved in the group of experts that authored the current EU EQS dossier for diclofenac.

Data availability

Data will be made available on request.

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The conclusions expressed in this paper represent the expert judgement of the authors, but not necessarily the opinion of their affiliation.

CRediT authorship contribution statement

Alexandra Kroll conceived the concept, managed the project, analysed data, wrote the manuscript.

Peter C. von der Ohe contributed to the effects assessment on zebrafish and the concept of logD-based adjustment, wrote the manuscript.

Heinz-R. Köhler coordinated the effects assessment on zebrafish and the concept of logD-based adjustment, wrote the manuscript.

Odile Sellier performed data research and assessment.

Marion Junghans assisted the concept definition and project management, reviewed the manuscript.

Appendix A. Supplementary data

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References

Acuña, V., Ginebreda, A., Mor, J.R., Petrovic, M., Sabater, S., Sumpter, J., et al., 2015. Balancing the health benefits and environmental risks of pharmaceuticals: diclofenac as an example. Environ. Int. 85, 327–333.

Alessandretti, I., Rigueto, C.V.T., Nazari, M.T., Rosseto, M., Dettmer, A., 2021. Removal of diclofenac from wastewater: a comprehensive review of detection, characteristics and tertiary treatment techniques. J. Environ. Chem. Eng. 9, 106743.

Altmann, J., Ruhl, A.S., Zietzschmann, F., Jekel, M., 2014. Direct comparison of ozonation and adsorption onto powdered activated carbon for micropollutant removal in advanced wastewater treatment. Water Res. 55, 185–193.

Avdeef, A., Box, K.J., Comer, J.E., Hibbert, C., Tam, K.Y., 1998. pH-metric logP 10. Determination of liposomal membrane-water partition coefficients of ionizable drugs. Pharm. Res. 15, 209–215.

Avdeef, A., Fuguet, E., Llinas, A., Rafols, C., Bosch, E., Volgyi, G., et al., 2016. Equilibrium solubility measurement of ionizable drugs - consensus recommendations for improving data quality. Admet Dmpk 4, 117–178.

Barbato, F., Caliendo, G., La Rotonda, M.I., Silipo, C., Toraldo, G., Vittoria, A., 1986. Distribution coefficients by curve fitting: application to ionogenic nonsteroidal antiinflammatory drugs. Quant. Struct.-Act. Relat. 5, 88–95.

Barceló, D., Petrovic, M., 2007. Pharmaceuticals and personal care products (PPCPs) in the environment. Anal. Bioanal. Chem. 387, 1141–1142.

Bittner, L., Teixido, E., Seiwert, B., Escher, B.I., Klüver, N., 2018. Influence of pH on the uptake and toxicity of β-blockers in embryos of zebrafish, *Danio rerio*. Aquat. Toxicol. 201, 129–137.

Bittner, L., Klüver, N., Henneberger, L., Mühlenbrink, M., Zarfl, C., Escher, B.I., 2019. Combined ion-trapping and mass balance models to describe the pH-dependent uptake and toxicity of acidic and basic pharmaceuticals in zebrafish embryos (Danio rerio). Environ. Sci. Technol. 53, 7877–7886.

Bundschuh, M., Weyers, A., Ebeling, M., Elsaesser, D., Schulz, R., 2016. Narrow pH range of surface water bodies receiving pesticide input in Europe. Bull. Environ. Contam. Toxicol. 96, 3–8.

Buser, H.-R., Poiger, T., Müller, M.D., 1998. Occurrence and fate of the pharmaceutical drug diclofenac in surface waters: rapid photodegradation in a lake. Environ. Sci. Technol. 32, 3449–3456.

Carvalho, R.N., Ceriani, L., Ippolito, A., Lettieri, T., 2015. Development of the First Watch List Under the Environmental Quality Standards Directive.

De Vivo, B.A., Louise, Bidovec, M., Lima, A., Pirc, S., Reeder, Shaun, Siewers, U., Smith, Barry, Albanese, S., Batista, M.J., Bel-lan, A., Birke, M., Breward, Neil, Demetriades, A., De Vos, W., Duris, M., Gravesen, P., Gregoraukiene, V., Halamic, J., Jordan, G., Lax, K., Locutura, J., O'Connor, P.J., Pasieczna, A., Slaninka, I., Tarvainen, T., 2006. Distribution of elements in stream water. In: De Vos .T., W.T, Reeder, S. (Eds.), Geochemical Atlas of Europe. Part 2: Interpretation of Geochemical Maps, Additional Tables, Figures, Maps and Related Publications.

Doležalová Weissmannová, H., Pavlovský, J., Fišerová, L., Kosárová, H., 2018. Toxicity of diclofenac: cadmium binary mixtures to algae Desmodesmus subspicatus using normalization method. Bull. Environ. Contam. Toxicol. 101, 205–213.

Du, J., Mei, C.F., Ying, G.G., Xu, M.Y., 2016. Toxicity thresholds for diclofenac, acetaminophen and ibuprofen in the water flea Daphnia magna. Bull. Environ. Contam. Toxicol. 97, 84–90.

EC, 2011. Nickel EQS Dossier, Prepared by the Sub-Group on Review of the Priority Substances List (Under Working Group E of the Common Implementation Strategy for the Water Framework Directive).

EC, 2018. Technical Guidance for Deriving Environmental Quality Standards Environment, Guidance Document No. 27, Updated version 2018, Document Endorsed by EU Water Directors at Their Meeting in Sofia on 11–12 June 2018.

EC, 2022. Proposal for a DIRECTIVE OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL amending Directive 2000/60/EC establishing a framework for Community action in the field of water policy, Directive 2006/118/EC on the protection of groundwater against pollution and deterioration and Directive 2008/105/EC on environmental quality standards in the field of water policy. Brussels, 26.10.2022 COM(2022) 540 final 2022/0344 (COD).

Ecotox Centre, 2023. Quality Criteria for Surface Waters and Sediments.
EMA, 2019. List of nationally authorised medicinal products. Active substance: diclofenac (systemic formulations). https://www.ema.europa.eu/en/documents/psusa/diclofenac-systemic-formulations-list-nationally-authorised-medicinal-products-psusa/00001048/201809_en.pdf.
Fini, A., Laus, M., Orienti, I., Zecchi, V., 1986. Dissolution and partition thermodynamic functions of some nonsteroidal anti-inflammatory drugs. J. Pharm. Sci. 75, 23–25.
GalPro, 2023. Active ingredient losses from galenic pharmaceutical plants via operational wastewater - GalPro. https://www.eawag.ch/en/department/uchem/projects/galpro/.
Hartmann, J., Lauerwald, R., Moosdorf, N., 2019. GLORICH - global river chemistry database. Supplement to: Hartmann, J et al. (2014): a brief overview of the GLObal RIver Chemistry Database, GLORICH. Procedia Earth Planet. Sci. 10, 23–27. https://doi.org/10.1016/j.proeps.2014.08.005. PANGAEA.
Joachim, S., Beaudouin, R., Daniele, G., Geffard, A., Bado-Nilles, A., Tebby, C., et al., 2021. Effects of diclofenac on sentinel species and aquatic communities in semi-natural conditions. Ecotoxicol. Environ. Saf. 211, 111812.
JRC, 2022a. EQS Datasheet Environmental Quality Standard Diclofenac, EC Joint Research Centre. https://circabc.europa.eu/ui/group/9ab5926d-bed4-4322-9aa7-9964bbe8312d/library/1910d2be-da0c-4354-89e1-4737b8a04536?p=1&n=10&sort=modified_DESC.
JRC, 2022b. EQS Datasheet Environmental Quality Standard Ibuprofen, EC Joint Research Centre. https://circabc.europa.eu/ui/group/9ab5926d-bed4-4322-9aa7-9964bbe8312d/library/cc88646f-1a58-45da-8b93-9f25a6564013?p=1&n=10&sort=modified_DESC.
Kincl, M., Meleh, M., Veber, M., Vrečer, F., 2004. Study of physicochemical parameters affecting the release of diclofenac sodium from lipophilic matrix tablets. Acta Chim. Slov. 51, 409–425.
Köhler, H.-R., Gräff, T., Schweizer, M., Blumhardt, J., Burkhardt, J., Ehmann, L., et al., 2023. LogD-based modelling and ΔlogD as a proxy for pH-dependent action of ionizable chemicals reveal the relevance of both neutral and ionic species for fish embryotoxicity and possess great potential for practical application in the regulation of chemicals. Water Res. 235, 119864.
Korkaric, M., Junghans, M., Pasanen-Kase, R., Werner, I., 2019. Revising environmental quality standards: lessons learned. Integr. Environ. Assess. Manag. 15, 948–960.
La Rotonda, M.I., Amato, G., Barbato, F., Silipo, C., Vittoria, A., 1983. Relationships between octanol-water partition data, chromatographic indices and their dependence on pH in a set of nonsteroidal anti-inflammatory drugs. In: Quantitative Structure-Activity Relationships, 2, pp. 168–173.
Leverett, D., Merrington, G., Crane, M., Ryan, J., Wilson, I., 2021. Environmental quality standards for diclofenac derived under the European Water Framework Directive: 1. Aquatic organisms. Environ. Sci. Eur. 33, 133.
Lonappan, L., Brar, S.K., Das, R.K., Verma, M., Surampalli, R.Y., 2016. Diclofenac and its transformation products: environmental occurrence and toxicity - a review. Environ. Int. 96, 127–138.
Maack, G., Schudoma, D., 2014. In: UBA (Ed.), UQN-Datenblatt: ENVIRONMENTAL QUALITY STANDARD DICLOFENAC (DRAFT).
Manallack, D.T., 2007. The pK(a) distribution of drugs: application to drug discovery. Perspect. Med. Chem. 1, 25–38.
McGettigan, P., Henry, D., 2013. Use of non-steroidal anti-inflammatory drugs that elevate cardiovascular risk: an examination of sales and essential medicines lists in low-, middle-, and high-income countries. PLoS Med. 10, e1001388.
Moermond, C.T., Kase, R., Korkaric, M., Ågerstrand, M., 2016. CRED: criteria for reporting and evaluating ecotoxicity data. Environ. Toxicol. Chem. 35, 1297–1309.
NORMAN, 2023. NORMAN Ecotoxicology Database.
OECD, 2011. Test No. 201: Freshwater Alga and Cyanobacteria, Growth Inhibition Test.
OECD, 2019a. Guidance Document on Aquatic Toxicity Testing of Difficult Substances and Mixtures.
OECD, 2019b. Test No. 203: Fish, Acute Toxicity Test.
Panchal, N., Kaur, M., Tharmatt, A., Thakur, S., Jain, S.K., 2020. Development, characterization and evaluation of parenteral formulation of diclofenac sodium. AAPS PharmSciTech 21, 219.
Peltzer, P.M., Lajmanovich, R.C., Martinuzzi, C., Attademo, A.M., Curi, L.M., Sandoval, M.T., 2019. Biotoxicity of diclofenac on two larval amphibians: assessment of development, growth, cardiac function and rhythm, behavior and antioxidant system. Sci. Total Environ. 683, 624–637.
Perlovich, G.L., Surov, A.O., Hansen, L.K., Bauer-Brandl, A., 2007. Energetic aspects of diclofenac acid in crystal modifications and in solutions—mechanism of solvation, partitioning and distribution. J. Pharm. Sci. 96, 1031–1042.
Peters, A., Wilson, I., Merrington, G., Schlekat, C., Middleton, E., Garman, E., 2022. Assessing the extent of environmental risks from nickel in European freshwaters: a critical reflection of the European Commission's current approach. Environ. Toxicol. Chem. 41, 1604–1612.
Rendal, C., Kusk, K.O., Trapp, S., 2011. Optimal choice of pH for toxicity and bioaccumulation studies of ionizing organic chemicals. Environ. Toxicol. Chem. 30, 2395–2406.
Sathishkumar, P., Meena, R.A.A., Palanisami, T., Ashokkumar, V., Palvannan, T., Gu, F. L., 2020. Occurrence, interactive effects and ecological risk of diclofenac in environmental compartments and biota - a review. Sci. Total Environ. 698, 134057.
SCHEER, 2023. Scientific Opinion on "Draft Environmental Quality Standards for Priority Substances under the Water Framework Directive" - Ibuprofen Final Opinion - CORRIGENDUM of 26 January 2023. https://health.ec.europa.eu/publications/scheer-scientific-opinion-draft-environmental-quality-standards-priority-substances-under-water-6_en.
Schwarz, S., Schmieg, H., Scheurer, M., Köhler, H.-R., Triebskorn, R., 2017. Impact of the NSAID diclofenac on survival, development, behaviour and health of embryonic and juvenile stages of brown trout, Salmo trutta f. fario. Sci. Total Environ. 607-608, 1026–1036.
Schweizer, M., von der Ohe, P.C., Gräff, T., Kühnen, U., Hebel, J., Heid, C., et al., 2021. Heart rate as an early warning parameter and proxy for subsequent mortality in Danio rerio embryos exposed to ionisable substances. Sci. Total Environ. 151744.
Simon, E.W., Beevers, H., 1952. The effect of pH on the biological activities of weak acids and bases I. The most usual relationship between pH and activity. New Phytol. 51, 163–190.
Trombini, C., Hampel, M., Blasco, J., 2016. Evaluation of acute effects of four pharmaceuticals and their mixtures on the copepod Tisbe battagliai. Chemosphere 155, 319–328.
van den Brandhof, E.-J., Montforts, M., 2010. Fish embryo toxicity of carbamazepine, diclofenac and metoprolol. Ecotoxicol. Environ. Saf. 73, 1862–1866.
Vlaardingen, PlAv, Traas, T.P., Wintersen, A.M., Aldenberg, T., 2004. ET X 2.0 A Program to Calculate Hazardous Concentrations and Fraction Affected, Based on Normally Distributed Toxicity Data; RIVM Report 601501028/2004.