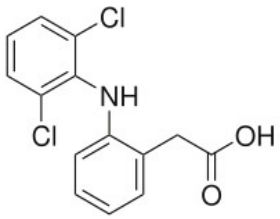
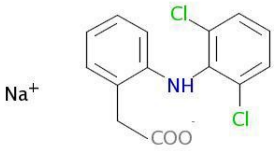
Miljøministeriet
Miljøstyrelsen

Fastsættelse af kvalitetskriterier for vandmiljøet

Diclofenac

CAS nr. 15307-86-5 (Diclofenac)
CAS nr. 15307-79-6 (Diclofenac natrium salt)





Vandkvalitetskriterium	$VKK_{ferskvand}$	0,04 µg/l
Vandkvalitetskriterium	$VKK_{saltvand}$	0,004 µg/l
Korttidsvandkvalitetskriterium	$KVKK_{ferskvand}$	246 µg/l
Korttidsvandkvalitetskriterium	$KVKK_{saltvand}$	25 µg/l
Sedimentkvalitetskriterium	$SKK_{ferskvand}$	Ikke relevant
Sedimentkvalitetskriterium	$SKK_{saltvand}$	Ikke relevant
Biota-kvalitetskriterium, sekundær forgiftning	$BKK_{sek.forgiftn.}$	1,16 µg/kg vådvægt (musling)
Biota-kvalitetskriterium, human konsum	HKK	61,35 µg/kg vådvægt

December 2022

Dansk resumé og konklusioner

Diclofenac er et organisk stof, der tilhører stofgruppen af derivater afledt af stoffet phenyl-eddikesyre. Stoffet anvendes farmaceutisk som et anti-inflammatorisk middel mod smerter i muskler og led, herunder blandt andet mod leddegigt og slidgigt.

Stoffets fysisk-kemiske egenskaber, dets fordeling imellem forskellige miljøer, dets skæbne via abiotisk og biotisk nedbrydning, samt dets biologiske effekter i det eksterne miljø er sammenfattet og vurderet af det Fælles Europæiske Forskningscenter JRC (JRC, 2022)[1], der på det fremlagte datagrundlag har bearbejdet data og beregnet miljøkvalitetskrav. Arbejdet og rapporteringen har været kommenteret af Europa-Kommissionens videnskabelige komite for sundhed og miljø, (SCHEER, 2022)[2].

Metodikken, der anvendes til udarbejdelse af miljøkvalitetskrav, er harmoniseret i EU og baserer sig på Europa-Kommissionens vejledning til fastsættelse af kvalitetskriterier i vandmiljøet (EC, 2018)[3].

Indledningsvist indeholder rapporten en sammenfatning af grundlag og viden om forekomsten af stoffet Diclofenac i relevante eksterne miljøer. Baseret på indrapporterede koncentrationer af Diclofenac i det eksterne miljø, viser den gennemførte screening og statistiske analyse følgende: de påviste og dokumenterede koncentrationer af stoffet Diclofenac i de europæiske staters ferske indlands overfladevande, sat i forhold til tentative kvalitetskriterier baseret på oplysninger om forventet nul-effekt niveau (PNEC: Predicted No Effect Concentration), viser at stoffet Diclofenac udgør en risiko for alle EU-landes indlands overfladevande.

En tilsvarende vurdering af risiko for Diclofenac i de europæiske marine overfladevande kan ikke foretages, idet screeningen viser at de tilvejebragte data fremstår opdelte og utilstrækkelige. Det konkluderes derfor, at datagrundlaget ikke er fuldt udviklet til at vurdere den konkrete risiko for marine overfladevande.

Stoffet er prioriteret til fastlæggelse af relevante kvalitetskriterier på baggrund af screeningen for stoffets tilstedeværelse og koncentration i det eksterne miljø.

Relevante data for stoffets økotoksikologiske effekter er præsenteret og beskrevet i rapporten fra JRC (JRC, 2022). Der er fastsat kvalitetskriterier for relevante specifikke miljøer og biota, for akutte påvirkninger og kroniske effekter, samt for afledte effekter gennem fødekæder, og for relevante indtag og human konsum. Kvalitetskriterier er fastsat på baggrund af resultater,

[1] Joint Research Center (JRC) of the Commission of the European Union: Diclofenac – Final Dossier after SCHEER final opinion – dated September 2022

[2] Scientific committee on Health, Environmental and Emerging Risks (SCHEER) of the Commission of the European Union: final opinion on azithromycin (Publication date 6 May 2022), available on-line at: https://health.ec.europa.eu/publications/scheer-scientific-opinion-draft-environmental-quality-standards-priority-substances-under-water-0_en

[3] European Commission (EC): Technical Guidance for Deriving Environmental Quality Standards – Guidance Document No. 27. Updated version 2018

datakvalitet og bredde i forhold til undersøgte akutte og kroniske effekter på specifikke organismer, trofiske niveauer og forskellige miljøer.

Diclofenac er undersøgt for økotoksikologiske effekter i en lang række studier, der rummer både akutte og kroniske effekter overfor arter indenfor såvel det ferske som det marine miljø på flere end de grundlæggende 3 taksonomiske grupper (alger, krebsdyr og fisk). Studierne er indledningsvist gennemgået for relevans og troværdighed (kvalitet), og tildelt en score i henhold til kriterier fastsat af Klimisch et al. (1997) – R1: troværdig uden restriktioner; R2 – troværdig med restriktioner; R3 – ikke troværdige; R4 – ikke anvendelige. Alene studier med score R1/R2 er præsenteret i rapporten og medtaget i udarbejdelsen af kvalitetskriterierne.

I dette grundlæggende datamateriale af studier med høj kvalitet og troværdighed (R1/R2) for stoffet Diclofenac, findes der mange relevante og solide studier af såvel akutte som kroniske effekter, der dækker minimum 3 taksonomiske grupper, og tillige et modeløkosystem (mesokosmos studie), men der findes alene få studier på arter fra det marine miljø.

Datasættet udgør et omfattende fagligt grundlag for fastsættelse af kvalitetskriterier, men indeholder også data fra mere sensitive arter. På dette grundlag er der for det kroniske datasæt foretaget en undersøgelse af specifikke arters følsomhed ved en statistisk bearbejdning af datasættet ved en SSD-analyse (Sensitive Species Distribution).

Tilgangen til at fastlægge kvalitetskriterier omfatter således en vurdering af datasættet ud fra en deterministisk tilgang og en statistisk tilgang, samt vurdering af resultater i forhold til data fra et modeløkosystem. Samlet set er tilgangen baseret på Europa-Kommissionens vejledning til fastsættelse af kvalitetskriterier i vandmiljøet (EC, 2018).

På dette grundlag er der foretaget vurderinger i henhold til fremgangsmåden fastsat i Europa-Kommissionens vejledning til fastsættelse af kvalitetskriterier i vandmiljøet (EC, 2018). Grundlag og metode for fastsættelse af kvalitetskriterier er generelt beskrevet for de konkrete miljøer og medier.

Korttidsvandkvalitetskriterium (KVKK)
Datagrundlaget for fastsættelse af KVKK er som udgangspunkt studier af de akutte effektniveauer for et stof, og herfra etablering af en acceptabel maksimal koncentration i relevante eksterne miljøer, der over kort tid ikke fører til uønskede effekter i disse miljøer.

Det samlede datasæt for den anvendte deterministiske metode indeholder relevante studier af akutte effekter, der omfatter en række studier som også går ud over det fastsatte minimum af tre trofiske niveauer (alger, krebsdyr og fisk). Datasættet omfatter taksonomiske grupper af potentielt sensitive arter, men for det marine miljø er datasættet relativt svagt, hvorfor datasættet for ferskvand og saltvand slås sammen. Den anvendte usikkerhedsfaktor er på baggrund heraf sat til 10 for ferskvand og 100 for saltvand jf. vejledningen (EC, 2018).

Med udgangspunkt i laveste LC_{50} værdi på 2.462 µg/l for et 96-timers studie af dødelighed hos padden *Physalaemus albonotatus* kan der, med afsæt i den deterministiske metode, fastlægges følgende KVKK-værdier:

$KVKK_{ferskvand}$ = 2.462 µg/l / 10 = 246,2 µg/l (afrundet til 246 µg/l)

$KVKK_{saltvand}$ = 2.462 µg/l / 100 = 24,62 µg/l (afrundet til 25 µg/l)

Det bør noteres at der formentlig er fejl i rapportens tabel 6.4. I tabellen fremgår at $KVKK_{ferskvand}$ og $KVKK_{saltvand}$ er fastsat på baggrund af test på *Dugesia japonica* (Li, 2013) med usikkerhedsfaktor på hhv. 10 og 100, resulterende i værdi på hhv. 420 og 42.Jævnfør afsnit 3.1 og beregningsafsnit 6.4.1.1 og 6.4.1.2 fremgår, at test udført med *Physalaemus albonotatus* (Peltzer et al., 2019) er anvendt resulterende i værdierne på hhv. 246 og 25 µg/l.

Vandkvalitetskriterium (VKK)
Datagrundlaget for fastsættelse af VKK er som udgangspunkt studier af de kroniske effektniveauer for et stof, og herfra etablering af en acceptabel koncentration i relevante eksterne miljøer, der ikke fører til uønskede langtidseffekter i disse miljøer.

Det samlede datasæt af relevante studier af kroniske effekter er omfangsrigt, og omfatter mange studier ud over det fastsatte minimum af 3 trofiske niveauer (alger, krebsdyr og fisk). Datasættet omfatter taksonomiske grupper af potentielt sensitive arter, men for det marine miljø er datasættet relativt svagt, hvorfor datasættet for ferskvand og saltvand slås sammen.

Den statistiske analyse af det kroniske datasæt ved statistisk SSD-analyse viser, at data er grupperede og fordelingerne i de tre grupper er forskellige (figur 6.2). På denne baggrund er tilgangen med fastsættelse af kvalitetskriterier ved anvendelse af SSD fravalgt.

Det gennemgåede studie af et modeløkosystem (mesokosmos studie) strækker sig over en periode på fem måneder, og indeholder data for NOEC (No Observed Effect Concentration) på både arts-, populations- og samfundsniveauer. Der er rapporteret om udfordringer med at fastholde ensartede forhold for konkrete miljøparametre i det anvendte modeløkosystem under hele forsøgets varighed, samt om problemer med dødelighed i upåvirkede kontrolsystemer. SCHEER anbefaler på dette grundlag, at der ikke anvendes NOEC data frembragt for specifikke arter (SCHEER, 2022). SCHEER finder dog overordnet, at de fremlagte data og konklusioner for et NOEC-niveau på 0,44 µg/l for populations- og samfundsniveauer kan anvendes som sigtelinje for fastlæggelse af et vandkvalitetskriterium.

På artsniveau er den laveste EC_{10}-værdi fundet til en værdi på 1,7 µg/l for et studie af vækst i plantekulturer hos arten *Lemna minor.* Denne værdi fravælges i forhold til den lavere NOEC-værdi fra mesokosmos studiet dækkende populations- og samfundsniveauer jf. anbefalingen fra SCHEER.

Med udgangspunkt i NOEC-værdien på 0,44 µg/l fra mesokosmos studiet og anvendelse af den deterministiske metode med en usikkerhedsfaktor på 10 for ferskvand og 100 for saltvand jf. vejledningen (EC, 2018) kan der fastsættes følgende VKK-værdier:

$VKK_{ferskvand}$ = 0,44 µg/l / 10 = 0,044 µg/l (afrundet til 0,04 µg/l)
$VKK_{saltvand}$ = 0,44 µg/l / 100 = 0,0044 µg/l (afrundet til 0,004 µg/l)

Kvalitetskriterium for sediment (SKK)
I henhold til retningslinjer i Europa-Kommissionens vejledning til fastsættelse af kvalitetskriterier i vandmiljøet (EC, 2018), skal der kun udarbejdes kriterier for sediment med henblik på at beskytte det bundlevende dyreliv mod forgiftning, såfremt der er evidens for, at et stof har potentiale for at kunne adsorbere til suspenderede stoffer og sediment.

Diclofenac har estimerede og eksperimentelt bestemte værdier for log Koc omkring 1 – 4 l/kg og tilsvarende for log Kow omkring 1 – 4 l/kg. Den store variation i binding og fordeling til organisk materiale skyldes overvejende stoffets egenskaber, herunder især at Diclofenac er en svag syre med en pKa værdi på 4, og derfor overvejende findes på ioniseret form under miljørelevante pH-forhold. De mest miljørelevante log Koc og log Kow værdier ligger derfor i intervallet 2 – 3 l/kg.

Der er i det tilvejebragte datagrundlag ikke fremkommet oplysninger om særlige effekter på bundlevende (bentiske) organismer, og miljørelevante log Koc og log Kow værdier ligger i intervallet 2 – 3 l/kg. Derved er kravet om fastsættelse af kriterium for sediment ved at værdierne overskrider den udløsende værdi på 3, ikke opfyldt.

Der er ikke tilvejebragt konkrete data fra undersøgelser af toksicitet for stoffet Diclofenac i sediment, men da adsorption til organisk stof knyttet til sedimenter vurderes at være lille, vurderes sedimentlevende organismer at være beskyttet ved kvalitetskriterierne for vand.

$SKK_{ferskvand}$ = - µg/kg tørvægt
$SKK_{saltvand}$ = - µg/kg tørvægt

Kvalitetskriterium for biota, sekundær forgiftning ($BKK_{sek.\ forgiftn.}$)
I henhold til retningslinjer i Europa-Kommissionens vejledning til fastsættelse af kvalitetskriterier i vandmiljøet (EC, 2018), skal der kun udarbejdes kriterier for biota med henblik på at beskytte dyrelivet mod sekundær forgiftning, såfremt der er evidens for, at et stof har et potentiale for at kunne bioakkumulere.

Diclofenac har estimerede og eksperimentelt bestemte værdier for log Kow omkring 3 – 4 l/kg, men under miljørelevante pH-forhold omkring 2 – 3 l/kg. Stoffet viser relativt lille tendens til at bioakkumulere (BAF) og lav opkoncentrering gennem fødekæder (TMF). I rapporten er gennemgået resultater fra en række studier af stoffets evne til at bioakkumulere og opkoncentrere gennem fødekæder, og grundlæggende fastslås, at bløddyr vurderes at være den mest kritiske artsgruppe ud fra feltbaserede undersøgelser af bioakkumulation. En BAF-værdi på 216 l/kg som geometrisk middelværdi fra relevante studier vurderes repræsentativ.

Disse oplysninger udløser beregning af kvalitetskriterier for biota baseret på indtag, der kan føre til sekundær forgiftning for biota ($BKK_{sek.\ forgiftn.}$).

Der er gennemgået en længere række af undersøgelser fokuseret på erkendt forgiftning med Diclofenac af gribbe på det indiske kontinent. Undersøgelserne frembringer data fra såvel nært beslægtede fuglearter som fugle af andre slægter. Datagrundlaget af frembragte LD_{50}-værdier viser, at gribbe er de mest sensitive for Diclofenac med en værdi på 0,225 mg/kg kropsvægt, men tillige at enkelte andre fuglearter også har tilsvarende sensibilitet.

Beregningsgrundlaget i Method A i Europa-Kommissionens tekniske vejledning (EC, 2018) er anvendt:

Det daglige energibehov (DEE) er bestemt ved anvendelse af den gennemsnitlige kropsvægt for Bengalgribben (*Gyps bengalensis*) på 4,75 kg. Formlen for DEE følger den angivet i Komen (1992):

DEE [kJ/d] = 826,7 x kropsvægt[kg]^{0,61} = 826,7 x 4,75[kg]^{0,61} = 2139 kJ/d

Dosis (LD_{50}) for Bengalgribben er angivet til 0,225 mg/kg kropsvægt. Det noteres at denne dosis er for to dage, hvorfor værdien divideres med en faktor 2, LD_{50} = 0,225 mg/kg lgv / 2 = 0,112 mg/kg lgv/dag. Den energinormaliserede koncentration af føden er bestemt på baggrund af dosis, DEE og kropsvægt:

$K_{energi\ normaliseret}$ [mg/kJ] = dosis x (kropsvægt[kg] / DEE) = 0,112 mg/kg lgv/dag x (4,75[kg] / 2139 kJ/d) = 0,000249 mg/kJ foder = 0,249 µg/kJ foder

Denne værdi korrigeres yderligere da varigheden af et akut fuglestudie generelt er fem dage. Ud fra halveringstid estimeres 88% af koncentrationen efter fem dage at være relevant for et 2-dags studie:

$K_{energi\ normaliseret}$ [mg/kJ] = 0,249 µg/kg foder – 12% = 0,219 µg/kJ foder

En LD_{10} på 0,074 mg/kg kropsvægt for Bengalgribben ved samme studie, er også præsenteret. Denne vil ved samme overstående beregninger resultere i en energinormaliseret værdi på 0,0722 µg/kJ foder. Da Bengalgribben er den mest sensitive art, vurderes det er gå videre med denne værdi.

Den energinormaliseret værdi skal konverteres til en koncentration i det kritiske fødeemne. For Diclofenac er $BKK_{sek.\ forgiftn.}$ bestemt for både akvatiske planter, leddyr, musling og fisk.
JRC (2022) er ikke tydelig på den videre beregning af kriterierne, men efter korrespondance med JRC oplyses at de anvendte værdier for energi- og vandindhold stammer fra EFSA (2009)[4] og Scheepmaker et al. (2005)[5].

$K_{akvatiske\ planter}$ [µg/kg_{vv}] = 0,0722 µg/kJ x 15.000 kJ/kg x (1-0,814) = 201,44 µg/kg_{vv}

K_{leddyr} [µg/kg_{vv}] = 0,0722 µg/kJ x 20.900 kJ/kg x (1-0,763) = 357,63 µg/kg_{vv}

$K_{musling}$ [µg/kg_{vv}] = 0,0722 µg/kJ x 19.300 kJ/kg x (1-0,917) = 115,66 µg/kg_{vv}

K_{fisk} [µg/kg_{vv}] = 0,0722 µg/kJ x 21.000 kJ/kg x (1-0,737) = 398,76 µg/kg_{vv}

[4] I rapporten af JRC (2022) med miljøkvalitetskrav for Diclofenac er værdier for energi- og vandindhold for de fire fødeemner ikke angivet. Ved kontakt til JRC blev det givet at værdierne for akvatiske planter, leddyr og fisk er fra appendix G i følgende reference: European Food Safety Authority; Guidance Document on Risk Assessment for Birds & Mammals on request from EFSA. EFSA Journal 2009; 7(12):1438. doi:10.2903/j.efsa.2009.1438.
[5] I rapporten af JRC (2022) med miljøkvalitetskrav for Diclofenac er værdier for energi- og vandindhold for de fire fødeemner ikke angivet. Ved kontakt til JRC blev det givet at værdierne for musling er fra tabel 4-5 i følgende reference: Scheepmaker, J.W.A., Smit, C.E. & van Raaij, M.T.M. (2005). Factsheets for the (eco)toxicological risk assessment strategy of the National Institute for Public Health and the Environment. Part V. RIVM report 601516013/2005.

Med en usikkerhedsfaktor på 100 baseret dels på anvendelse af en kronisk værdi fra et akut-studie (faktor 10) og dels på ekstrapolation til det eksterne miljø fra toksikologiske studier i laboratorier (faktor 10), er der beregnet følgende kvalitetskriterier for biota:

$BKK_{sek.\ forgiftn.}$ = 201,44 µg/kg_{vv} / 100 = 2,01 µg/kg vådvægt (akvatiske planter)
= 357,63 µg/kg_{vv} / 100 = 3,58 µg/kg vådvægt (leddyr)
= 115,66 µg/kg_{vv} / 100 = 1,16 µg/kg vådvægt (musling)
= 398,76 µg/kg_{vv} / 100 = 3,99 µg/kg vådvægt (fisk)

Hvoraf laveste beregnede $BKK_{sek.\ forgiftn.\ ferskvand}$ for musling (1,16 µg/kg vådvægt[6]) sættes som endelig værdi for $BKK_{sek.\ forgiftn}$.

Kvalitetskriterium for human konsum af vandlevende organismer (HKK)
Kvalitetskriteriet for biota til human konsum skal sikre mennesker mod sundhedsskadelige påvirkninger fra indtag af forurenede fiskeriprodukter. Principielt er kvalitetskriteriet (HKK) fastsat på baggrund af toksikologiske studier af pattedyr og bestemmelse af en NO(A)EL (No Observable Adverse Effect Level) for oralt indtag, oftest fastlagt som en tærskelværdi for et acceptabelt eller tolerabelt dagligt humant indtag eller en referencedosis. På grundlag af en beregningsformel med standard human konsum af vandlevende organismer kan der bestemmes et kvalitetskriterium for biota til human konsum (EC, 2018).

REACH registrering fastslår, at Diclofenac forårsager skader på organer ved længerevarende og gentagen eksponering, er skadelig ved indtag og mistænkt for at være skadelig for fertilitet og foster. På dette grundlag anbefaler SCHEER udarbejdelse af et kvalitetskriterium for human konsum af vandlevende organismer (HKK).

Der er i det tilvejebragte datagrundlag oplysninger om en ADI (Acceptable Daily Intake) på 0,5 µg/kg kropsvægt/dag[7] baseret på en LOEL-værdi (Lowest Observed Effect Level) på 0,1 mg/kg kropsvægt/dag bestemt for rotter, og anvendelse af en usikkerhedsfaktor på 200.

Ved anvendelse af beregningsgrundlaget fastsat i Europa-Kommissionens tekniske vejledning (EC, 2018), er der beregnet følgende kvalitetskriterium for human konsum af vandlevende organismer:

HKK = 0,2 x 0,5 µg/kg kropsvægt/dag / 0,00163 = 61,35 µg/kg biota vådvægt

Supplerende kan det fastslås, at der på baggrund af studier i forsøgsdyr for stoffet Diclofenac ikke er konstateret indikationer på at stoffet er kræftfremkaldende eller mutagent, og der er i laboratorie-undersøgelser af reproduktionsskadende virkninger hos dyr ikke set effekter på fertilitet, udvikling af fostre eller nyfødtes udvikling.

[6] Bemærk at JRC (2022) noterer enheden for musling på 1,16 forkert i tabel 3.2. Enheden er ikke mg/kg, men µg/kg.
[7] Bemærk at JRC (2022) noterer enheden flere steder som mg/kg kropsvægt/dag, hvor den anvendte reference noterer denne som µg/kg kropsvægt/dag.

Vandkvalitetskriterium baseret på $BKK_{sek.\ forgiftn.}$ og HKK
Der er beregnet et kvalitetskriterium for sekundær forgiftning af vandlevende organismer (biota) for beskyttelse af dyrelivet ($BKK_{sek.\ forgiftn.}$) i henholdsvis muslinger og fisk, og for samme type vandlevende organismer er der beregnet et kvalitetskriterium for human konsum (HKK). Vurderingsgrundlaget er en konvertering af begge værdier ($BKK_{sek.\ forgiftn.}$ og HKK) til en sammenlignelig koncentration i vandsøjlen ved beregning baseret på tilvejebragte data for bioakkumulationsfaktorer (BAF).

I ferskvand er det for de frembragte $BKK_{sek.\ forgiftn.}$-værdier fastslået, at der med en BAF-værdi på 216 l/kg for bløddyr er beregnet en koncentration af stoffet Diclofenac i vand på 5,4 ng/l for muslinger. Ved anvendelse af samme BAF-værdi på 216 l/kg for bløddyr svarer værdien for HKK til en koncentration af stoffet Diclofenac i vand på 284 ng/l.

Kvalitetskriteriet for biota til human konsum (beskyttelse af mennesker) er derved noget højere end kvalitetskriterium for biota fastsat for at beskytte dyrelivet mod sekundær forgiftning ($BKK_{sek.\ forgiftn.}$), når disse omregnes til en koncentration i vandsøjlen.

Det bemærkes, at den beregnede værdi for koncentrationen af Diclofenac i vandsøjlen på 5,4 µg/l, baseret på $BKK_{sek.\ forgiftn.}$, er mindre end det generelle vandkvalitetskriterium (VKK) på 40 ng/l. Dog ændres VKK ikke til 5,4 µg/l, da denne værdi er baseret på, at $BKK_{sek.\ forgiftn.}$ er bestemt ud fra akut toksicitet og ikke som forventet en kronisk effekt.

Kvalitetskriterium for human konsum af drikkevand ($HKK_{Drikkevand}$)
Kvalitetskriteriet for drikkevand skal sikre mennesker mod sundhedsskadelige påvirkninger fra et almindeligt dagligt indtag af drikkevand. For stoffet Diclofenac er der hverken fastsat en gældende EU kvalitetsstandard for drikkevand eller en retningsgivende koncentrationsværdi fra verdenssundhedsorganisationen WHO.

Principielt er kvalitetskriteriet for human konsum af drikkevand ($HKK_{Drikkevand}$) fastsat på baggrund af toksikologiske studier af pattedyr og bestemmelse af en NO(A)EL for oralt indtag, oftest fastlagt som en tærskelværdi for et acceptabelt eller tolerabelt dagligt humant indtag eller referencedosis. På grundlag af en beregningsformel med standard human konsum af drikkevand kan der bestemmes et kvalitetskriterium i henhold til beregningsgrundlaget fastsat i Europa-Kommissionens tekniske vejledning (EC, 2018).

Der er i det tilvejebragte datagrundlag oplysninger om en ADI på 0,5 µg/kg kropsvægt/dag baseret på en LOEL-værdi på 0,1 mg/kg kropsvægt/dag bestemt for rotter, og anvendelse af en usikkerhedsfaktor på 200.

Ved anvendelse af standardværdier for kropsvægt og indtag af drikkevand, kan der udledes et kvalitetskriterium for human konsum af drikkevand jf. beregningsgrundlaget fastsat i Europa-Kommissionens tekniske vejledning (EC, 2018)

$HKK_{Drikkevand}$ = (0,2 x 0,5 µg/kg kropsvægt/dag x 70 kg) / 2 l = 3,5 µg/l

Effekter af stoffets ionisering ved relevante pH værdier i det eksterne miljø
Stoffet Diclofenac er et ikke-ladet molekyle, der dog som en svag syre kan protolysere med en pKa værdi omkring 4. Stoffet forekommer derfor under miljørelevante forhold med pH værdier mellem 5 og 9, som et negativt ladet stof.

Konklusion
Følgende kvalitetskriterier for vandmiljøet er udregnet for Diclofenac:

Vandkvalitetskriterium

$VKK_{ferskvand}$	0,04 µg/l
$VKK_{saltvand}$	0,004 µg/l

Korttidsvandkvalitetskriterium

$KVKK_{ferskvand}$	246 µg/l
$KVKK_{saltvand}$	25 µg/l

Sedimentkvalitetskriterium

$SKK_{ferskvand}$	Ikke relevant
$SKK_{saltvand}$	Ikke relevant

Biotakvalitetskriterium, sekundær forgiftning

$BKK_{sek.forgiftn.}$	1,16 µg/kg vådvægt musling

Biotakvalitetskriterium, human konsum

HKK	61,35 µg/kg biota vådvægt

EQS DATASHEET

ENVIRONMENTAL QUALITY STANDARD

Diclofenac

Expert group, generating this dossier:

Member state representatives:
BE
CH
DE
DK
FI
FR
IT
NL
SW
UK
JRC

Stakeholder
EurEau
GSK
Swedish Water

Contact
German Environment Agency
Department of Pharmaceuticals

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Changes on the Dossier after the SCHEER final Opinion (2022)

Following the SCHEER final opinion published on the 3rd of August 2022 (SCHEER, 2022)[8], the JRC updated the Diclofenac Dossier.

For the MAC-QS derivation, the JRC selected as starting point the 96h-LC_{50} of 2,462.29 µg.L^{-1} for the amphibian *Physalaemus albonotatus* (Peltzer et al., 2019), agreeing with the SCHEER Opinion (2022). Applying an AF of 10, the **MAC-$QS_{fw, eco}$ was set at of 246 µg.L^{-1}. Applying an AF of 100, a MAC-$QS_{sw, eco}$ of 25 µg.L^{-1} was derived**.

According to the SCHEER, "*neither the deterministic approach, using the mesocosm study by Joachim et al (2021), nor the probabilistic approach using the SSD could be said to be satisfactory*". For this reason, the SCHEER supported the **AA-$QS_{fw,eco}$ of 0.04 µg.L^{-1} and a AA-$QS_{sw,eco}$ of 0.004 µg.L^{-1}**, derived using the NOEC of 0.44 µg.L^{-1} of the community response from the mesocosm study of Joachim et al. (2012).

Considering that diclofenac is an acid which dissociates at neutral pH into an anion, it would be unlikely to bind to sediment. For this reason, there was no necessity to derive a $QS_{sediment}$. This decision was supported by the SCHEER (2022).

For secondary poisoning, an LD_{50} of 0.25 mg.kg_{bw}^{-1}.d^{-1} for the most-sensible group, vultures, was used. The biota standard was calculated following the energy-normalized method reported in the EQS Technical Guidance (EC, 2018). The **$QS_{biota, secpois}$** was derived for different food items (fish, bivalves, arthropods, vegetation). The lowest value was obtained for bivalves, and it was equal **to 1.16 µg.kg^{-1}_{diet}** (rounded 1.2 µg.kg^{-1}_{diet}).

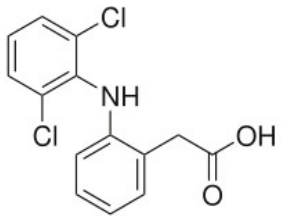
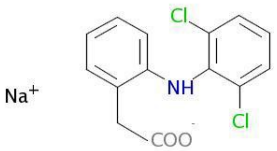
Furthermore, for the back-calculation to water, the JRC used a BAF of 216 L.kg^{-1} for molluscs, agreeing with the SCHEER Opinion. The $QS_{biota, secpois}$ of 1.16 µg.kg^{-1}_{diet} was thus divided by the BAF of 216 L.kg^{-1} to generate a $QS_{water, secpois}$ of 5.4 ng.L^{-1} (0.0054 µg.L^{-1}). Although this standard is lower than the AA-$EQS_{fw, eco}$ of 40 ng/L, it has not been selected as final chronic freshwater standard since the $QS_{biota, secpois}$ was derived with an acute study. Furthermore, the EQS Technical Guidance (EC, 2018) discourages the use of acute toxicity studies for the QS_{biota} derivation. Therefore, the $QS_{water, sec\ pois}$ of 5.4 ng/L cannot be considered statistically robust.

Furthermore, the SCHEER asked the JRC to derive the $QS_{biota, hh}$ using a diclofenac ADI of 0.5 µg.kg_{bw}^{-1}.d^{-1} provided by EMA in (2003). Using this starting point, a **$QS_{biota, hh}$ of 61.35 µg.kg^{-1}** (rounded 61 µg.kg^{-1}) was derived. Applying a BAF of 216 L.kg^{-1}, the back-calculation to water gives a value of **0.28 µg.L^{-1}**. To protect human health from drinking water, the ADI of 0.5 µg.kg_{bw}^{-1}.d^{-1} was once again used, leading to a **$QS_{dw, hh}$ of 3.5 µg.L^{-1}**. All these values were endorsed by the SCHEER.

[8] SCHEER final opinion on diclofenac: https://health.ec.europa.eu/publications/scheer-scientific-opinion-draft-environmental-quality-standards-priority-substances-under-water-0_en

1 Chemical Identity

Table 1.1: Chemical identity of Diclofenac

Common name	Diclofenac
Chemical name (IUPAC)	2-(2,6-Dichloroanilino)phenylacetic Acid
Synonym(s)	Proprietary names of pharmaceuticals containing Diclofenac or Diclofenac sodium salt: Acoflam; Arthrotec; Cataflam; Dicloflex; Diclomax; Diclotard; Diclovol; Diclozip; Econac; Flamatak; Flamrase; Flexotard; Isclofen; Lofensaid; Motifene; Pennsaid; Rheumatac; Rhumalgan; Slofenac; Solaraze; Volraman; Volsaid; Voltaren(e); Voltarol
Chemical class (when available/relevant)	Phenylacetic acid derivates
CAS number	15307-86-5 15307-79-6 (Diclofenac sodium salt)
EU number	239-348-5 239-346-4 (Diclofenac sodium salt)
Molecular formula	C14H11Cl2NO2
Molecular structure	 
Molecular weight (g.mol^{-1})	296.15 318.13 (Diclofenac sodium salt)

The relation of the Molecular weight Diclofenac / Diclofenac sodium salt is 0.9309, consequently no difference between Diclofenac / Diclofenac sodium salt was assumed for effect data and no recalculation of the test results was undertaken because of the small difference in the molecular weight of both compounds.
Independent of this, Diclofenac is normally completely dissociated and available only as Diclofenac-anion only, if used at the normal pH range; see also 6.1 for more details.

2 Existing evaluations and Regulatory information

Table 2.1: Existing regulatory information

Annex III EQS Dir. (2008/105/EC)	Not Included
Existing Substances Reg. (793/93/EC)	Not applicable
Pesticides(91/414/EEC)	Not relevant
Biocides (98/8/EC)	Not relevant
PBT substances	Not investigated
Substances of Very High Concern (1907/2006/EC)	No
POPs (Stockholm convention)	No
Other relevant chemical regulation (veterinary products, medicament, ...)	Directive 2004/27/EC (European Directive for approval of medicinal products)
Endocrine disrupter	Not investigated

3 Proposed Quality Standards (QS)

Diclofenac belongs to the more data rich pharmaceutical substances in terms of fate and effect studies available in the public literature. Using the Scopus databank and searching for the term “diclofenac” in the subject area “Environmental Science” leads to 3,274 document results (30th. May 2021). However, most of this literature is not sufficient in terms of reliability and/or relevance for deriving an EQS, and consequently was not assessed. Literature assessed but found not usable for EQS setting is listed in Annex IV, Chapter 12.

3.1 Environmental Quality Standard (EQS)

	Value	**Comments**
Proposed AA-EQS for [freshwater] [µg L^{-1}]	0.04	See section 6.4.2
Corresponding AA-EQS in [marine water] [µg L^{-1}]	0.004	
Proposed MAC-EQS for [freshwater] [µg L^{-1}]	246	See section 6.4.1
Proposed MAC-EQS for [marine waters] [µg L^{-1}]	25	

3.2 Specific Quality Standard (QS)

Protection objective9	Value	Comments
Pelagic community (freshwater)	0.04 µg/l	See section 6.4
Pelagic community (marine waters)	0.004 µg/l	
Benthic community	Not evaluated	See section 6.5
Predators (secondary poisoning)	1.16 mg/kg 5.4 ng/L	See section 6.6
Human health via consumption of fishery products	61.35 µg/kg 0.28 µg/l	See section7
Human health via consumption of water	3.5 µg/L	

9 Please note that as recommended in the Technical Guidance for deriving EQS (EC 2018), "EQSs […] are not reported for 'transitional and marine waters', but either for freshwater or marine waters". If justified by substance properties or data available, QS for the different protection objectives are given independently for transitional waters or coastal and territorial waters.

4 Measured Environmental Concentrations

4.1 Freshwater

Note: This section is updated after the final adoption of QS values by the SCHEER committee in the plenary meeting on 2 August 2022. The term Predicted No Effect Concentration (PNEC) is utilised sometimes in the text as a more general term in risk assessment and for keeping approach used in the prioritisation exercise, started 2014 (Carvalho et al., 2016), consequently assuming that the PNEC is equal to the freshwater AA-EQS=0.04 µg/L.

4.1.1 Data availability and data scenarios

In regard to the information on diclofenac's exposure, the JRC has used disaggregated data existing at the beginning of current prioritisation exercise, which started in 2014 (Carvalho et al., 2016), and recent data (after 2014) which were officially reported to the EEA (Watch List and WISE) by the EU Member States (MS). In addition, the latest available data in the WISE database (version released in 2022) also have been retrieved and used to check the current temporal trend of diclofenac's concentrations in inland surface waters and in the risk assessment.
The collected disaggregated raw data for measured environmental concentrations (MECs) in the inland surface water are summarised in Table 4.1 showing the source, dataset and corresponding periods of monitoring. A short description of each of the referred datasets is provided thereafter below.

Table 4.1: Sources, dataset and available disaggregated raw monitoring data for measured environmental concentrations (MECs) in the inland surface water compartment. For confidentiality, coded instead of real names of MS are used by the JRC.

Source/Dataset	Available disaggregated raw data
JRC, Prioritisation dataset (2014)	10682 samples (77.9% quantified) from 685 sites in 13 MS (2006 – 2015).). Range of LOQs of non-quantified samples 0.001 – 0.05 µg/L.
EEA, Watch List (2019)	12382 samples (68.6% quantified) from 872 sites in 26 MS (2014 - 2019). Range of LOQs of non-quantified samples 0.00085 – 0.06 µg/L.
EEA, WISE (2020)	14378 samples (63.8% quantified) from 831 sites in 25 MS (2008 – 2019). Range of LOQs of non-quantified samples 0.00085 – 0.1 µg/L.
Data received or retrieved after the 18th meeting of WFD CIS WG Chemicals (held in October 2020)	CWPharma project (2020) https://www.lansstyrelsen.se/4.f2dbbcc175974692d268b9.html 48 quantified and 7 non-quantified samples from 25 sites in 6 MS (2017 – 2018). Range of LOQs 0.5 – 1.2 ng/L. Range of measured concentrations 0.00025 – 2.2 µg/L. Statistics assuming that the unquantified samples are equal to ½ LOQ: Mean=0.23 µg/ (StDev=0.45 µg/L) Median = 0.033 µg/L 90th percentile = 0.58 µg/L 95th percentile = 0.92 µg/L 99th percentile = 2.15 µg/L

	WISE 2022 (EEA) 8458 samples (26.6% quantified) from 1389 sites in 10 MS (2019 – 2021). Range of LOQs of non-quantified samples 0.00036 – 0.1 µg/L. MS#12 is overrepresented holding about 73.8% of all samples. The data for 2021 were not considered since in this year the MS#29 reported only 10 non-quantified samples. The descriptive statistics, shown below, is estimated by Kaplan-Meier nonparametric method (ProUCL 5.1 tool of the US EPA). Range of concentrations 0.00036 – 5.3 µg/L Mean=0.028 µg/ (StDev=0.119 µg/L) Median = 0.02 µg/L 90th percentile = 0.067 µg/L 95th percentile = 0.134 µg/L 99th percentile = 0.45 µg/L Note: These data are not included in the combined dataset but are used to evaluate the current temporal trend of exposure in inland surface waters and also in the risk assessment.

The Prioritisation dataset (2014) includes monitoring data collected at the beginning of the second prioritisation exercise (Carvalho et al., 2016; https://circabc.europa.eu/w/browse/52c8d8d3-906c-48b5-a75e-53013702b20a) which are taken from following sources:

- SoE - monitoring data reported by MS under the State of the Environment (SoE) WISE (Water Information System for Europe) managed by the European Environment Agency (EEA).
- MSDAT – monitoring data directly submitted to the JRC by EU member states following a request of DG ENV to the EU Water Directors (on 21 March 2014). In addition, some monitoring data have been submitted on behalf of the European drinking water companies.
- EMPODAT - a database of geo-referenced monitoring data managed by NORMAN (Network of reference laboratories, research centres and related organisations for monitoring of emerging environmental substances) https://www.norman-network.net/). The EMPODAT data were provided to the JRC in March 2015.
- JDS - monitoring data from the third Joint Danube Survey (JDS) from the year 2013 https://www.icpdr.org/
- IPCheM - the Information Platform for Chemical Monitoring data, managed by the JRC was downloaded in January 2015 (https://ipchem.jrc.ec.europa.eu).

The Watch List (WL) dataset includes monitoring data from several reporting cycles of the WL (2015-2019) and this dataset is in detail described in a dedicated report (Marinov and Lettieri, 2020; https://circabc.europa.eu/ui/group/9ab5926d-bed4-4322-9aa7-9964bbe8312d/library/deabbcb4-c001-4855-b503-04f27996ca7d/details).

The monitoring data from the WISE database, managed by the EEA, has been received in November 2020 (information about WISE data could be found on the following link https://www.eea.europa.eu/data-and-maps/data/waterbase-water-quality-icm-1).

During discussions in the sub-group of review (SG-R) of the priority substances list, the GlaxoSmithKline plc (GSK) has proposed additional monitoring data, which are publicly available and have been collected by the GSK, to be included in the analysis of diclofenac (overall 26790 samples including an extensive dataset of France). The JRC has considered this proposal, but comparing the sources (references) of data, provided by GSK, it was concluded that the data having the same sources and monitoring periods should be presented in both datasets. This includes, for instance, the data from 18 MS reported to the WL and

WISE4 dataset from the EEA and/or the measurements for Danube (ICPDR) and Rhine (ICPR) rivers. At the end, the major difference between the datasets of the JRC and GSK is the vast set of measurements from France (21472 samples from Naiades database), which represent about 80% of the GSK dataset for diclofenac, but are missing in the JRC dataset. The French data, proposed by the GSK, are summarised in Table 4.2 and they were included in the combine dataset for exposure.

Table 4.2: Source, dataset and summary statistics of additional publicly available monitoring data for diclofenac proposed by the GSK to be included in the JRC dataset. The descriptive statistics is estimated by Kaplan-Meier nonparametric method (ProUCL 5.1 tool of the US EPA).

Country/Source	**MECs and LOQs** (µg/L)
France Time period: 2016 - 2018 http://www.naiades.eaufrance.fr/acces-donnees#/physicochimie	21472 samples (31% quantified) Range of LOQs: 0.006 - 0.05 Median = 0.02 Mean = 0.0248 90th percentile = 0.058 95th percentile = 0.11 99th percentile = 0.317

Further, the JRC acknowledged the point raised by the stakeholders that despite the constant improving of sensitivity of analytical techniques, any set of measured concentrations may contain a portion of non-detected or non-quantified samples, called often “less than” values or censored concentrations (Helsel 2006; Gardner 2011; Helsel 2012; Shoari and Dubé, 2018; Merrington et al., 2021). The censored or less than values are measurements for which the observed concentration is less than the limit of detection (LOD) or limit of quantification (LOQ) and for them, the true sample concentration is somewhere between zero and the reporting limit (Helsel, 2006; Gardner, 2011). Three approaches exist for tackling the censored data problem: i) ignoring less than data, ii) substituting less than data and, the third one iii) comprehensive mathematical techniques (Helsel 2006; Gardner 2011; Helsel 2012; Shoari and Dube, 2018). The practice of analysing datasets with censored data in regulatory agencies, US EPA and EFSA is summarised in Shoari and Dube (2018) showing that either substitution or mathematical techniques are applied according to levels of censoring. Accordingly, the JRC has adopted to deal with the uncertainty from censored data, when deriving statistics of MECs, by using the Kaplan-Meier nonparametric method and/or as alternative, if feasible, the substitution approach. The latter follows the guideline of the European Food Safety Authority (EFSA, 2010) which suggests making the calculations of statistics twice, once for a lower bound by substituting non-detects with null and once for an upper bound by substituting non-detects with the LOD or LOQ. If the difference between the upper and lower bound of the estimated parameter is negligible, then substitution with the LOD or LOQ is recommended (this is the worst-case scenario but other scenarios are also possible, i.e. ½ LOQ). When the difference is not negligible or the upper bound estimate is in the range of (eco)toxicological threshold, then alternative estimation techniques should be used. A similar approach is applied also by the US EPA (Shoari and Dube, 2018). As a software tool dealing with dataset including censored data (in particular deriving statistics by the Kaplan-Meier method which is especially useful because avoids assumptions about the

data distribution) the JRC is using ProUCL v5.1 of US EPA (https://www.epa.gov/land-research/proucl-software).
Moreover, in monitoring datasets the usage of non-quantified samples is a challenge when not all Limit of Quantifications (LOQs) of applied analytical methods are adequate to the Predicted No Effect Concentration (PNEC). For this reason, and also following the experience from the latest review of the Priority Substances (PS) list (Carvalho et al, 2016), three data scenarios are considered in this dossier (Table 4.3).

Table 4.3: Data scenarios considered in the data analyses and risk assessment (please note that the scenario indicated as Sc3 was called Sc2-PNEC-QC in the last monitoring-based prioritisation exercise, Carvalho et al., 2016).

Data scenario	Description
Scenario 1 (Sc1)	**Only quantified** monitoring samples
Scenario 2 (Sc2)	**All monitoring samples** (quantified and non-quantified). Only when applying the substitution approach, the non-quantified samples are set equal to a half of LOQ as stipulated in Directive 2009/90/EC
Scenario 3 (Sc3)	**Quantified** monitoring samples **plus non-quantified** samples when **½ LOQ ≤ PNEC** (or EQS) Sc3 is a more relevant data scenario for making a risk assessment according the sub-group on review (SG-R) of the priority substances list (Carvalho et al., 2016).

Scenario 1 (Sc1) includes only quantified samples, thus clearly overestimating the risk. If application of the substitution approach for censored data is feasible, then in both Scenario 2 (Sc2) and Scenario 3 (Sc3) the non-quantified samples are set to half LOQ[10]. However, Sc2 comprises all monitoring records, thus could lead to non-confirmed exceedances when ½LOQ>PNEC, while Sc3 takes into account quantified monitoring samples and non-quantified samples only when ½LOQ≤PNEC, thus avoiding any non-confirmed exceedances. According to the sub-group on review (SG-R) of the priority substances list, Sc3 is the most relevant scenario to assess whether the substance poses a risk at EU-level (Carvalho et al., 2016). Anyway, information for Sc1 and Sc2 data scenarios is also presented for completeness.
Then, the records from the datasets, shown in Tables 4.1 and 4.2, have been combined in a single dataset (called thereafter COMBI dataset). However, it should be noted that duplicated records are possible between the individual datasets in particular between the Watch List and WISE datasets. Thus, after removal of duplicates from COMBI dataset, the latter is used for making a union wide risk assessment. A summary information about the numbers of participating MS, monitoring sites and collected samples is presented in Table 4.4 for Sc1 and Sc2 data scenarios (info about Sc3 is given after the data quality check).
Furthermore, the detailed statistics per country for Sc2 and Sc3 scenarios is provided in a complementary Excel file entitled ***MEC_Diclofenac_dossier*** (including the number of sites, number of samples, fraction from all samples, number of quantified samples, info about LOQ values, statistics of MECs, etc.). The staistics evidenced that three MS are overrepresented in

10 Under the QA/QC Directive and EQS Directive, MS are required to replace the non-quantified samples by half LOQ to assess compliance with the EQS for individual substances. However, the amended EQSD mentions that "when the calculated mean value of a measurement, when carried out using the best available technique not entailing excessive costs, is referred to as "less than limit of quantification", and the limit of quantification of that technique is above the EQS, the result for the substance being measured shall not be considered for the purposes of assessing the overall chemical status of that water body".

the combined dataset holding together about 95.6% of all samples (MS#06 contributed with about 35.6%, MS#07 with 16.1% and MS#12 with 43.9% of all records).

Table 4.4: Available disaggregated data for the measured environmental concentrations (MECs) in inland surface water compartment across EU MS (jointly data from all countries after the elimination of duplicated records) for the period 2006 – 2019 in the Sc1 and Sc2 data scenarios of the combined dataset (called thereafter COMBI dataset).

Scenario	Member States (MS)	Sites	Samples	Quantified samples (%)
Sc1	24	2411	25785	100
Sc2	26	3448	49003	52.6

4.1.2 Quality of data

The quality of measured environmental concentrations (MECs) is essential for making a proper risk assessment analysis. The applied general requirements for data quality and the procedures for treatment of outliers and duplicates in the exposure datasets are described in two JRC reports (Carvalho et al, 2016; and Loos et al., 2018).
The records in the COMBI dataset fulfil the general requirements for appropriate data reporting (where, when, what, how was measured, etc.). The dataset is also free of duplicates and outliers. Therefore, a special attention is paid here on the fulfilment of the LOQ-PNEC condition, union representativeness of data and uncertainty (bias) related to non-quantified (censored) concentrations.
For instance, considering the data from all MS together, Figure 4.1 shows the range of LOQs of non-quantified samples per country while Figure 4.2 informs how many non-quantified samples fulfilled the LOQ-PNEC condition (½ LOQ≤PNEC) in each of the reporting MS. It was found that MS have monitored with sufficiently sensitive analytical methods and practically all non-quantified samples, except 19 samples from MS#06 and 8 samples from MS#10 (totally 27), fulfilled the LOQ-PNEC criterion. The detailed information about the LOQ values per MS for non-quantified samples in Sc2 dataset is provided in the accompanying Excel file.

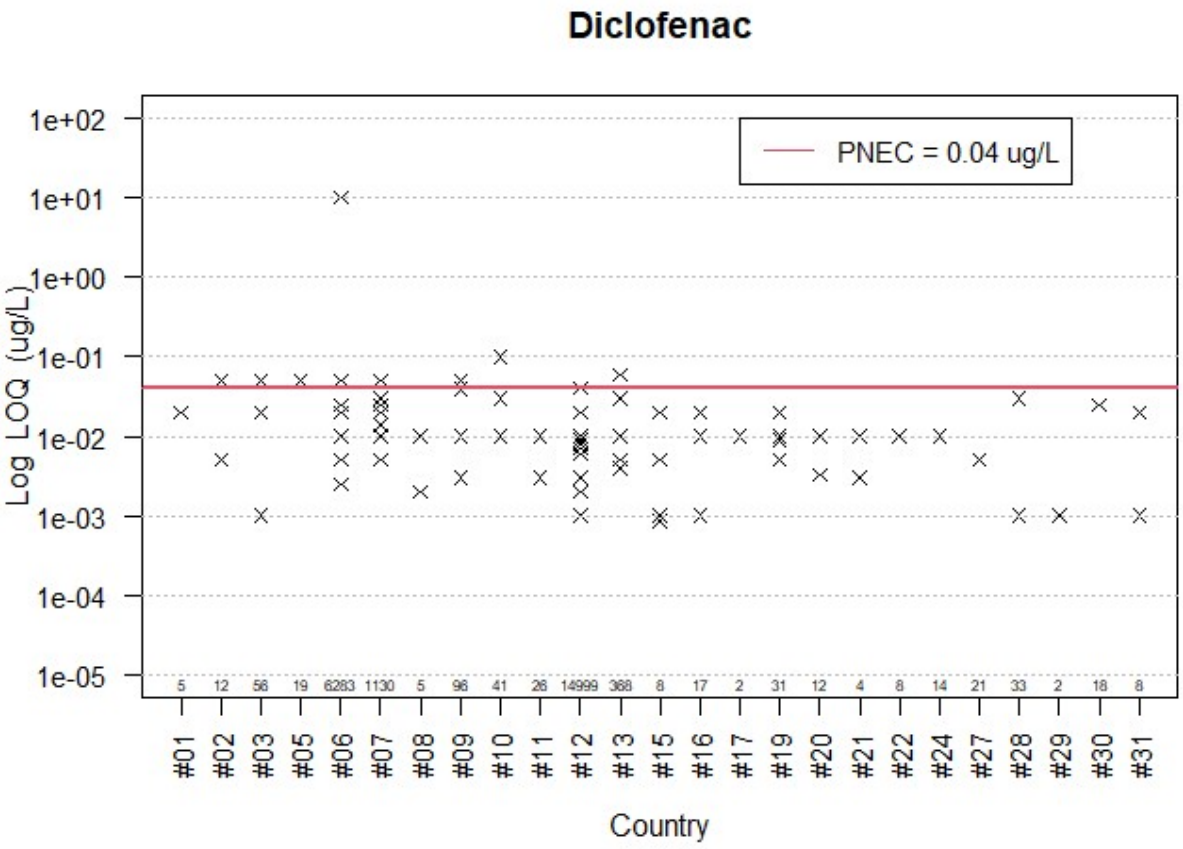


Figure 4.1: Range of LOQs for non-quantified samples in Sc2 scenario of combined dataset per country. The lowermost line of the figure shows the overall number of non-quantified samples in each reporting MS. For confidentiality the countries' names are coded. The red line indicates the PNEC value.

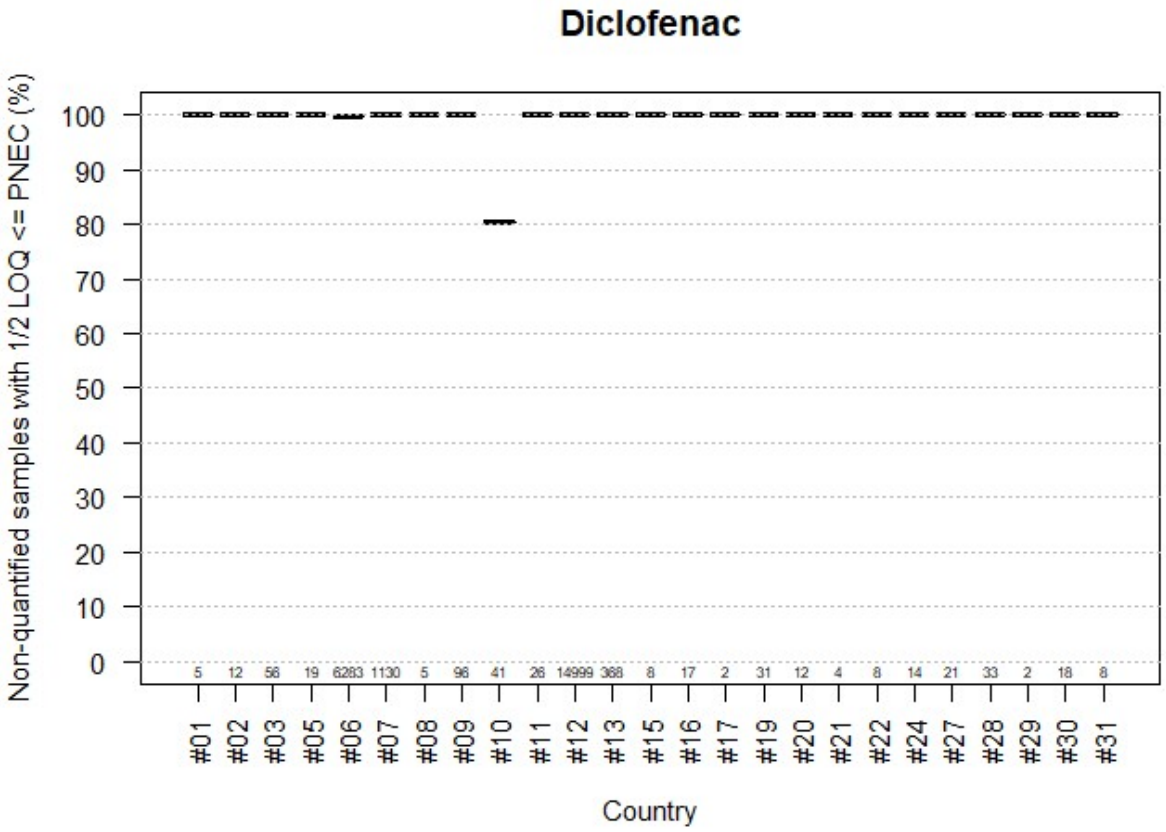


Figure 4.2: Number of non-quantified samples fulfilled LOQ-PNEC condition (½ LOQ≤PNEC) as percentage from all reported non-quantified samples per country in Sc2 scenario of the combined dataset. The lowermost line of the figure shows the overall number of non-quantified samples in each reporting MS. For confidentiality the countries' names are coded.

After the LOQ-PNEC check the decisive Sc3 data scenario is developed considering PNEC=0.04 µg/L. The basic information for this scenario is presented in Table 4.5. Moreover, the detailed statistics for Sc3 dataset is provided in the complementary Excel file. It was concluded that there are sufficient amount of data with a good quality for making a union-wide risk assessment.

Table 4.5: Available disaggregated data for the measured environmental concentrations (MECs) across EU MS (jointly data from all countries after the elimination of duplicated records) for the period 2006 – 2019 in Sc3 data scenario of the combined dataset (PNEC=0.04 µg/L).

Scenario	Member States (MS)	Sites	Samples	Quantified samples (%)
Sc3	26	3438	48976	52.63

Thereafter, the plots of histogram (Figure 4.3) and cumulative frequency (Figure 4.4) have been prepared for measured concentrations (data from all MS together) in Sc3 data scenario of the combined dataset undertaking a substitution by ½ LOQ for censored data. The histogram (Figure 4.3) showed a presence of lot non-quantified samples with concentration 0.005 µg/L (about 19.7% of all) corresponding to LOQ=0.01 µg/L and with concentration 0.01 µg/L (about 21% from all) corresponding to LOQ=0.02 µg/L. The cumulative frequency (Figure 4.4) is compared to a log-normal distribution with the same mean and standard deviation. It was found that the empirical distribution is not far away from the log-normal distribution.

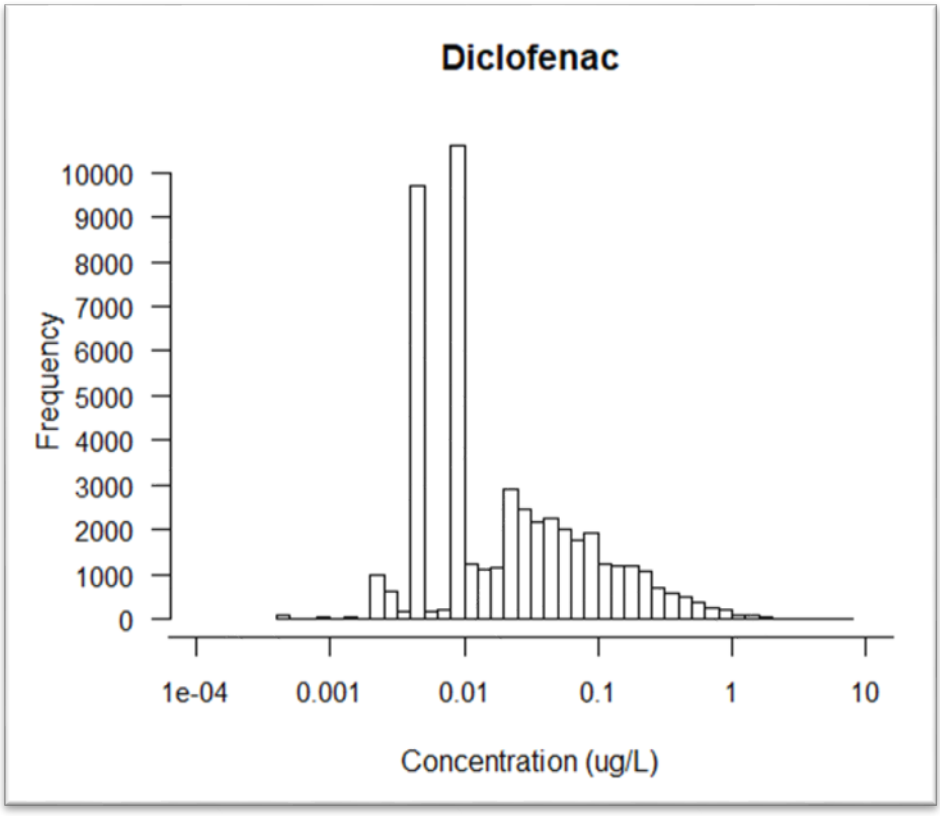


Figure 4.3: Histogram of concentrations (data from all MS together) for Sc3 of the combined dataset undertaking a substitution by a half of LOQ for censored data.

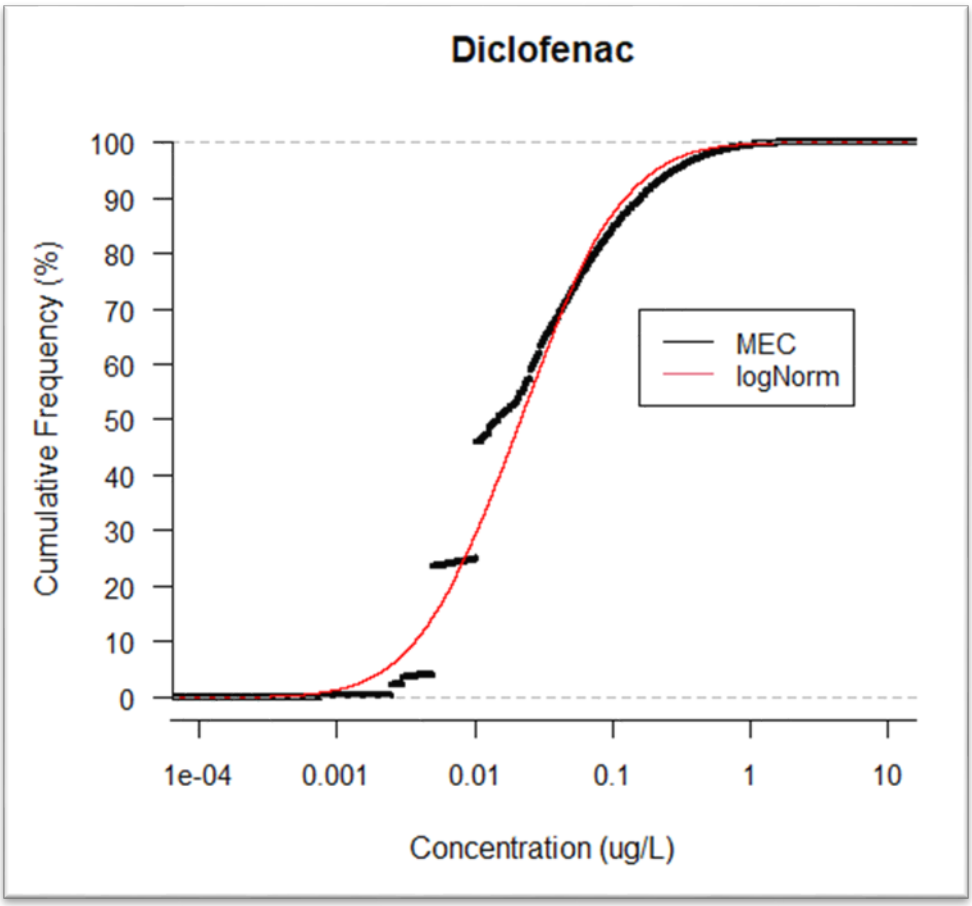


Figure 4.4: Cumulative frequency of concentrations (data from all MS together) for Sc3 of the combined dataset undertaking a substitution by half of LOQs for censored data. The red curve represents a cumulative frequency of log-normal distribution with the same mean and standard deviation.

4.1.3 Summary statistics of measured concentrations

The summary statistics of measured concentrations in compartment inland surface water for Sc3 (min, average, standard deviation (StDev), median, 90th percentile (P90), 95th percentile (P95), 99th percentile (P99) and max) is estimated considering together the data from all MS and using Kaplan-Meier (KM) nonparametric method (ProUCL 5.1 tool) of the US EPA (https://www.epa.gov/land-research/proucl-software). The obtained results are presented in Table 4.6. For completeness, the table shows also statistics for Sc3 with the substitution approach taking into consideration two extreme cases (lower bound 1% of LOQ and upper bound 99% of LOQ) alongside with the common "central" approach (50% of LOQ). One could observe that the mean concentration, found by Kaplan-Meier method, is between the estimates of lower bound and middle substitution (i.e. 1% and 50% of LOQ), while the median is identical to the upper bound of replacement (99% of LOQ). The nonparametric method and substitution approximation showed equal values for higher percentiles (for example ≥90).
According to ProUCL 5.1 tool, the assessed variance in Sc3 by KM method is about 0.0233 µg/L. The 95% upper confidence limit (95% UCL) of mean concentration, estimated by KM, is 0.0698 µg/L through bootstrapping and 0.0716 µg/L according Chebyshev method (ProUCL 5.1). The 95% upper tolerance limit with 95% coverage (i.e. 95% UCL of the 95th percentile) is 0.316 µg/L by KM approach assuming normal distribution and higher, 0.729 µg/L, according Chebyshev method (ProUCL 5.1).

Table 4.6: Summary (descriptive) statistics of measured concentrations for Sc3 scenario (jointly data from all MS) estimated by Kaplan-Meier nonparametric method for dataset containing censored data (ProUCL 5.1 tool of the US EPA). For completeness, the statistics for Sc3 derived by the substitution approach for censored data considering two extreme cases (lower bound 1% of LOQ and upper bound 99% of LOQ) alongside with the common "central" approach (50% of LOQ) is also presented.

Concentration (µg/L)	Kalpan-Meier method (ProUCL 5.1)	Scenario 1% LOQ	Scenario 50% LOQ	Scenario 99% LOQ
Min	5.3E-04	8.50E-06	4.25E-04	5.3E-04
Mean	0.0633	0.06106	0.06482	0.06858
StDev	0.153	0.1536	0.1522	0.151
Median	0.02	0.011	0.015	0.0198
P90	0.16	0.16	0.16	0.16
P95	0.28	0.28	0.28	0.28
P99	0.73	0.7302	0.7302	0.7302
Max	7.1	7.1	7.1	7.1

In addition for completeness, Table 4.7 compares the summary (descriptive) statistics of measured environmental concentrations for Sc3 scenario (jointly data from all MS) estimated by Kaplan-Meier nonparametric method for dataset containing censored data (ProUCL 5.1 tool) with the statistics for Sc1 and Sc2 data scenarios (Sc1 includes only quantified samples; in Sc2 scenario a substitution by half of LOQ is applied for censored data).

Table 4.7: Comparison statistics of measured concentrations for Sc3 scenario (jointly data from all MS) estimated by Kaplan-Meier nonparametric method for dataset containing censored data (ProUCL 5.1 tool of the US EPA) with statistics for Sc1 and Sc2 data scenarios (Sc1 includes only quantified samples; in Sc2 scenario a substitution by half of LOQ is applied for censored data).

Concentration (µg/L)	**Scenario Sc1**	**Scenario Sc2**	**Scenario Sc3 Kalpan-Meier method (ProUCL 5.1)**
Min	5.30E-04	4.25E-04	5.3E-04
Mean	0.1158	0.0667	0.0633
StDev	0.1962	0.1806	0.153
Median	0.052	0.015	0.02
P90	0.27	0.16	0.16
P95	0.438	0.28	0.28
P99	0.97	0.748	0.73
Max	7.1	7.1	7.1

Besides, since three MS (#06, #07 and #12) are overrepresented in the combined dataset holding together about 95.6% of all samples (see the supporting Excel file), the Table 4.7 differentiates the summary statistics if all MS are presented in the Sc3 dataset versus a hypothetical scenario of excluding the data-rich countries. In this exercise, the statistics are estimated also by Kaplan-Meier nonparametric method for dataset containing censored data (ProUCL 5.1 tool). Generally, compatible results were obtained when comparing the complete Sc3 dataset (all MS presented) and the scenario "excluding the three overrepresented MS" from Sc3 (i.e. #06, #07 and #12). Oppositely, an increase of descriptive statistical parameters (excluding the min and max concentrations) was found in case of elimination from Sc3 only the data of MS#12 (on average a rise of about 43.9%; range of raising from 22% to 85%).

Table 4.8: Comparison statistics for measured environmental concentrations in Sc3 data scenario considering either jointly data from all MS or excluding the most data-rich countries (without MS#06, MS#07 or only without MS#12) from the combined dataset. The statistics are estimated by Kaplan-Meier nonparametric method for dataset containing censored data (ProUCL 5.1 tool of the US EPA).

Concentration (µg/L)	Sc3 KM ProUCL (all MS)	The most data-rich MS excluded from Sc3 (without #12)	The three data-rich MS excluded from Sc3 (without #06, #07 and #12)
Min	5.3E-04	5.3E-04	6.5E-04
Mean	0.0633	0.0933	0.059
StDev	0.153	0.187	0.18
Median	0.02	0.037	0.03
P90	0.16	0.23	0.12
P95	0.28	0.39	0.23
P99	0.73	0.917	0.864
Max	7.1	7.1	3.25

Finally, for a sake of completeness, the Table 4.9 presents the statistical parameters for Sc3 data scenario calculated as unweighted means of values from all reporting MS (see the complementary Excel file). The statistics of each individual country is estimated by Kaplan-Meier nonparametric method for dataset containing censored data (ProUCL 5.1 tool of the US EPA).

Table 4.9: Comparison of statistics of measured environmental concentrations for Sc3 scenario of the combined dataset considering either together measurements from all MS or estimated as unweighted means of values from individual MS (the min and max concentrations, shown in the table, are not average values). The statistics of each individual country is estimated by Kaplan-Meier nonparametric method for dataset containing censored data (ProUCL 5.1 tool of the US EPA).

Concentration (µg/L)	Sc3 (all MS)	Sc3 (unweighted means from all MS)
Min	5.3E-04	5.3E-04
Mean	0.0633	0.071
StDev	0.153	0.134
Median	0.02	0.028
P90	0.16	0.148
P95	0.28	0.284
P99	0.73	0.537
Max	7.1	7.1

4.1.4 Temporal trend

The temporal trend of exposure in the period 2006-2020 is verified according to the annual variability of the 95th percentiles (P95) of MECs (inland surface water) according to the procedure adopted by the sub-group of revision of the Priority Substances list (Carvalho et al., 2016). The analysis includes as well the latest data from WISE 2022. The P95 of MECs are estimated by Kaplan-Meier nonparametric method (ProUCL 5.1 tool of the US EPA).
The trend of exposure is analysed, firstly, considering the data from all MS together (see Figure 4.5). In the period 2007-2014 there is a gradual increase of P95 from 0.249 µg/L to 0.645 µg/L. Afterwards, although the general diminished values of P95, no clear temporal trend of exposure and oscillating P95 were observed from 2015 up to 2020. However, worth mentioning that the 95th percentiles of MECs were higher than the PNEC (0.04 µg/L) in the entire time period.
Then, in order to check a possible impact of the most data-abundant MS on the trend of exposure, Figure 4.6 shows the 95th percentiles of MECs per year for Sc3 scenario if the MS#12 is excluded from the combined WL dataset. In this case onwards 2015, one could observe almost stable P95 (a very low fluctuating P95) which are lower than the peak-value in 2014 but still exceed the PNEC (0.04 µg/L).

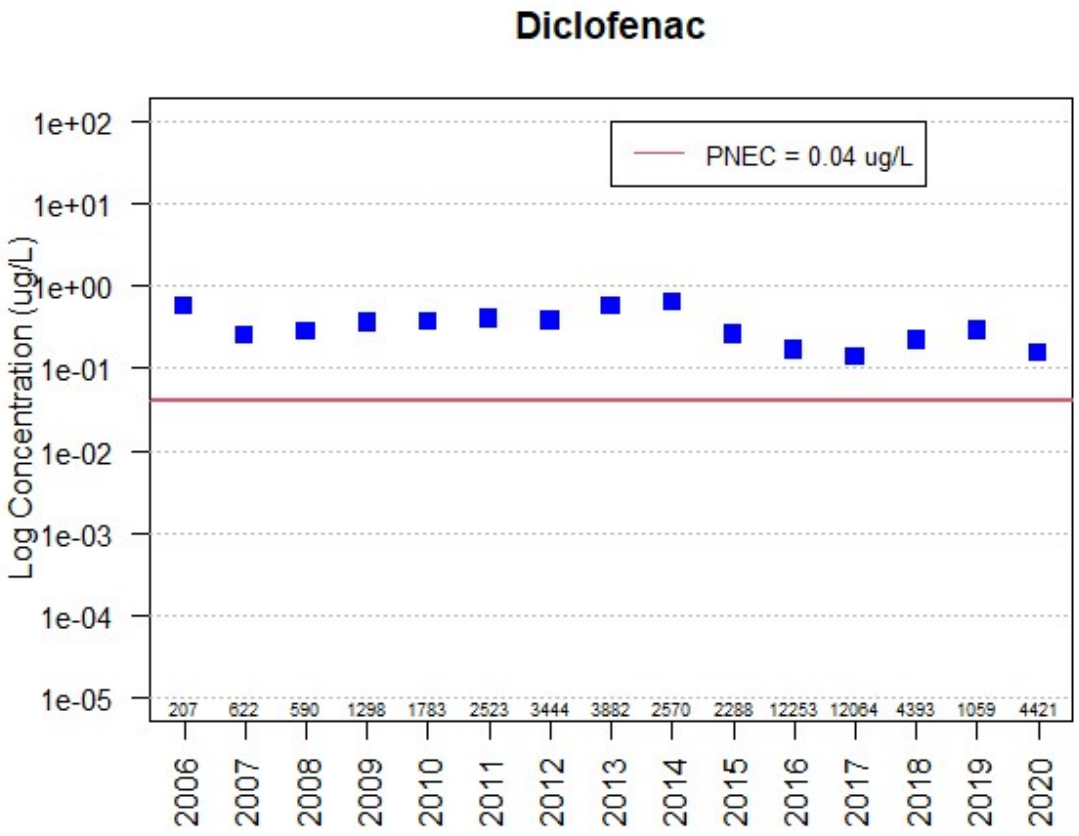


Figure 4.5: Plot of 95th percentiles of measured environmental concentrations per year for Sc3 scenario of the combined WL dataset considering data from all MS.

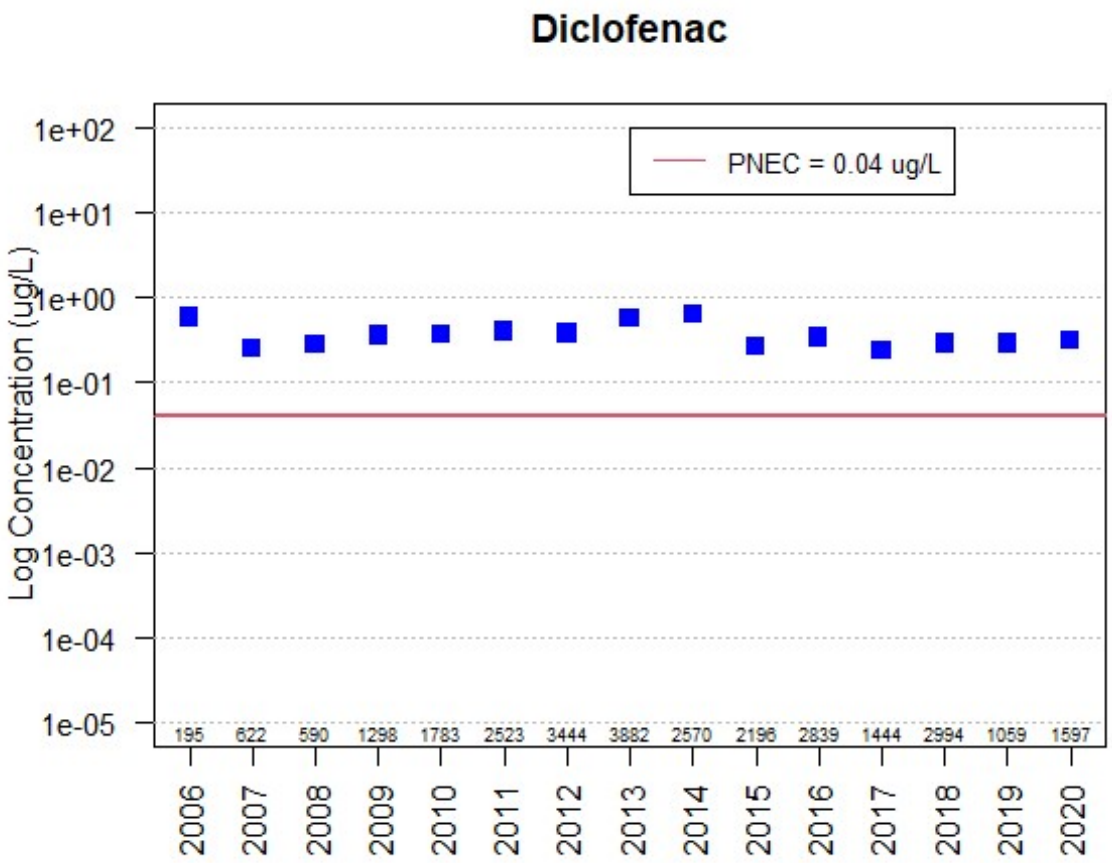


Figure 4.6: Plot of 95th percentiles of measured environmental concentrations per year for Sc3 scenario if the most data-rich country (MS#12) is excluded from the combined WL dataset.

4.1.5 Risk assessment

The Risk Assessment (RA) analysis, developed after the adoption EQS values by the SCHEER committee, includes two components – first, a screening of overall risk for inland surface water compartment and second, a compliance check in regard to the freshwater AA-EQS.

Screening of risk

The screening of overall risk was elaborated following the procedure adopted by the sub-group of revision of the Priority Substances list (Carvalho et al., 2016; https://circabc.europa.eu/w/browse/52c8d8d3-906c-48b5-a75e-53013702b20a). Accordingly, the risk screening is based on MECs in Sc3 data scenario of the combined dataset and utilizes PNEC equal to the freshwater AA-EQS=0.04 µg/L. The risk screening takes into account the Risk Quotient RQ(P95), the Spatial, Temporal and Extent of PNEC exceedances (STE score) and number of exceeding MS (see Table 4.10).

The Risk Quotient RQ(P95) is estimated by the 95th percentile (P95) of concentrations considering measurements in Sc3 from all MS and for the entire time period. A given country is specified as "Exceeding MS" if the 95th percentile of its own measured concentrations is higher than the freshwater AA-EQS. The STE (Spatial, Temporal and Extent of PNEC exceedances) is assessment tool developed in-house by the JRC. The STE method is widely described and discussed in Carvalho et al., 2016 (https://circabc.europa.eu/w/browse/52c8d8d3-906c-48b5-a75e-53013702b20a). The STE calculates for each substance a risk score by summing the Spatial, Temporal and Extent of PNEC exceedance factors (indexes) using P95 of MECs at monitoring sites. The range of STE scores is between 0 and 3 since the individual factors vary from 0 to 1, where a STE score of 0 indicating null concern, while a score of 3 showing an extremely high concern.

The relevant P95 of MECs (see Table 4.6) is estimated by Kaplan-Meier nonparametric method for datasets containing censored data (ProUCL 5.1 tool of the US EPA). The P95 of reporting MS, respectively exceedances in each MS, are evaluated also with the Kaplan-Meier method and ProUCL tool (see the complementary Excel file). However, the STE score is calculated in a traditional manner using the substitution by half of LOQs for non-quantified (censored) data.

Table 4.10: Risk assessment screening results. The evaluation is based on measured concentrations in Sc3 scenario of the combined dataset and PNEC=0.04 µg/L. The Risk Quotient RQ(P95) is estimated by the 95th percentile of concentrations considering altogether measurements from all MS whereas the P95 is estimated by Kaplan-Meier nonparametric method for datasets containing censored data (ProUCL 5.1 tool of the US EPA). A given country is specified as "Exceeding MS" if the 95th percentile of its measured concentrations is higher than the PNEC value. The P95 of reporting MS, respectively exceedances in each MS, are evaluated also with the Kaplan-Meier method and ProUCL tool.

Scenario	RQ (P95)	Fspat	Ftemp	Fext	STE score	Exceeding MS (% from total)	Total number of reporting MS
Sc3 (all MS)	7	0.37	0.527	0.18	1.077	22 (84.6%)	26

The performed screening indicated a presence of risk for inland surface waters at EU level because the overall RQ(P95)=7, viz. it is considerably higher than one, the STE score is elevated (>1) and 22 out of the 26 reporting MS in Sc3 showed exceedances (about 84.6% from all MS).

Notes:

1. The EU-wide concern for freshwaters is confirmed even by the mean concentrations estimated for different variants of Sc3 scenario (see the descriptive statistics given in Tables 4.6 – 4.9), because the mean concentrations in all data scenarios exceeded the PNEC=0.04 µg/L.
2. According to the additionally provided monitoring data by CWPharma project and GSK (Naiades dataset) the 95th percentiles of MECs exceeded PNEC=0.04 µg/L respectively 23 and 2.75 times (see Tables 4.1 and 4.2), which supports also the concern of risk existence.
3. The available latest data for exposure from WISE 2022 (see Table 4.1) likewise confirmed that diclofenac continues to pose an EU-wide risk in the recent years since RQ(P95)=3.35 and 7 reporting MS showed exceedances.

Compliance check

The compliance check, which is a core part of the developed risk assessment, was performed according to the EQS Directive[11] (amended by the Directive 2013/39/EU). The compliance is based on MECs in Sc3 data scenario of the combined dataset and is considered to be fulfilled (not failed) if the annual average measured concentrations at monitoring sites in the participating MS do not exceed the AA-EQS (according to the available exposure data the max concentrations in all reporting MS did not exceed the freshwater MAC-EQS=246 µg/L). In the compliance analysis the non-quantified concentrations in the Sc3 dataset were assumed to be equal to a half of LOQs[12] i.e. the substitution approach, adopted by the Directives 2009/90/EC and 2013/39/EU, was applied.

At first, a boxplot of annual average concentrations at monitoring sites (Sc3 data scenario) for the considered time period is shown on Figure 4.7 comparing to the freshwater AA-EQS=0.04 µg/L.

Thereafter, a relevant statistics about the number of monitoring sites in Sc3 dataset which annual mean concentrations exceeded the freshwater AA-EQS (given also as a percentage from the total number of sites) is presented in Table 4.11. For instance, recently (onwards 2015), yearly from 85 up to 353 monitoring sites, corresponding to 20%-40.1% (on average 28.7%) of all sampling locations, showed annual mean concentrations higher than the freshwater AA-EQS (in the period 2006-2014 the averaged percentage of exceeding annual mean concentrations at sites is 56.3%).

[11] Directive 2008/105/EC Annex I Part B

Paragraph 1 "For any given surface water body, applying the AA-EQS means that, for each representative monitoring point within the water body, the arithmetic mean of the concentrations measured at different times during the year does not exceed the standard'' and

Paragraph 2 "For any given surface water body, applying the MAC-EQS means that the measured concentration at any representative monitoring point within the water body does not exceed the standard''.

[12] Directive 2009/90/EC Article 5 Paragraph 1 states "Where the amounts of physico-chemical or chemical measurands in a given sample are below the limit of quantification, the measurement results shall be set to half of the value of the limit of quantification concerned for the calculation of mean values".

According to the available latest data for exposure from WISE 2022 (see Table 4.1) the annual percentages of exceeding mean concentrations at sites vary from 11% to 16%. Therefore, the above observations confirm distinctly the failure of compliance in regard to the freshwater AA-EQS.

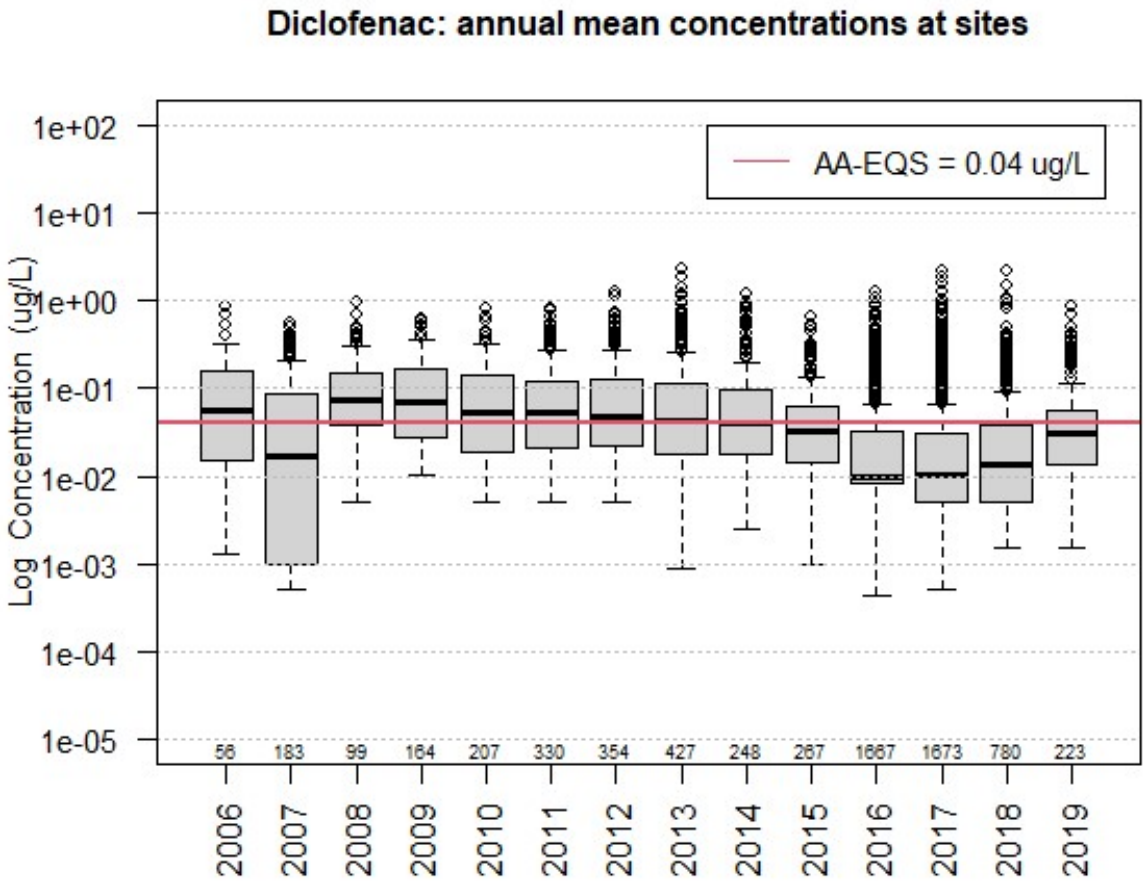


Figure 4.7: Boxplot of annual average values of measured concentrations at monitoring sites in Sc3 scenario for the considered time period. In this analysis the non-quantified concentrations are assumed to be equal to a half of LOQ (Directives 2009/90/EC and 2013/39/EU). The lowermost line of the figure gives the overall number of monitoring sites in each year. The red line indicates the PNEC equal to the freshwater AA-EQS.

Table 4.11: Number of monitoring sites in Sc3 dataset which annual mean concentrations exceeded the freshwater AA-EQS (given also as a percentage from the total number of sampling locations). In this analysis the non-quantified concentrations are assumed to be equal to a half of LOQ (Directive 2009/90/EC and 2013/39/EU).

Year	Number of reporting MS	Total number of sites	Number of exceeding sites	% of exceeding sites from all
2006	4	56	31	55.36
2007	9	183	72	39.34
2008	3	99	73	73.74
2009	4	164	104	63.41
2010	4	207	118	57.00
2011	3	330	187	56.67
2012	4	354	210	59.32
2013	10	427	218	51.05
2014	5	248	125	50.40
2015	10	267	107	40.07
2016	25	1667	353	21.18
2017	25	1673	334	19.96
2018	21	780	190	24.36
2019	5	223	85	38.12

Conclusion:
The performed risk screening and the observed failures of compliance in regard to the freshwater AA-EQS=0.04 µg/L, estimated through the monitoring data available in the combined dataset described in this dossier, showed that diclofenac poses an EU-wide risk for inland surface waters.

4.2 Coastal/Transitional water

This section is not fully developed because currently only a small amount of disaggregated monitoring data exists for the coastal/transitional water compartment. The available raw data from the EEA (Watch List and WISE database) are described in Table 4.12. These raw data were merged in a combine dataset (Sc2 scenario) in which the duplicated records were eliminated. Then, a summary information for the Sc2 dataset is provided in Table 4.13.

Table 4.12: Source and available disaggregated raw monitoring data for measured environmental concentrations in coastal/transitional water.

Source/Dataset	Available disaggregated raw data
EEA, Watch List (2019)	28 samples (25% quantified) from 7 MS for the period 2015-2019
EEA, WISE (2020)	38 samples (18.4% quantified) from 6 MS for the period 2016-2019

Table 4.13: Available raw data for the measured environmental concentrations from several MS (after the elimination of duplicated records) for the period 2015 – 2019 in the combined dataset for Sc2 scenario (coastal/transitional water)

Scenario	Member States (MS)	Sites	Samples	Quantified samples (% of all)
Sc2	9	19	53	17

Regarding the quality of available monitoring data in Sc2 scenario, the range of LOQs of non-quantified samples is from 0.001 µg/L to 0.039 µg/L. About 47.7% of non-quantified samples (21 out of 44 samples) are taken with LOQs≥0.009 µg/L which might indicate an insufficient sensitivity of applied analytical methods. Moreover, the total amount of data is scarce for making a reliable risk assessment. However for a sake of completeness, the descriptive statistic of measured concentrations was estimated and it is presented in Table 4.14. In the statistical analysis the non-quantified concentrations are assumed to be equal to a half of LOQs.

Table 4.14: Summary statistics of measured environmental concentrations for Sc2 scenario of combined dataset for coastal/transitional water. In this analysis the non-quantified concentrations are assumed to be equal to a half of LOQs.

	Min	Mean	StDev	Median	P90	P95	P99	Max
Concentration (µg/L)	$5*10^{-4}$	0.0136	0.0368	0.005	0.0195	0.057	0.172	0.24

5 Environmental Behaviour

5.1 Environmental distribution

Table 5.1: Summary of Environmental Distribution Data of Diclofenac

		Reference
Water solubility (mgl^{-1})	2.37 at 20°C (Diclofenac)	Fini et al., 1999
Volatilisation	1500 at 20°C (Diclofenac sodium)	Caleo, 2010
Vapour pressure (Pa)	6,14 10^{-8} mm Hg 1,59 x 10^{-7} Torr	Nelly and Blau, 1985 ACS-Datenbank, 2005
Henry's Law constant ($Pa\ m^3\ mol^{-1}$)	4.8 10^{-7}	US EPA (2021)
Organic carbon – water partition coefficient (K_{OC})	1450 L/kg (pH=1, calculated) 874 L/kg (pH=4, calculated) 2,30 L/kg (pH=7, calculated) 1 L/kg (pH=8-10, calculated)	ACS-Datenbank, 2005
Suspended matter – water partition coefficient ($K_{susp-water}$)	Sludge K_{oc} = 47 - 1310 L/Kg Sludge $logK_{ow}$ = 4.51 Sludge $logK_{oc}$ = 0.78 Sludge K = 41 ± 3 cm^3/g	Ternes et al. 2004 Urase and Kikuta, 2005 BLAC, 2003 Drillia,et al . 2005
	Soil K_{oc} = 200 – 631 L/kg	Chefetz et al. 2008
	Soil K_{oc} = 107.3 – 167.3 cm^3/g (0-5 cm soil layer)	Scheytt et al 2005a
	Soil K_{oc} = 121.0 - 2310.0 cm^3/g	Xu et al. 2009
	Soil K = 61.7 – 83.2 cm^3/g (15-25 cm soil layer) Sediment $logK_{oc}$ = 2.45 - 3.74	Scheytt et al 2005b
Octanol-water partition coefficient (Log Kow)	logKow = 4.02 logP = 3.28 ± 0.36 (calculated) LogP = 1.12	Syracuse-Science- Center, 2002 Ternes 1998
	logKow = 4.51 (pH ~ 3) logD = 1.31 (pH = 7.4)	Avdeef et al. 1998
	logKow = 4.6	Ternes et al. 2004

The pK_a value for diclofenac of approximately 4 indicates that a log K_{OW} value for the unionised form is not relevant for environmental fate. The log K_{OW} value based on the dissociated form of diclofenac of 0.68 indicates a low affinity for non-aqueous phases. The physico-chemical properties of diclofenac indicate that the substance is water soluble, ionised in aqueous environmental media, and is unlikely to undergo significant environmental

partitioning due to its presence in an anionic form in the environment. Diclofenac may undergo some partitioning to cationic adsorbent phases in the environment, including some clay minerals such as kaolinite, under some pH conditions. The empirical partitioning data are consistent with the indications from physico-chemical data that adsorption of diclofenac to both soils and sewage sludges is relatively limited, suggesting a relatively high level of mobility in the environment.

5.2 Abiotic and Biotic degradations

Table 5.2: Summary of Abiotic and Biotic Degradation of Diclofenac

		Reference
Photolysis	Rapid degradation of DCF to a level <1% of the initial concentration after 4 days exposure to sunlight (DT50 < 4d) DT50= 2.4 days (in salt and organic-free water, 50° N in winter) DT50= 39 min (in natural water and Milli-Q water	Buser, et al. 1998 Andreozzi, et al. 2003 Latch et al. 2003
Biodegradation	DT_{50} (type of water) = 5.5 – 18.6 d Significant depletion by sediment microbial activity (93 % depletion of diclofenac after 5 days) $T_{½}$ = 5.5 – 18.6 days in sediment systems (bench-scale annular flume; flat sediment surface vs moving	Groning et al. 2007 Kunkel and Radke, 2008

6 Effects and Quality Standards

The studies were evaluated and assessed according to Moermond et al. (2016) in accordance with recommendations from TGD 27 (EC, 2018). This assessment includes a set of 20 reliability and 13 relevance criteria, whereby the classes assigned (R1-4) match those of Klimisch et al. (1997):

R1 Reliable without restrictions: All critical reliability criteria for this study are fulfilled. The study is well designed and performed, and it does not contain flaws that affect the reliability of the study.

R2 Reliable with restrictions: The study is generally well designed and performed, but some minor flaws in the documentation or setup may be present.

R3 Not reliable: Not all critical reliability criteria for this study are fulfilled. The study has clear flaws in study design and/or how it was performed.

R4 Not assignable: Information needed to assess the study is missing. This concerns studies that do not give sufficient experimental details and that are only listed in abstracts or secondary literature (books, reviews, etc.) or studies of which the documentation is not sufficient for assessment of reliability for one or more vital parameters.

In considering the toxicity data for diclofenac, both the reliability and the ecological relevance of the endpoints have been taken into account, according to TGD 27.

Some of the key acute and chronic toxicity studies for diclofenac are outlined in the tables below. In considering the ecotoxicity data for diclofenac both the reliability and the ecological relevance of the endpoints have had to be taken into account.

6.1 PH-Effects

Physico-chemical features of natural fresh waters, including pH, temperature, oxygen, carbon dioxide, divalent cations, anions, carbonate alkalinity, salinity and dissolved organic matter, can affect the environmental risk to aquatic wildlife of pollutant chemicals. Physico-chemistry directly and/or indirectly affect the solubility, speciation, bioavailability and uptake of chemicals, including via alterations in the trans-epithelial electric potential (TEP) across the gills or skin (Pinheiro et al 2021). The authors emphasise that a better understanding of chemical toxicity and more accurate environmental risk assessment requires greater consideration of the natural water physico-chemistry in which the organisms we seek to protect live.

Boström and Berglund (2015) found significant differences in pH among countries with a median range from 7.0 (Sweden) to 8.3 (Cyprus). Within-country pH variations ranged from 0.4 pH units (Switzerland) to 5.9 pH units (Spain). This is in line with Bundschuh et al. (2016), who reported a mean pH of 7.8 in European rivers (without the Scandinavian countries) with a maximum pH of 12.4 and a minimum pH of 4.3. This huge variability is summarized in the GLObal RIver Chemistry database GLORICH, which combines an assemblage of hydrochemical data from varying sources with catchment characteristics of the sampling locations. The data base comprises 1.27 million samples distributed over 17,000 sampling locations to demonstrate the huge variability (often >1000-fold) (Hartmann et al. 2019)

In this context, it is important to note that around 80% of all pharmaceuticals are ionisable (Manallack 2008). This means that aquatic environmental pH can affect their chemical

specification, i.e., the fraction of ionic or uncharged forms (Boström and Berglund 2015). Diclofenac is chemically a weak acid, i.e., small changes in the test pH can significantly alter the balance between the dissociated and non-dissociated form of the substance. These altered dissociation equilibria are expected to significantly affect the partition coefficient of diclofenac (i.e., the pH dependent log Dow), and thus also its bioavailability and measurable toxicity, according to OECD guideline 23 on the test of difficult substances (OECD 2019). The reason for this is that for the most part only the neutral, uncharged form can pass the biological membranes. It is, therefore, essential that the relevant dissociation constant (i.e., the pKa) and the respective log D values are considered in the environmentally relevant pH-range of approximately 5 to 9 (see figure 6.1) prior to the commencement of testing. In fact, differences of more than one order of magnitude in the acute toxicity of ionic substances have been observed due to alterations of the test pH in the environmentally relevant range (Anskjær 2013), which is in line with most OECD guidelines. In case of Diclofenac, this also means that bioaccumulation is increasing with lower pH levels. As seen in figure 6.1 below, the log D is changing quite considerably between pH 7 and pH 8.5, which is according to Bundschuh et al. (2016), and Boström and Berglund (2015) the pH range of more than 90 % of the surface water in Europe. And this variation can occur quite naturally, due to diurnal variation, but also due to small scale variations of abiotic and biotic factors, like lightening conditions and the potential of photosynthesis; differences between interstitial and open water; particular organic matter, Redox-Potential.

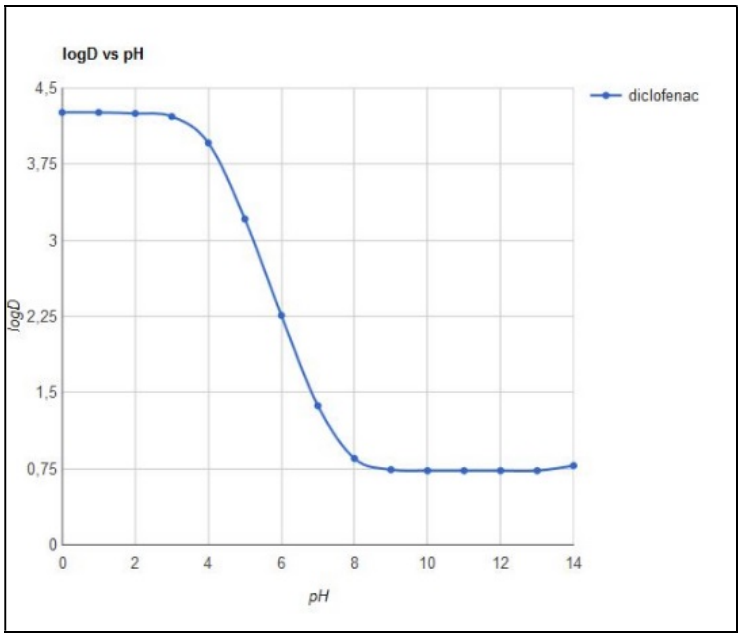


Figure 6.1: Prediction of the pH dependence of the octanol-water coefficient (log D) of Diclofenac (Chemaxon 2016).

6.2 Acute aquatic ecotoxicity

6.2.1 Acute Data

In general, acute values are considered not relevant for human pharmaceuticals, due to their (pseudo)- chronic exposure pattern. In addition, the concentrations of diclofenac measured in surface waters receiving only wastewater discharges would not be expected to be sufficiently high to cause acute effects.

However, there may be situations (e.g., combined sewer outfall discharges during storm events, and waters receiving untreated hospital or pharmaceutical manufacturing effluents) where intermittent elevated concentrations may occur in the receiving environment. The reliable acute dataset for diclofenac is shown in Table 6.1. Many of these studies, did not include any analytical verification of diclofenac exposure concentrations and results are therefore reported as nominal concentrations only. Nevertheless, they are considered to be reliable and relevant for the derivation of EQS (Klimisch et al. 1997, Moermond et al. 2016) considering that exposure remained short term and that therefore degradation might have not occurred.

The reliable acute toxicity dataset for diclofenac covers freshwater algae, freshwater and marine crustaceans, two other freshwater invertebrate taxa (a ciliate and a platyhelminth worm), and freshwater fish and amphibians.

Literature assessed but found not usable for EQS setting is listed in Annex IV, Chapter 12.

6.2.2 Acute Effects

Table 6.1: Selected acute data from different taxa exposed to Diclofenac.

Taxonomic Group	Organism	Effect	Exposure Type	Duration	IC/EC/LC50 (µg L^{-1})	Analytical measurement	Reference	Reliability
Algae	*Desmodesmus subspicatus*	Population Growth	Static	72 hours	135400	Yes	Meden-Kunkel and Maletzki 2010	2
Algae	*Desmodesmus subspicatus*	Population Growth	Static	72 hours	60440	Yes	Weissmannova et al. 2018	2
Algae	*Haematococcus pluvialis*	Population Growth	Static	14 days#	29000	No	Bacsi et al. 2018	2
Crustacean	*Daphnia magna*	Immobility	Static	48 hours	22430	Yes	Ferrari et al. 2003	2
Crustacean	*Daphnia magna*	Immobility	Static	48 hours	53700	No	Gheorghe et al. 2016	2
Crustacean	*Daphnia magna*	Immobility	Static	48 hours	96600	No	Gomez-Olivan et al. 2014	2
Crustacean	*Daphnia magna*	Immobility	Static	48 hours	>10000	No	Fekete-Kertesz et al. 2016	2
Crustacean	*Daphnia magna*	Immobility	Static	48 hours	60700	Yes	Lee et al. 2011	2
Crustacean	*Daphnia magna*	Immobility	Static	48 hours	123300	No	de Oliveira et al. 2016	2
Crustacean	*Daphnia magna*	Immobility	Static	72 hours	6230	Yes	Du et al. 2016	2
Crustacean	*Moina macrocopa*	Immobility	Static	48 hours	142600	Yes	Lee et al. 2011	2
Crustacean	*Ceriodaphnia dubia*	Immobility	Static	48 hours	22700	Yes	Ferrari et al. 2004	2
Crustacean	*Ceriodaphnia silvestrii*	Immobility	Static	48 hours	37900	No	de Oliveira et al. 2018	2
Crustacean	*Gammarus fossarum*	Mortality	Static	48 hours	58000	Yes	Triebskorn et al. 2017	1
Crustacean	*Atyaephyra desmarestii*	Mortality	Semi-static	96 hours	6300	Yes	Nieto et al. 2016	2
Crustacean	*Tisbe battagliai*	Mortality	Static	48 hours	9500	Yes	Trombini et al. 2016	2
Crustacean	*Siriella armata*	Mortality	Static	96 hours	2919	No	Perez et al. 2015	2
Ciliate	*Tetrahymena pyriformis*	Population growth	Static	24 hours	26560	No	Lang and Kohidai 2012	2
Platyhelminth	*Dugesia japonica*	Mortality	Static	96 hours	4200	No	Li 2013	2
Fish	*Cyprinus carpio*	Mortality	Static	96 hours	70980	No	Saucedo-Vence et al. 2015	2
Fish	*Cyprinus carpio*	Mortality	Static	96 hours	109640	No	Gheorghe et al. 2016	2
Fish	*Danio rerio*	Mortality (embryos)	Semi-static	144 hours	6110	Yes*	Praskova et al. 2011	2
Fish	*Danio rerio*	Mortality (juveniles)	Semi-static	96 hours	166600	Yes*	Praskova et al. 2011	2
Fish	*Danio rerio*	Mortality (embryos)	Static	48 hours	14150	No	Zhou et al. 2019	2
Fish	*Danio rerio*	Mortality (embryos)	Semi-static	72 hours	7800	Yes	van den Brandhofet al. 2010	2
Fish	*Oryzias latipes*	Mortality	Static	96 hours	10100	No	Nassef et al. 2009	2
Amphibian	*Lithobates catesbeianus*	Mortality (embryos)	Static	96 hours	12100	No	Cardoso-Vera et al. 2017	2
Amphibian	*Xenopus laevis*	Mortality	Static	96 hours	9560	No	Cardoso-Vera et al. 2017	2

Taxonomic Group	Organism	Effect	Exposure Type	Duration	IC/EC/LC50 (µg L^{-1})	Analytical measurement	Reference	Reliability
		(embryos)						
Amphibian	*Trachycephalus typhonius*	Mortality (embryos)	Semi-static	96 hours	2828.43	Yes*	Peltzer et al. 2019	2
Amphibian	*Physalaemus albonotatus*	Mortality (embryos)	Semi-static	96 hours	2462.29	Yes*	Peltzer et al. 2019	2

Bacsi et al. (2018) report EC50 values for 96 hours, 7 days and 14 days. The 14-day EC50 is taken as the lowest (worst case) value despite the time period being longer than most acute algal tests.

* Paper reports that exposure concentrations were measured, but the results are not reported, and LC50 values are based on nominal concentrations.

6.3 Chronic aquatic ecotoxicity

The reliable, population-relevant freshwater chronic ecotoxicity data for diclofenac are given in Table 6.2. All ecotoxicity data for EQS derivation were subjected to reliability and relevance assessment according to accepted methodologies (EC 2018, Moermond et al. 2016, Klimisch et al. 1997). It should be noted that for some of the studies listed in Table 6.2 the detailed reliability and relevance outcome relates directly to the study endpoint given in the table; some of these studies feature additional endpoints that were considered to be not reliable and/or not relevant. Literature assessed but found not usable for EQS setting is listed in Annex IV, Chapter 12.

Table 6.2: Selected reliable chronic data for species exposed to diclofenac.

Taxonomic Group	Organism	Effect	Exposure Type	Duration	NOEC/ EC10 (µg/L)	Analytical measurement	Reference	Reliability
Algae	*Desmodesmus subspicatus*	Population growth	Static	3 days	52600	Yes	Meden-Kunkel and Maletzki 2010	2
Algae	*Desmodesmus subspicatus*	Population growth	Static	3 days	15540	Yes	Weissmannova et al. 2018	2
Algae	*Dunaliella tertiolecta*	Population growth	Static	4 days	25000		DeLorenzo and Fleming 2008	2
Aquatic plant	*Lemna minor*	Growth	Static	10 days	1.7	No	Kummerova et al. 2016	2
Aquatic plant	*Lemna minor*	Growth	Static	7 days	3140		Markovic et al. 2021	2
Aquatic plant	*Azolla filiculoides*	Growth	Static	10 days	24000	No	Vannini et al. 2018	2
Rotifer	*Plationus patulus*	Population growth	Static	25 days	1400	No	Sarma et al. 2013	2
Rotifer	*Lecane papuana*	Population growth	Static	5 days	590		Tovar-Agullar 2019	2
Crustacean	*Moina macrocopa*	Population growth	Static	10 days	788	No	Sarma et al. 2013	2
Crustacean	*Moina macrocopa*	Reproduction	Static	7 days	16750	Yes	Lee et al. 2011	2
Crustacean	*Daphnia magna*	Reproduction	Semi-static	21 days	120	Yes	Du et al. 2016	2
Crustacean	*Daphnia magna*	Reproduction	Semi-static	21 days	1900	Yes	Triebskorn et al. 2017	1
Crustacean	*Daphnia magna*	Reproduction	Static	21 days	8300	Yes	Lee et al. 2011	2
Crustacean	*Daphnia magna*	Reproduction	Semi-static	21 days	72000	No	de Oliveira et al. 2015	2
Crustacean	*Daphnia magna*	Reproduction			18		Liu et al. 2017	2
Crustacean	*Ceriodaphnia silvestrii*	Reproduction	Semi-static	8 days	1000	No	de Oliveira et al. 2018	2
Crustacean	*Gammarus fossarum*	Reproduction	Semi-static	35 days	790	Yes	Triebskorn et al. 2017	1
Crustacean	*Palaemon longirostris*	Development			40		Gonzalez-Ortegon et al. 2015	2
Gastropod Mollusc	*Lymnaea stagnalis*	Reproduction	Semi-static	28 days	1540	Yes	Scymaris 2020a	1
Bivalve Mollusc	*Mytilus edulis trossulus*	Byssus strength			3.2		Ericson et al. 2010	2
	Dreissena polymorpha	Mortality	Flow Through (mesocosm)	171 days	0.25		Joachim et al. 2021	2
Echinoderm	*Paracentrotus lividus*	Larval length	Static		5.2		Ribeiro et al. 2015	2
Echinoderm	*Paracentrotus lividus*	Fertilisation and Embryo Development	Flow-through (adult) and Static (fertilisation, embryo-development)	4 days (adult), 4 hours (fertilisation) 48 hours (embryo development)	>1000	Yes	Scymaris 2020b	1
Fish	*Oryzias latipes*	Reproduction	Semi-static	14 days	25	No	Yokota et al. 2016	2
Fish	*Oryzias latipes*	Reproduction	Semi-static	14 days	7.8	Yes	Yokota et al. 2017	1
Fish	*Oryzias latipes*	Jaw malformation	Semi-static	90 days	12.6	Yes	Yokota et al. 2018	1
Fish	*Oryzias latipes*	2nd generation hatching	Semi-static	3 months	7100	Yes	Lee et al. 2011	2

Taxonomic Group	Organism	Effect	Exposure Type	Duration	NOEC/ EC10 (µg/L)	Analytical measurement	Reference	Reliability
Fish	*Salmo trutta*	Mortality	Semi-static	127 days	3.5	Yes	Schwarz et al. 2017	2
Fish	*Gasterosteus aculeatus*	Jaw malformation	Flow through	21-28 days	7.2	Yes	Naslund et al. 2017	1
Fish	*Oncorhynchus mykiss*	Hatching, larval development, mortality, growth	Flow through	60 days	>1084	Yes	Memmert et al. 2013	1
Fish	*Oncorhynchus mykiss*	Eye malformation	Flow through	28 days	5	Yes	Birzle 2015	2
Fish	*Danio rerio*	Growth	Flow through	30 days	8.6	Yes	Memmert et al. 2013	1
Fish	*Danio rerio*	Growth	Semi-static	28 days	5000	Yes	Praskova et al. 2014	2
Fish	*Danio rerio*	Hatching	Semi-static	80 hours	1250	No	Ribeiro et al. 2015	2
Fish	*Cyprinus carpio*	Larval mortality	Semi-static	30 days	674	Yes	Stepanova et al. 2013	2

* Paper reports that exposure concentrations were measured, but the results are not reported, and LC50 values are based on nominal concentrations.

6.3.1 Derivation of the AA-$QS_{freshwater,eco}$

The available reliable and relevant chronic toxicity data for Diclofenac includes studies on algae, plants, crustaceans, rotifers, gastropod molluscs, bivalve molluscs, echinoderms and fish. The studies have examined a wide range of endpoints and been undertaken over a range of exposure durations.
Limited data are available on marine species, but there is no reason to expect a difference and therefore it is proposed to pool the freshwater and marine data, see also sections 6.4.1.2 and 6.4.3.

6.3.1.1 Deterministic approach

The deterministic approach involves the application of an assessment factor to the lowest reliable and relevant NOEC/EC10 where the dataset influences the size of the assessment factor applied depending on its content (data quality and species representativity). NOECs are available *inter alia* for algae, invertebrates, and fish, which based on the EU EQS guidance (EC 2018) enables an assessment factor of 10 to be applied.
The most sensitive chronic study assessed were *Dreissena polymorpha*, as part of the mesocosm conducted by Joachim et al. 2021. As the mussels were exposed in cages, this study can be used a single species study and used for the deterministic approach. The calculated EC10 value is 0.25 µg/L.
Nevertheless, in the final opinion on diclofenac dossier (2022), the SCHEER rejected the use of the EC_{10} of 0.25 µg/L for *D. polymorpha* (Joachim et al., 2021): Indeed, "*As the mussels were exposed in cages, this study can be used as a single species study and used for the deterministic approach. In reviewing this part of the Joachim et al. (2021) study, the SCHEER noted diclofenac seemed to have little or no impact on end-points, such as condition, energy reserve, amylase activity or immune function except at the highest concentration. However, a relatively higher mortality was highlighted at the effect concentration of 0.44 µg L^{-1} with 40.6%+/-6.0% mortality compared to 29.7% +/-9.6% for the control. The authors report this as significant at $p<0.05$. On this basis, an EC_{10} value of 0.25 µg L^{-1} is offered. It was confusing that in Annex I, chapter 9 of the dossier, an EC_{10} of 0.37 µg L^{-1} rather than 0.25 µg L^{-1} is reported. The SCHEER does not consider that the high loss of mussels (almost 1/3rd) in the control was acceptable and they were sceptical that the difference was statistically significant. Therefore, the SCHEER does not endorse the proposed starting point for a deterministic AA-$QS_{fw,eco}$ nor the AA-$QS_{sw,eco}$ proposed on the same conceptual basis*".

The second lowest chronic value reported is the EC_{10} of 1.7 µg/L for growth in the higher plant *Lemna minor* (Kummerova et al. 2016). This datum was used for further calculations.

6.3.1.2 Species Sensitivity Distribution (SSD) approach

Chronic toxicity data for Diclofenac is available for a range of species including algae, plants, crustaceans, rotifers, amphibians, and fish. Results of 21 studies were found to be usable for the SSD approach. These studies were assessed according to the CRED criteria (Moermond et al. 2016). A summary of these studies is listed in Annex 1- Chapter 9 .

Table 6.3: Studies suggested for the SSD approach.

Major taxonomic group	Species	EC10 (or NOEC) µg/l	Study
Algae	*Dunaliella tertiolecta*	25000	DeLorenzo & Fleming 2008
	Desmodesmus subspicatus	15540	Weissmannová et al. 2018
Higher plants	*Lemna minor*	1.7	Kummerova et al. 2016
	Azolla filiculoides	24000	Vannini et al. 2018
Rotifera	*Plationus patulus*	1400	Sarma et al. 2014
	Lecane papuana	590	Tovar-Aguilar 2019
Bivalvia	*Mytilus edulis*	3.2	Ericson et al. 2010
	Dreissena polymorpha from mesocosm	0.25	Joachim et al. 2021
Gastropoda	*Lymnaea stagnalis*	1540	Scymaris 2020a
Crustacea, Branchiopoda	*Daphnia magna*	18	Liu et al. 2017
	Ceriodaphnia silvestrii	1000	de Oliveira et al. 2018
	Moina macropoda	788	Sarma et al. 2014
Crustacea, Amphipoda	*Gammarus fossarum*	790	Triebskorn et al. 2017
Crustacea, Decapoda	*Palaemon longirostris*	40	González-Ortegón et al. 2015
Echinodermata	*Paraentrotus lividus*	5.2	Rebeiro et al. 2015
Pisces	*Oncorhynchus mykiss*	5	Birzle 2015
	Oryzias latipes	7.8	Yokota et al. 2017
	Danio rerio	8.6	Memmert et al. 2013
	Gasterosteus aculeatus	7.2	Naslund et al 2017
	Cyprinus carpio	674	Stepanova et al. 2013
	Salmo trutta	3.5	Schwarz et al 2017

However, the data set of EC10 and NOEC for Diclofenac is clustered in three with values below 40, between 590 and 1600 and 15000, which leads to three steps in the percentiles (Figure 6.2). Normality of the log sensitivities was rejected (Shapiro-Wilk's test, p=0.0143). In a similar way, log-triangular distributions also do not appear to be appropriate.
The distribution of log EC10 or log NOEC appears to be multimodal according to the histogram. The goodness of fit of a normal distribution, and of mixtures of 2, 3, or 4 normal distributions were compared using the AIC (R packages fitdistr and mixtools) (R Core Team 2020 http://www.r-project.org/index.html). The AIC was lowest with a trimodal distribution (AIC=77.4, 65.6, 50.0, and 51.0 respectively), indicating a higher goodness of fit.

A view of the violin- boxplot plot (Figure 6.3) showed that the data is bimodally distributed. The question arose if the sample consists of two samples from two different populations. Therefore, the residuals of the modelled distribution functions (exemplary for loglogistic model) and the observations have been investigated.

The distributions of the two samples are different as the boxplots showed (Figure 6.3). A two-sided t-test was used to proof the hypotheses of the different distributions (pre-test of normal distribution and homoscedasticity were conducted if the t test criteria are fulfilled) and confirmed a significant difference:
Two Sample t-test [t = -4.064, df = 19, p-value = 0.0006619]
alternative hypothesis: true difference in means is not equal to 0.
95 percent confidence interval: -0.18135718 -0.05805642
sample estimates:
mean in group high: 0.06286658 mean in group low: 0.05684022.

A full detailed statistical analysis can be found in Annex II, Chapter 10.

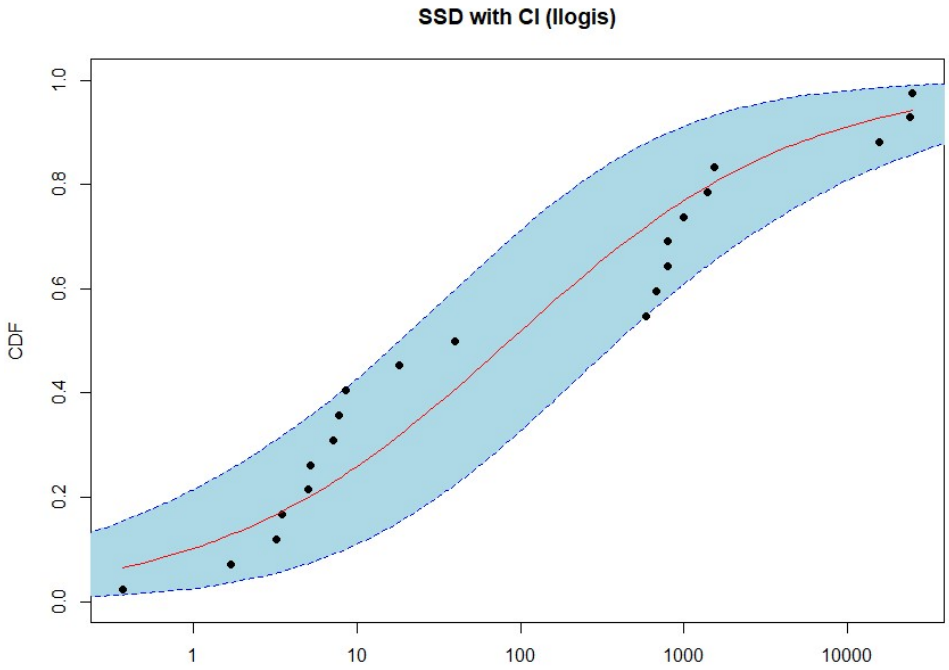


Figure 6.2: cumulative distribution of EC10/NOEC [µg/l], observed data and simulated loglogistic function with confidence intervals.

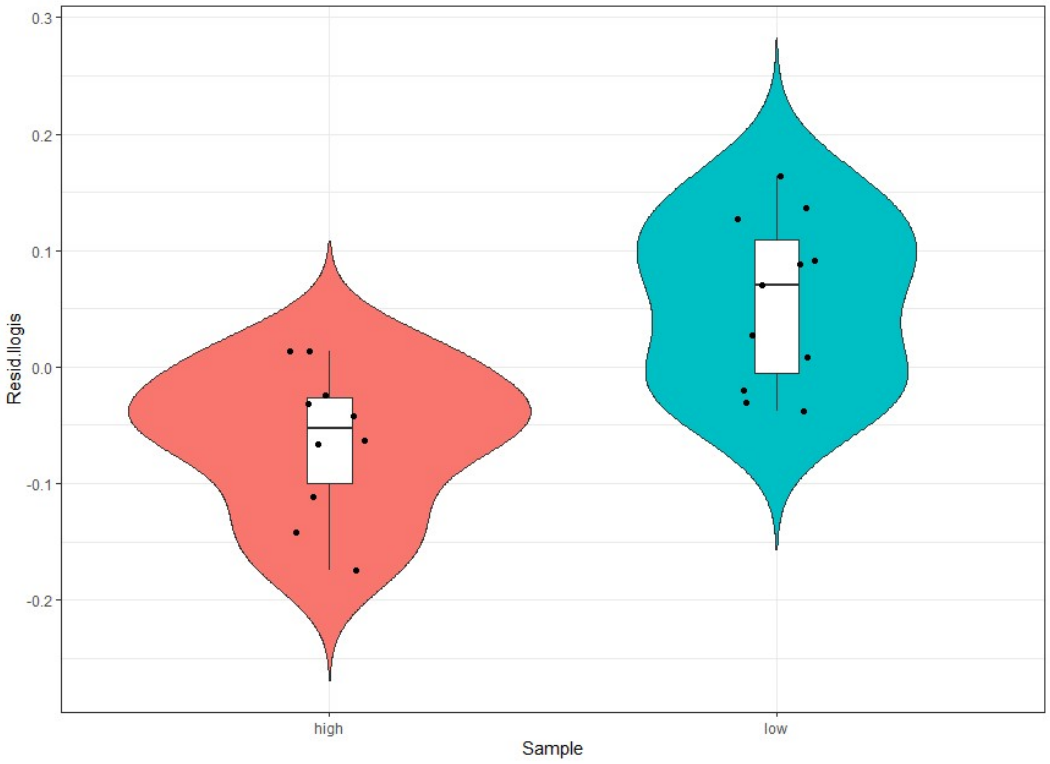


Figure 6.3: Violin- boxplot of the residuals of the loglogistic model and the observed data

In contrast to e.g., substances with an estrogenic mode of action like Estradiol and Ethinylestradiol, for Diclofenac there are no clear taxonomic related differences found in the distribution of the SSD. For example, two autotrophic species (*Dunaliella tertiolecta* and *Desmodesmus subspicatus)* are on the higher end of the distribution but the duckweed (*Lemna minor)* is also shown to be the second most sensitive species. Moreover, fish toxicity data ranged from 3.5 µg/L for *Salmo trutta* up to 674 µg/L for *Cyprinus carpio*. Consequently, it was considered there were no ecological or taxonomic reason to use one part of the SSD only and exclude other studies.
These results suggest the SSD approach may not be applicable to the whole dataset. However, no mechanistic explanation for a sensitive subgroup could be identified and the SSD may also not be applicable to the sensitive subgroup.
In line with the TGD guidance document (EC2018), it is suggested not to use the SSD at all for setting the EQS for Diclofenac:
"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.".

6.3.1.3 Mesocosm Study

According to the TGD 27, if a mesocosm study is available, and it fulfils the criteria regarding reliability and relevance as defined below, the corresponding toxicity data may be used either as the

basis of QSfw,eco derivation or, when an SSD is used, to help select the size of AF applied to the HC5. (EC 2018).
Such a mesocosm study was conducted by Joachim et al. (2021). The authors tested three concentrations of diclofenac in outdoor freshwater mesocosms with continuous exposure of 171 days (ca. 5.5 months). The scientific reliability of the study was evaluated according to the criteria of De Jong et al. (2008).

- The test system represents a realistic freshwater community, since natural populations of algae, zooplankton and macroinvertebrates were present, as well as macrophytes and fish. Caged zebra mussels were included as bioindicators.
- The description of the experimental set-up is adequate and unambiguous, and sufficient details are reported in the paper and supplementary information.
- The exposure regime is adequately described, and measured concentrations are available, but analytical results are not reported in detail.
- The investigated endpoints are sensitive and in accordance with the working mechanism of the compound. In addition to the effects on the aquatic ecosystem, effects on zebra mussel and stickle backs were studied, both on an individual level and for population relevant parameters.
- Although raw data are not available, the details of the statistical analyses are presented in the supplementary information and data are analysed according to up-to-date methods.

The study is to be considered reliable since all criteria for such a study are fulfilled. However, since a GLP statement is lacking, the study is judged as reliable with restrictions (Ri 2).

Summary of the results of the mesocosm study:
Test system
Twelve artificial streams, flow through, 20 m length, 1 m width, three sections, upper, slope and lower. Upper part 5 cm, lower part 10 cm sediment, slope with 10-20 cm rocks. Artificial fine grain sediment, 80% sand and 20% clay. Location: North of France (INERIS, Verneuil-en-Halatte, France). Flow through with denitrified and dechlorinated tap water at 800 L/h.

Biological composition
Mesocosm were set up from October 2012 till March 2013. Watercress *Nasturnium offinicale*, starwort *Callitriche platycarpa* and Water milfoil *Myriophyllum spicatum* were added in October and November. Origin not described. In November, zooplankton and periphyton were added from an unpolluted artificial pond. In addition, shredders (200 individuals of *Asellus aquaticus*, and 80 grams i.e., 2500- 3000 individuals of *Gammarus pulex*) and gastropods (200 *Potamopyrus antipodorum*, 17 *Planorbis carinatus* and 50 *Radix balthica*) were introduced into each mesocosm. Finally, invertebrate predators were released into each mesocosm during the same month. Each mesocosm received 8 Notonecta, 6 *Glossiphonia complanata* and 4 *Erpobdella octoculata*. Mesocosms were then left to settle until March 2013. Then 15 mature female and 10 male sticklebacks (*Gasterosteus aculeatus*) were added to each mesocosm and 2 cages with 120 zebra mussels (*Dreissena polymorpha*) were added to each mesocosm before the start of the exposure.

Exposure
Treatments, 0, 0.1, 1 and 10 µg/L (nominal), in triplicate. Continuous flow. Treatment from 16.4.2013 (day 0) until 4.10.2013 (day +171).

Analytical sampling

Concentration was measured monthly (6 sampling dates, at 2, 36, 63, 99, 125 and 171 dpt), with 3 samples at 0, 5 and 19 m from the inlet for treated canals (27 samples) and with one sample at 10 m from the inlet for control canal (3 samples). This makes a total of 180 samples. Average concentrations are presented for the three streams per treatment. Nine metabolites and transformation products were measured in watercress at the end of the experiment.
Average effective concentrations (AEC) were then calculated for each treatment using the mean values of the three replicates between 5 and 19 meters. An integration method from Van Wijngaarden et al. (1996) was used.

Physico-chemical parameters.
Water temperature was measured every 10 min. (at 5 and 15 m); pH, conductivity and dissolved oxygen were measured weekly.

Effect sampling
Volume score of macrophytes and filamentous green algae were estimated every two weeks. Cages with zebra mussels were harvested after 2 and 5 months. Survival was estimated and biochemical parameters (digestive enzyme activities, energy quantification, and Electron Transport System activity i.e., ETS), immune parameters, oxidative activity, and genotoxicity (DNA strand breaks) were estimated.
At the end of the experiment, 30 fish were selected. Condition factor, liver somatic index and gonadal somatic index were estimated. Liver oxidative stress was estimated as were biomarkers as protein concentration, hepatic activities of lipidic lipoperoxidation (TBARS), superoxide dismutase (SOD), glutathion total (GST), glutathione peroxydase (GPx). Energy allocation was determined with liver lipid analyses. The spleen was used for immunomarker analyses. Leucocyte distribution, cellular mortality (apoptotic and necrotic leucocytes), leucocyte respiratory burst, lysosomal membrane integrity (LMI) and phagocytosis activity were performed.
For fish effect on larvae drift was determined (daily, expressed as larvae/week/mesocosm). Fish population structure was determined at the end of the experiment (all fish populations were killed, and then length and weight were measured).
Zooplankton was measured every 4 weeks in the upper and lower section, invertebrates were sampled on artificial substrates. Monthly from 48 days before treatment till 148 d after treatment.

Statistical analysis
Univariate and multivariate analyses, PRC.

Results
Chemical analysis
Mean measured concentrations at the inlet of the mesocosms were 0.06 ± 0.02, 0.46 ± 0.13, and 4.36 ± 1.29 µg/L, respectively for the 0.1, 1, and 10 µg/L treatments during the entire experiment. Average effective concentrations over the mesocosms are **0.041** ± 0.016, **0.44** ± 0.05, **3.82** ± 0.47 µg/L. AECs for each time step is provided in the supplementary appendix of the publication.
Diclofenac (DCF) and three transformation products were found in watercress, in the high treatment. DCF, 4' OH-DCF, and DCF-lactam were found in macrophyte tissue in all replicates, with concentrations ranging between 9.43 and 31 ng/g, 6.3–12.8 ng/g, and 0.3–1.3 ng/g, respectively. The metabolite 5' OH-DCF was found at a concentration of 0.9 ng/g in only one replicate. Results in the control and the low and medium treatments were below the limit of detection.

Physico-chemical parameters.
Clear and long-lasting significant effects were found for dissolved oxygen in the highest treatment, so NOEC is set at 0.44 µg/L.

Biological observations
Macrophytes
The volume scores of watercress, water starwort and Eurasian water milfoil were significantly affected by the treatment. Clear negative effects are found for watercress, NOEC 0.44 µg/L. For water milfoil, significant effects were seen at the lowest treatment level on some occasions towards the end of the study.

Zebra mussel
Enzyme and condition parameters were not significantly affected. Immunomarkers hemocyte distribution, hemocyte mortality were significantly affected in the two highest dosages. Genotoxicity, measured as DNA strand breaks was found in all treatments (NOEC <0.041 µg/L). Mortality was significantly increased on the second sampling date (5 months) in all treatments by 12, 16 and 40% as compared to the control. The authors conclude to a NOEC of 0.041 µg/L. However, a significant increase of mortality was also found at the lowest dose. Control mortality after 5 months was 30%. This percentage mortality in control canals may not be considered high compared to other experiment with similar design (e.g. Palais et al., 2012). This may be attributed to the fact that mussels suffer from long-term holding in cages (maybe after 2 months). However, it should be noted that there was a clear concentration-effect in mortality.

For mortality of caged zebra mussel, a re-calculated EC10 of 0.25 µg/l is suggested.

Fish
For the biomarkers, clear effects were seen for the ROS basal level (decrease) which could be correlated with other biomarkers of oxidative stress (TBARs measured in the liver, leucocyte oxidative stress). NOEC < 0.041 µg/L.
At the population level, high mortality of founder fish was observed in two of the three mesocosms in the highest treatment concentration (from +36 dpt to +46 dpt). In these two replicates no founder fish were found at end of the experiment. In the third replicate, founder fish were found at the end of the experiment but in fewer numbers compared to the control. This effect was not found in the other treatments. Overall population effects on F0 and F1 generation were seen in the highest treatment.
The detailed statistical analysis of the mortality of the sticklebacks (*Gasterosteus aculeatus*) is presented in Chapter 11 Annex III.
For mortality of the female founder fish, a re-calculated EC10 of 0.20 µg/L is suggested.

Zooplankton
Significant treatment related community effects were found on one sampling date (38 days post treatment, all treatments). For the main groups, a NOEC of 0.44 µg/L was found for cladocerans on two consecutive sampling dates.

Macroinvertebrates
Community responses showed significant effects. At 95 and 148 dpt significant effects were found in the highest treatment, but also the 0.44 µg/L treatment seemed to be affected. For the main groups, a NOEC of 0.44 µg/L was found for “scrapers”.

Conclusion
The authors concluded that: "*In consideration of all the results, the NOEC value is <0.041 µg/L at the individual level and 0.44 µg/L at the population and community levels.*"

It is suggested to use mortality of the female founder fish as the overall endpoint for the mesocosm, with an EC10 value of 0.20 µg/L.
In addition, it is suggested to include the results of the mussel study (EC10 = 0.25 µg/L) in the SSD calculation, because this data was deemed assimilated to a laboratory data as mussels were caged and thereby not subjected to any trophic interaction.

6.4 Tentative QSwater

Table 6.4: Tentative QS_{water} for Diclofenac

	Relevant study for derivation of QS	Assessment factor	Tentative QS
$MAC_{freshwater, eco}$	*Dugesia japonica* (Li 2013)	10	420 µg/L
$MAC_{marine\ water, eco}$		100	42 µg/L
AA-$QS_{freshwater, eco}$	*Gasterosteus aculeatus* (Joachim et al. 2021)	5	0.04 µg/L
AA-$QS_{marine\ water, eco}$		50	0.004 µg/L
AA-$QS_{freshwater, sed.}$	Not triggered and no sufficient data		
AA-$QS_{marine\ water, sed.}$			

6.4.1 Derivation of the MAC-QS

6.4.1.1 Derivation of the MAC-$QS_{freshwater,eco}$

The short-term toxicity dataset for diclofenac covers freshwater algae, freshwater, and marine crustaceans, two other freshwater invertebrate taxa (a ciliate and a platyhelminth worm), and freshwater fish and amphibians. Acute marine data is only available for crustaceans. As diclofenac is ionised at pH values above four (see Chapter 6.1) no difference in ionisation behaviour would be expected between freshwater and seawater. According to the TGD 27, the default position is to combine freshwater and marine datasets unless a significant difference between them can be statistically demonstrated (EC 2018).

The default position is to combine freshwater and marine datasets unless a significant difference between them can be statistically demonstrated (EC 2018). Only 3 of the 16 species in the acute dataset for diclofenac are for marine species (all crustaceans). Despite the difference in the number of data points for freshwater and marine species, and the fact that the acute marine dataset only covers a single taxonomic group, a statistical comparison of the freshwater and marine acute datasets has been attempted. The variances of the freshwater and marine datasets are not significantly different (p=0.187), and the sensitivities are not significantly different (p=0.095). Whether or not the variances in the datasets are different only influences the choice of test used to assess differences in sensitivity (i.e., a t-test with either equal, or unequal variances). The acute ecotoxicity datasets can therefore be combined.

The lowest value in the combined acute dataset is the 96h-LC_{50} of 2,462.29 µg/L for the amphibian *Physalaemus albonotatus* (Peltzer et al., 2019), which is used for deriving the MAC-EQS for freshwater and marine waters.

As short-term tests from three trophic levels are available, an Assessment Factor (AF) of 10 could be applied, which lead to a **$MAC\text{-}QS_{fw,\ eco}$ = 246 µg/L.**

6.4.1.2 Derivation of the MAC-$QS_{saltwater,eco}$

The higher diversity in marine species and the fact that no species is represented calls for a higher AF in the derivation of the $QS_{saltwater}$ compared to the AF for the $QS_{freshwater}$. Consequently, an additional assessment factor of 10 is suggested for diclofenac.
Applying an additional AF of 10 for marine waters, will lead to a

$MAC\text{-}QS_{sw,\ eco}$ = 25 µg/L.

6.4.2 Derivation of the AA-$QS_{freshwater,eco}$

The available chronic toxicity data for Diclofenac includes studies on algae, higher plants, crustaceans, rotifer, bivalves, gastropods, and fish. The majority of the data however relates to studies on fish.
Limited data are available on marine species. Therefore, as data is insufficient to demonstrate any statistically significant difference between freshwater and marine species sensitivity, both datasets are pooled in accordance with TGD 27 (EC, 2018). See also section 6.4.3.

6.4.2.1 Derivation of the AA-$QS_{freshwater\ eco,}$ using the Deterministic approach.

The deterministic approach involves the application of an assessment factor to the lowest reliable and relevant NOEC or EC10 with the size of the dataset influencing the size of the assessment factor applied. NOECs are available for algae, higher plants, crustaceans, rotifer, bivalves, gastropods, and fish, which based on the TGD 27 (EC 2018) enables an assessment factor of 10 to be applied.
The most sensitive chronic species assessed was the zebra mussel (*Dreissena polymorpha)*, as part of the mesocosm conducted by Joachim et al. 2021. Nevertheless, as discussed above, this value was rejected by the SCHEER (2022).

The second lowest chronic value reported is the EC_{10} of 1.7 µg/L for growth in the higher plant *Lemna minor* (Kummerova et al. 2016). This datum was used for further calculations. Applying an AF of 10 on the EC_{10} of 1.7 µg/L, the **$AA\text{-}QS_{fw,\ eco}$ is equal to 0.17 µg/L.**

6.4.2.2 Derivation of the AA-$QS_{freshwater\ eco,}$ using the Species Sensitivity Distribution (SSD) approach

An EQS, using the SSD approach is not suggested, as the data for the SSD are clustered and the distributions of the two samples are different (see chapter 6.3.1.2 and Annex II).

6.4.2.3 Derivation of the AA-$QS_{freshwater\ eco,}$ using the Mesocosm results.

The guidance (EC 2018) states: “the AF applied to mesocosm studies or (semi-)field data will need to be reviewed on a case-by-case basis, but no guidance is given with respect to the *range* of AFs to be applied.

Brock et al. (2008) compared micro/mesocosm experiments for several chemicals in which long-term exposure was simulated. They estimated a geographical extrapolation factor based on the ratio of the upper and lower limit of the 95% confidence interval of NOECs for toxic effects. These factors ranged between 1.4 and 5.4. *This suggests that, where there is (a) only a single model ecosystem study, and (b) sensitive taxa are included in the study of a compound with a specific mode of action, an assessment factor of 5 would account for variation in the NOECs*. When additional, confirmative mesocosm studies are available, the AF may be lowered. Further discussion around the selection of AFs on mesocosm studies is to be found in Giddings et al (2002). In determining the size of AF to be applied, the following should be considered:

- What is the overall quality of the micro- or mesocosm study/studies from which the EC10 or NOEC has been derived?
- What is the relationship between the mode of action of the investigated substance and the species represented in the available micro- or mesocosm studies? Are sensitive species represented?
- Do the available micro- or mesocosm studies include vulnerable species or representatives of taxonomic groups (e.g., families, orders) of vulnerable species that are part of the aquatic ecosystems to be protected?
- Do the available micro- or mesocosm studies represent the range of flow regimes that should be protected by the EQS? Consider specific populations of species inhabiting the lotic and lentic water types to be protected.
- How representative are the mesocosm studies: do they represent the range of trophic statutes of waterbodies that should be protected by the EQS?"

According to the SCHEER final Opinion (2022), the mesocosm study of Joachim et al. (2021), is considered a useful piece of work, since the experiment was conducted more than 5 months. However, according to the SCHEER: "*the authors themselves report they were unable to control variables like oxygen between the different treatments and there were problems with high mortalities in the controls. It is the opinion of the SCHEER that the NOECs estimated for parameters at individual level cannot be assumed to be fully reliable, while the NOEC at the population and community level proposed in the conclusion of the paper (0.44 µg/L) may be used as a line of evidence for confirming or revising the EQS derived with deterministic or probabilistic procedures*". Applying an AF of 10, the **AA-QS$_{fw,eco}$ is set at 0.04 µg/L**.

6.4.3 Derivation of the AA-QS$_{saltwater,\ eco}$

According to the TGD 27 (EC 2018), "*ecotoxicity data for freshwater and saltwater organisms should be pooled for organic substances…. The pooled datasets are then used to derive both freshwater and saltwater QSs. Where there are too few data (either freshwater or saltwater) to perform a meaningful statistical comparison and there are no further indications (spread of the data, read-across, expert judgement) of a difference in sensitivity between freshwater vs saltwater organisms, the data sets may be combined for QS derivation."*

6.4.3.1 Derivation of the AA-QS$_{saltwater\ eco,}$ using the Deterministic approach.

A QS$_{freshwater,eco}$ of 0.17 µg/L has been proposed based on use of the deterministic approach (See Section 6.4.2.1).

However, the higher diversity in marine species and the fact that only one marine crustacean species is available call for a higher AF in the derivation of the $QS_{saltwater}$ compared to the AF for the $QS_{freshwater}$
Application of an additional assessment factor of 5 to this value gives an **AA $QS_{saltwater, eco}$ of 0.034 µg/L**.

6.4.3.2 Derivation of the AA-$QS_{saltwater\ eco,}$ using the mesocosm approach.

An AA $QS_{freshwater,eco}$ of 0.04 µg/l has been proposed based on use of the mesocosm approach (See Section 6.4.2.3). As the mesocosm available is a freshwater mesocosm an additional assessment factor of 10 is applied for deriving a $QS_{saltwater,eco}$ of 0.004 µg/L.

6.5 Derivation of the $QS_{sediment}$

As the substance seems not to bind strongly to sediment, and we have no indications that sediment dwelling organisms should be especially sensitive compared to species living in the water column, the derivation of sediment QS is not triggered.

6.6 Derivation of a QS for Secondary poisoning ($QS_{biota,secpois}$)

6.6.1 Toxicity in avian species

Several toxicity studies with diclofenac are available, most notably the studies with vultures as a result of the massive intoxication on the Indian subcontinent.

Oral doses of diclofenac were administered to non-releasable captive Oriental white-backed vultures (*Gyps bengalensis*), in total 24 dosed vultures and 8 controls (Oaks et al 2004). Vultures were either dosed orally (at single doses of 2.5 and 0.25 mg/kg body weight to two juveniles each) or fed tissues from goats or buffaloes treated with diclofenac, a few hours before slaughter (resulting doses ranged from 0.007 to 0.940 mg/kg body weight administered to 20 vultures). All control birds (two for the oral dose and six for the dosing via meat) survived. Dosed birds that died showed renal failure with extensive visceral gout. Dead vultures collected from the field that showed visceral gout had concentrations of diclofenac in the kidneys ranging from 0.051 to 0.643 mg/kg, while vultures that died from other causes had diclofenac concentrations in the kidneys that were below the detection limit of 0.005-0.010 mg/kg.
One vulture (numbered 11) that received the lowest dose of 0.007 mg/kg body weight, died with visceral gout, despite the fact that histopathological examination showed that the bird had low uric acid concentration in the plasma (reported in Swan et al 2006), comparable with the other birds that received low doses. On the other hand, the concentration of diclofenac in the kidney was rather high (0.38 mg/kg). Although not suggested by the authors, it could be a possibility that this vulture was accidentally changed with vulture number B, which received a rather high dose of 0.600 mg/kg body weight, but survived and had a concentration of diclofenac in the kidneys that was below the detection limit (0.005-0.01 mg/kg).
The data for the Oriental white-backed vulture (*Gyps bengalensis*) by Oaks et al (2004) were analysed in two subsequent studies (Green et al 2007, Swan et al 2006). On basis of the log(ln)-normal distribution of the toxicity data determined by the maximum likelihood method, both studies calculated the LD50 and the mean and standard deviation of the distribution on the data set, both

including and excluding the outlier that died despite of a very low dose of diclofenac (vulture 11). The LD50 calculated by removing the outlier was 225 µg/kg body weight. With the outlier included, the LD50 was 98 µg/kg body weight. Based on the presented data for the mean and the standard deviation of the log-normal distribution, an LD10 could also be determined from the presented data. With the outlier included the LD10 is 8.7 µg/kg body weight. The LD10 without the outlier is 74 µg/kg body weight.

Swan et al (2006) examined if the European Griffon vulture (*Gyps fulvus*) and the African white-backed vulture (*Gyps africanus*) were equally sensitive. Two African white-backed vulture and three Griffon vultures received a single dose of 800 µg/kg body weight and died within two days of dosing, while all controls survived. A similar experiment was repeated by Naidoo et al (2009) with Cape Griffon Vulture (*Gyps coprotheres*). Both birds died after receiving a dose of 800 µg/kg body weight. These experiments confirmed the general susceptibility of all *Gyps* species to diclofenac.
To examine if American vultures would be equally sensitive as Eurasian vultures, Rattner et al (2008) exposed Turkey vultures (*Cathartes aura*) to increasing concentrations of diclofenac. Two control vultures were included and eight vultures were exposed to concentrations ranging from 0.08 mg/kg to 2.5 mg/kg body weight. All vultures survived the observation period of seven days. After three weeks, five previously exposed vultures were given a single oral dose of 2.5 to 25 mg/kg body weight, with inclusion of one extra control vulture. No mortality occurred and there were no signs of overt toxicity. Apparently, this species is much less sensitive for diclofenac than the species from the *Gyps* genus. This lower sensitivity goes hand in hand with lower uric acid levels in the plasma of Turkey vultures dosed with diclofenac in comparison with species from the *Gyps* genus.
Four other types of birds were tested in a study by Hussain et al (2008). Broiler chicks (*Gallus gallus*, 15 days old), pigeons (*Columba livia*, 3 months old), Japanese quail (*Coturnix japonica*, 4 weeks old) and mynah (*Acridotheres tristis*, independent young) were orally exposed to diclofenac at dose rates of 0 (control), 0.25, 2.5, 10 and 20 mg/kg body weight, for seven consecutive days. Mortality was observed until two weeks after exposure ended. The LD50 calculated with a log-logistic model from the presented results was 4.1 mg/kg body weight/day for broiler chicks. For pigeons this value was 15.6 mg/kg body weight/day. For Japanese quail and mynah there was an onset of toxicity at the two higher dosages, but the LD50s were higher than 20 mg/kg body weight for these species. For broiler chicks and pigeons, the LD50 was accompanied by a significant reduction in body weight at all doses.
Other studies with chicken resulted in similar or slightly higher LD50s. Naidoo et al (2007) applied single intramuscular doses to hens of 18 weeks of age at five dosages of 0.6 to 10 mg/kg body weight. The LD50 was 9.8 mg/kg body weight. Assuming 50% oral bioavailability, this would be equivalent to an oral dose of 19.6 mg/kg body weight. Reddy et al (2006) applied a single intramuscular dose of 5 mg/kg body weight in poultry of both sexes of 6 weeks of age. 40% mortality occurred. At the same dose in the study by Naidoo et (2007) 33% mortality occurred. In a recent study with White Leghorns of 6 weeks old diclofenac was administered at oral doses of 2 and 20 mg/kg body weight (Jain et al 2009). In the control group and 2 mg/kg body weight dose all six birds survived. At 20 mg/kg body weight, 3 out of six birds died within twelve hours. Apparently, the repeated dose for 7 consecutive days causes the LD50 to be about a factor of 5 lower than the LD50s from single dose studies.
An overview of the derived LD50s is presented in the table below. The LD50 for vultures is clearly the most critical endpoint, although it is useful to realize that at similar doses (0.25 mg/kgbw/d) reduced body weight was observed for broiler chicks and juvenile pigeons. It appears that chicken, although taxonomically not closely related to the vultures, are rather sensitive for diclofenac as well. To the contrary, another genus of vultures appears to be rather insensitive. It is important to note that it has been suggested that not only vultures, but also raptors, storks, cranes and owls may

be very sensitive to non-steroidal anti-inflammatory drug (NSAID), including diclofenac (Cuthbert et al 2007).

Table 6.5: Summary of LD50 values of different avian studies

Species name	Scientific name	LD50 [mg/kg$_{bw}$/d]	Reference
Oriental white-backed vulture	*Gyps bengalensis*	0.225	Green et al. 2007 Swan, et al. 2006
Griffon vulture	*Gyps fulvus*	<0.80	Swan et al 2006
African white-backed vulture	*Gyps africanus*	<0.80	Swan et al. 2006
Cape Griffon Vulture	*Gyps coprotheres*	<0.80	Naidoo et al 2009
Turkey vultures	*Cathartes aura*	>25	Rattner et al. 2008
Chicken	*Gallus gallus domesticus*	4.1	Hussain et al. 2008
Pigeon	*Columba livia domestica*	15.6	Hussain et al. 2008
Japanese quail	*Coturnix japonica*	>20 (55)	Hussain et al. 2008
Mynah	*Achridotheres tristis*	>20 (55)	Hussain et al. 2008

6.6.2 Derivation of $QS_{biota, sec\ pois}$

According to the Technical Guidance Document on EQS derivation acute toxicity data should preferably not be used to derive quality standards for secondary poisoning. However, in this case there are no other data than acute toxicity data, although the toxicity of diclofenac to birds is well established. Therefore, these acute toxicity data are taken as basis for the derivation of the $QS_{biota, sec\ pois}$. For the most sensitive species, the dose-response information is available.
The average weight of an oriental white-backed vulture is 4.75 kg, with a daily meat consumption of 341 g ungulate tissue per day (Green et al 2007). Based on the allometric relationship for non-passerine terrestrial birds an oriental white-backed vulture of 4.75 kg will have a daily energy expenditure (DEE) of 1995 kJ/d. An allometric relationship specific for the closely related species Cape vulture (*Gyps coprotheres*) is also available (Komen 1992): DEE [kJ/d] = 826.7*BW[kg]$^{0.61}$
This leads to a similar value of 2139 kJ/d. A daily consumption of ungulate tissue of 341 g/d as reported by Green et al (2007) corresponds to an energy content of ungulate tissue of 6272 kJ/kg, which is similar to the experimental value of 6200 kJ/kg reported by Komen (1992), and it might be that Green et al (2007) indirectly used these data by Komen (1992). Therefore, the value for DEE from the regression by Komen (1992) is used in further calculations. All data are very consistent, which increases the confidence of the calculations based on reported dose.
The LD10 and LD50 are 74 and 225 µg/kg body weight, respectively, which represents a dosing either given orally as single dose or consumed by the vultures via the meat. From the data by Oaks et al (2004) it appears that the ten vultures that were exposed to the highest concentration of diclofenac in meat (6.4 mg/kg), received a total dose of 0.82 to 0.94 mg/kg body weight per day, which equals on average 640 g meat if a body weight of 4.75 kg is assumed. Given the estimated daily consumption of 341 g/d, it appears that the vultures have been fed with contaminated food for two days. The total dose has thus to be divided by a factor of 2 days to obtain an LD10 of 37 µg/kg body weight per day and an LD50 of 112 µg/kg body weight per day.
The energy normalised effect concentration can be calculated according to two different methods according to the guidance document. Method 1: The dose can be recalculated as diet concentrations by the formula from the guidance document (LCx=LDx*BW/DEE), which yields an LC10 of 0.082

µg/kJ diet and an LC50 of 0.249 µg/kJ diet. Method 2: The diet concentration can be normalised to the energy content. Although no EC10 and EC50 are given in the studies, it follows from the study by Oaks et al (2004) that a concentration of 0.64 mg diclofenac per kg buffalo meat corresponds to an average total dose of 0.863 mg/kg body weight, which is 11.7 and 3.84 times higher than the LD10 and LD50 of 0.074 and 0.225 mg/kg body weight, respectively. If the concentrations in meat for the EC10 and EC50 are assumed to be lower by the same factor, the EC10 and EC50 are 0.548 and 1.67 mg/kg meat, respectively. With an energy content 6200 kJ/kg meat, these values become 0.088 and 0.269 µg/kJ diet for the LC10 and LC50, respectively. Both methods give very similar results, which indicates that the data are indeed very consistent. Further calculations are based on the results from method 1, because exact values for the EC10 and EC50 and the energy content of the buffalo meat, all needed as input for method 2 were not reported directly in the study.
To calculate a $QS_{biota,\ sec\ pois}$ from an LC50 by default a factor of 100 would be applied to extrapolate to chronic toxicity. However, the standard test duration for an acute bird study would be 5 days instead of 2 days. If it is assumed that the toxicity is mainly determined by the internal concentration, the half-life of diclofenac in the birds could be used to estimate how far from equilibrium the concentration is. For several species, including the vultures from the genus *Gyps* and for chickens, the half-life of diclofenac in the body is known. The half-life of diclofenac in two white-backed vultures (*Gyps africanus*) was examined and amounted to 14 and 18 hours. With a half-life of 16 hours, it can be estimated that after 2 days the concentration is 88% of the concentration after 5 days. The correction would thus be relatively small and the resulting LC50 0.219 µg/kJ diet. With an AF of 100 this value becomes 2.19 ng/kJ diet.
Finally, a factor 10 is default to extrapolate from the most sensitive species to the ecosystem. Given the fact that the tested species cover only 6 orders of avian species, this factor seems necessary in this case, because many orders of marine and inland water birds are not represented by the tested species, e.g. gannets, auks, gulls, waders, grebes, loons, storks, herons, flamingos, coots, moorhens, kingfishers and cormorants. Besides that, some well-known species for the European water systems belong to the most sensitive tested order (Accipitriformes) to which also the *Gyps* species belong, e.g. ospreys and sea eagles. The $QS_{biota,\ sec\ pois}$ then becomes 0.219 ng/kJ diet.
Part of the extrapolation from acute toxicity data to chronic toxicity accounts for the fact that the acute EC50 represents a 50% effect concentration, while the chronic NOEC/EC10 refers to a low effect concentration. For the oriental white-backed vulture the LD10 is available next to the LD50. If the same derivation of the $QS_{biota,\ sec\ pois}$ is performed with an AF factor of 10 instead of 100 for extrapolation from acute fo chronic, the resulting value is 0.722 ng/kJ diet. The factor of 10 (overall factor of 100) is normally applied only to a subacute toxicity study of 28 days for mammals. However, in this case, it is considered that an overall assessment factor of 100 on the LD10 is sufficiently conservative as the tested species is by far the most sensitive and the effects seen point toward an acute toxic effect rather than a long-term chronic effect on e.g. reproduction. However, a chronic toxicity tests with birds is still missing and would be highly relevant to assess the chronic toxicity on other endpoints than mortality only, such as growth and reproduction.

The resulting $QS_{biota,\ sec\ pois}$ in the relevant food items for aquatic food chains are calculated by multiplying with the energy content of these food items and shown in the table below.

Table 6.6: $QS_{biota,\ sec\ pois}$ in food items relevant for aquatic food chains.

Food item	$QS_{biota,\ sec\ pois}$ [µg/kg$_{diet}$]
Fish	3.99
Bivalves	1.16

Freshwater arthropods	3.58
Aquatic vegetation	2.01

6.6.3 Bioaccumulation

In general, for several taxonomic groups relatively large discrepancies exist between bioconcentration determined under laboratory conditions and bioaccumulation determined in field conditions. For this reason, the BAF values obtained from the laboratory studies are of little use for the field situation. A clear indication of differences in accumulation of diclofenac between laboratory exposure and field exposure comes from the studies by Mezzelani et al. (2016a, 2016b, 2018), who analysed the same organisms under laboratory and field conditions using the same analytical techniques. Mussels exposed to 0.5 µg/L in the laboratory had dry weight concentrations of 4.75 µg/kg dwt after 14 days of exposure (Mezzelani et al 2016b)-. Mussels exposed to 2.5 µg/L in the laboratory had dry weight concentrations of 1.63, 3.63, and 2.25 µg/kg dwt after 14, 30, or 60 days of exposure (Mezzelani et al 2018). Mussels exposed to 25 µg/L in the laboratory had dry weight concentrations of 14.9 µg/kg dwt after 14 days of exposure (Mezzelani et al 2016a). Mussels collected from the field at Portonovo Bay, located in the Central Adriatic Sea had concentrations of <1, 16.11 and <1 µg/kg dwt in July, August and September 2014, respectively. In a further study (Mezzelani et al 2020), mussels were captured at 6 sites in the Tyrrhenian Sea and 8 sites in the Adriatic Sea over three consecutive years and in several seasons. Half of the samples contained concentration at or below the limit of quantification of 1.4 µg/kg dwt. However, half of the samples contained higher concentrations with the upper 10% in excess of 100 µg/kg dwt. In about one third of the samples the concentrations are higher than the highest concentrations found in the laboratory at 25 µg/L. The field studies are not accompanied by water sampling, but from other monitoring data it follows that the water concentrations in the Mediterranean Sea and other coastal area do by far not reach concentrations as high as 25 µg/L (Mezzelani et al 2016b). Summarizing, the concentrations in the same mussel species from the field are higher than the concentrations in mussels exposed in the laboratory, while the water concentrations in the field are generally much lower than the concentrations used in the laboratory experiments (up to 25 µg/L).
As indicated by the guidance document, molluscs (bivalves) are often critical in food chains when substances do not biomagnify and show low bioaccumulation. Diclofenac has relatively low bioaccumulation factors and trophic magnification factors tends to be below 1. Trophic levels of biota were determined separately for the areas Meiliang Bay and East Coast of the large freshwater Lake Taihu in China (Xie et al 2017). Trophic levels for phytoplankton, zooplankton and zoobenthos were rather comparable for the two areas, although the trophic levels for shrimp and fish were 0.24 to 0.67 units higher for Meiliang Bay. Trophic magnification factor (TMF) were 0.52 for Meiliang Bay and 0.40 for East Coast, both with highly significant slopes. In an earlier study (Xie et al 2015) considering the whole lake as a whole a TMF of 1.06 was found, with no significant slope. In the ecosystem of the Qinhuai River in China (Yang et al 2020), the TMF for diclofenac was 0.39 (read from figure). These figures point at the highest concentrations in the lower part of the food chain.
Indeed, the field-derived bioaccumulation factor of molluscs seem to be critical if related to the biota standards from the table above. The values that are most relevant in this case are from the field study from the North Bosque River, TX, USA (Du et al 2014). Monitoring was performed over a relatively short time span of 3 days, in which daily average filtered water concentrations were very similar. The monitoring location was downstream of a major wastewater treatment plant (WWTP) and river flow at this point in the river is predominantly determined by the effluent flow of the WWTP. Samples were analysed by LC-MS. Diclofenac was not found in any of the fish

species. In contrast, almost all invertebrates had detectable concentrations of diclofenac. In the molluscs (snail, three size classes of Asian clam, pondhorn mussel, paper pondshell mussel) the BAF calculated from the results ranged from 140 to 419 L/kg with a geomeatric mean of 216 L/kg. Other field studies on bioaccumulation in molluscs show similar levels of BAF values. However, the data are often less reliable due to large spatial scale in combination with grouping of the data. The accumulation of pharmaceuticals was examined in two studies describing the food web of Lake Taihu in China (Xie et al. 2015, 2017).

In the first study (Xie et al. 2015), the food web was sampled at sixteen sites in May 2013. Next to water and sediment, phytoplankton, zooplankton, zoobenthos and fish were sampled. Diclofenac was detected in 75% of the filtered water samples and the concentrations from <0.03 to 17.6 ng/L with an average of 5.91 and a median value of 6.00 ng/L. Concentrations per location were lowest in the southeast and highest in the northwest. Concentrations were expressed on dry weight basis. Water content was presented for all organisms. Concentrations in biota were not reported per site and were thus insufficient to calculate the individual BAF values. Wet weight BAF calculated from the reported average dry weight concentrations, the reported moisture content and the average water concentration were 44 to 145% of the reported values for the different species. The differences between calculated and reported BAF values are not clear. Little information is given on the data used for the reported BAF values and it is not clear if these are averages or site-specific BAFs, or whether there are other reasons for the discrepancies. Reported wet weight median BAF values were 91 for phytoplankton, 145 for zooplankton, 70 for mussel, 142 for snail, 77 for bivalves, 307 for white shrimp, 157 for common carp, 98 for lake anchovy, 80 for crucian carp and 133 L/kg for yellow catfish. Due to the missing raw data, the differences between reported and calculated values and the aggregation of the data, this study could only be used as supporting information.

In the second study (Xie et al. 2017), the food web was sampled at sixteen sites in December 2014. Next to water and sediment, phytoplankton, zooplankton, zoobenthos and fish were sampled. Diclofenac was detected in 88% of the filtered water samples and the concentrations ranged from <0.02 to 26 ng/L with a median value of 4.4 ng/L. Concentrations per location were not presented for individual pharmaceuticals, but the total concentrations of pharmaceuticals detected in the water were lowest at one site on the west coast and in the lake centre. Concentrations were expressed on dry weight basis. Water content was presented for all organisms, however, for fish not for the individual organs. Data were not reported per site and were thus insufficient to construct the individual BAF values. Reported BAF values were only on the level of zoobenthos, shrimp and fish, and not for individual species. For phytoplankton and zooplankton, it was possible to calculate a wet weight BAF from the reported median dry weight concentrations, the reported moisture content and the median water concentration. These BAF values were within 10% of the reported BAF concentrations. However, zooplankton and phytoplankton were sampled at all sampling sites with the 64 samples evenly distributed over the sites, while this was not the case for the other species. Especially the fish species were not sampled in more than half of the sampling sites. BAF values for the other species could thus not be calculated due to missing representative water concentrations. Reported wet weight median BAF values were 318 for phytoplankton, 600 for zooplankton, 192 for zoobenthos (mussel, snail, bivalve), 386 for shrimp, 134 for fish muscle, 12 for fish gills, 128 for fish brain and 421 for fish liver. Based on the median concentrations in water and organisms and the reported water content, the calculated wet weight BAF values are 278 L/kg for mussels (*Anodonta* sp.), 390 L/kg for snails (*Bellamya* sp.) and 142 L/kg for bivalves (Corbiculidae). Due to the missing raw data and the aggregation of the data this study could only be used as supporting information. Nevertheless, the reported BAF value for zoobenthos, which consists of mussel, snail and bivalve, is 192 L/kg, which is very close to the geometric mean of 216 L/kg calculated from the study North Bosque River.

Another study examined the accumulation in the New Qinhuai River, Qinhuai River and a section of the Yangtze River in China during April to July 2018 (Yang et al. 2020). Next to water, suspended matter and sediment, phytoplankton, zooplankton, freshwater mussel (*Anodonta woodiana*), freshwater shrimp (*Macrobranchium nipponense*), snail (*Bellamya aeruginosa*), loach (*Paramisgurnus dabryanus*), grass carp (*Ctenopharyngodon idellus*), common carp (*Cyprinus carpio*), crucian carp (*Carassius auratus*), silver carp (*Hypophtha lmichthys molitrix*), bighead carp (*Hypophthalmichthys nobilis*), whitebait (*Reganisalanx brachyrostralis*), catfish (*Silurus asotus*) and yellow catfish (*Pelteobagrus fulvidraco*) were sampled. Water samples were filtered over an 0.45 µm filter (described in Yang et al 2019). Biota concentrations were expressed on wet weight basis. Water content was presented for all organisms. Diclofenac was detected in 100% of the water samples and the concentrations in water were 1.0±0.5 ng/L in the New Qinhuai River, 22.5±5.5 ng/L in the Qinhuai River and 1.8±0.7 ng/L in a section of the Yangtze River. Concentrations per location and time point were presented graphically for individual pharmaceuticals (Yang et al 2019). Both in time and space the concentrations of diclofenac and other pharmaceuticals fluctuated widely. Therefore, the aggregation into average exposure concentrations for a whole river section during four months seems too high. Data were not reported per site and were thus insufficient to construct the individual BAF values. Reported BAF values were only presented graphically in three categories. Calculated BAF values seem to match these data. Very high BAF values were calculated (and reported) for the New Qinhuai River, especially for the lower trophic levels with a BAF for zooplankton of 12400 L/kg. The BAFs were 3400 for snails and 5900 L/kg for shrimps and ranged for fish from <240 to 3200 L/kg. In the Qinhuai River, calculated BAF values were 416 and 564 for phytoplankton and zooplankton, <9 for snails and mussels, 208 for shrimps and <12 to 183 for fish. In the Yangtze River section, calculated BAF values were 833 to 1278 for phytoplankton and zooplankton, 1278 for snails and <133 to 222 for fish.
It must be noted that although biota concentrations are rather similar, the water concentrations are much lower in the New Qinhuai River and the Yangtze River section in comparison to the Qinhuai River. Species migration is not known, but at least for the invertebrates this might be limited. However, water concentrations seem to vary widely in space and time as can be concluded from the previous study (Yang et al 2019). Raw data are missing to construct BAF values specific for each sampling time and sample location. Because of these considerations, these data this study could only be used as supporting information.

6.6.4 Derivation of the $QS_{water, sec\ pois}$

The selected BAF value to calculate the $QS_{water, sec\ pois}$ is the geometric mean value of 216 L/kg for molluscs derived from the study by Du et al (2014). The $QS_{water, sec\ pois}$ is derived by dividing the $QS_{biota, sec\ pois}$ of 1.16 µg/kg for bivalves by this value.
The resulting $QS_{water, sec\ pois}$ is 5.4 ng/L.
Although this standard is lower than the AA-$EQS_{fw, eco}$ of 40 ng/L, it has not been selected as final chronic freshwater standard since the $QS_{biota, secpois}$ was derived with an acute study. Furthermore, the EQS Technical Guidance (EC, 2018) does not encourage the use of acute toxicity studies for the QS_{biota} derivation. Therefore, the $QS_{water, sec\ pois}$ of 5.4 ng/L cannot be considered statistically robust.

7 Human health

7.1 Human health via consumption of fishery products

Table 7.1: Human health via consumption of fishery products

		Master reference
Mammalian oral toxicity	Baboon / Oral / 12 months / Endpoint not specified. LOAEL: 5 $mg.kg^{-1}$ NOEC: $mg.kg^{-1}$ (CF=) biota ww Reliability: 4	Novartis internal data
	ADI: 0.5 $mg.kg_{bw}^{-1}.day^{-1}$	EMEA (2003)
CMR	Diclofenac sodium was found to be neither mutagenic nor carcinogenic, and reprotoxicity studies revealed no effects on fertility, embryonic development, or postnatal development. However, diclofenac sodium exposure should be avoided in late pregnancy due to the effect of prostaglandin inhibition, which may exert effects on the foetal cardiovascular system, e.g. premature closure of the ductus arteriosus.	Novartis internal data

7.1.1 Tentative $QS_{biota, hh}$

According to the REACH registrations this substance causes damage to organs through prolonged or repeated exposure, is harmful if swallowed and is suspected of damaging fertility or the unborn child. Indeed, diclofenac sodium exposure should be avoided in late pregnancy because of prostaglandin inhibition, which may exert effects on the foetal cardiovascular system, such as premature closure of the ductus arteriosus (Novartis internal data). Furthermore, as stated above, evidence of bioaccumulation for diclofenac exists. Hence, in agreement with SCHEER opinion (2022), the derivation of a biota standard for human health is performed on the basis of the hazardous properties of a substance.

The calculation of the $QS_{biota, hh\ food}$ is based on the following equation from the EQS Technical Guidance (E.C., 2018):

$QS_{biota, hh\ food} = 0.2 * TL_{hh} / 0.00163$

To represent the threshold level of human health (TL_{hh}), an Oral Reference Doses (RfD), Acceptable Daily Intake (ADI), Tolerable Daily Intake (TDI), or No Observable Adverse Effect Level (NOAEL) with appropriate assessment factor can be used.

An ADI of 0.5 µg/kg $_{bw}$/day was derived by the Committee for Veterinary Medical Products of the European Medical Agency (EMA, 2003), starting from an overall pharmacological LOEL of 0.1 mg/kg$_{bw}$/day to which an AF of 200 was applied. Indeed, the pharmacological NOEL for antiphlogistic and antipyretic activity after oral administration in rats was 0.1 mg/kg$_{bw}$/day as a single dose. Furthermore, the constriction of the ductus arteriosus in the foetal rats was also demonstrated at this concentration.

Therefore, the ADI of 0.5 µg/kg $_{bw}$/day (EMEA, 2003) was selected as threshold level for human health (TL_{hh}), 0.2 is the allocation factor and and 0.00163 kg$_{fish}$/kg $_{bw}$/day is 95th percentile of the daily intake of fish and seafood by adults (person of 70 kg). The obtained value for $QS_{biota,\ hh}$ food is therefore 61.35 µg/kg$_{biota,ww}$. The $QS_{water,hh}$ is calculated as follows by dividing the $QS_{biota,\ hh\ food}$ by the BAF of 216 L/kg for molluscs derived from the study by Du et al (2014), giving a value of 0.284 µg/L.

Tentative $QS_{biota,\ hh}$	**Relevant study for derivation of $QS_{biota,\ hh}$**	**Tentative $QS_{biota,\ hh}$**
Human health	ADI: 0.5 mg.kg$_{bw}^{-1}$.day^{-1}	61.35 µg/kg$_{biota,ww}$ Corresponding to 0.284 µg.l^{-1}

7.2 Human health via consumption of drinking water

Table 7.2 Human health via consumption of drinking water

Existing drinking water standard(s)	**no preferred regulatory standard**	**Master reference**
Health-related indication value [Gesundheitlicher Orientierungswert (GOW)]	0.3 µg/L	(German Environment Agency, 2020) (Value for Diclofenac is from 2008)
	ADI: 0.5 mg.kg$_{bw}^{-1}$.day^{-1}	EMEA (2003)

A drinking water standard for diclofenac from the German Environment Agency (2020) of 0.3 µg/L is reported. However, the EQS Technical Guidance (E.C., 2018) suggests to use an EU or a WHO standard. Standards from these institutions for diclofenac are not reported. Therefore, according to the EQS Technical Guidance (E.C., 2018) and in agreement with SCHEER opinion (2022), the $QS_{dw,\ hh}$ is calculated according to the following equation reported in the EQS Technical Guidance (EC, 2018):

$QS_{dw,\ hh} = (0.2*TL_{hh}*bw)/uptake_{dw}$

A human body weight (bw) of 70 kg and a daily uptake of drinking water ($uptake_{dw}$) of 2 litres were chosen according to the EQS Technical Guidance (E.C., 2018). The default value of 0.2 is the

fraction of the human TL_{hh} allocated to the intake of the substance via drinking water (E.C., 2018). The TL_{hh} chosen is the ADI of 0.5 µg/kg_{bw}/day (EMA, 2003). Therefore, **the tentative $QS_{dw, hh}$ is equal to 3.5 µg/L**.

8 Literature

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9 Annex I - Summary of chronic studies considered for SSD.

Birzle 2015

Oncorhynchus mykiss.

10 different types of eye damage after 28 days.

The report presents a table with the number of fish (out of 20) that had the different types of ocular lesions at the concentrations 0.1, 1, 5, 25 and 100 µg/l.

None of the fish in the control and at 0.1 and 1 µg/l showed any ocular lesions, and in most cases, effects were only seen at 25 and 100 µg/l.

The report does not give any information on EC_{10} or NOEC values. But EC_{10} values can be deduced from the table:

Thickening of Cornia Stroma	EC_{10} = 13 µg/l. Interpolation between 5 and 25 µg/l.
Keratitis	EC_{10} = 19 µg/l. Regression
Ulcer in Cornea	EC_{10} = 44 µg/l. Interpolation between 25 and 100µg/l.
Effects on prelateral membrane	EC_{10} = 25 µg/l. 10% effect measured at 25 µg/l.
Cataract	EC_{10} = 11 µg/l. Regression.
Missing lens	EC_{10} = 44 µg/l. Interpolation between 25 and 100µg/l.
Adherence of Iris to Cornea	EC_{10} = 6.7 µg/l. Regression.
Haemorrhage inside front part of eye	EC_{10} = 44 µg/l. Interpolation between 25 and 100µg/l.
White veil	EC_{10} = 5 µg/l. 10% effect measured at 5 µg/l.

It might be uncertain to which degree each of these effect types are relevant, i.e., will lead to impairment of vision, and thus if you should just choose the lowest EC_{10}. The lowest value is 5 µg/l for "white veil". The implication of this for vision is not straightforward but the effect might be population relevant if such an alteration of the vision might lead to consequences on avoidance capacity. Moreover, iritis and adherence of iris to cornea have EC_{10}s at approximately the same level (6.5 and 6.7 µg/l), and it is proposed to apply **EC_{10} = 5 µg/l.**

DeLorenzo & Fleming 2008

Dunaliella tertiolecta

96h algal test, cell density

EC_{50} = 185690 µg/l

EC_{10} or NOEC is not given, but the degree of effect at the lowest exposure concentration of 25000 µg DCF/L is described as a "significant effect" by the authors and close to 10%. So, the EC_{10} can be set at **EC_{10} = 25000 µg/l**

Ericson et al. 2010

Mytilus edulis trossulus

Byssus strength, byssus abundance, and "scope for growth".

Scope for growth did not show a clear dose- response.

For byssus abundance there was a statistically significant reduction at the highest tested concentration, so the NOEC corresponds to 100 µg/l. At 100 µg/l the abundance was 102% of that at the control, and it does not seem feasible to calculate an EC_{10}.

For byssus strength there was a statistically significant and monotonous dose-response, and the effect seen at the highest concentration is statistically significant.
The NOEC for byssus strength is therefore 100µg/l.
However, the effect seen at 100 µg/l corresponds to 17% effect compared to the control, and the estimated EC_{10} is:
EC_{10} = 3.2 µg/l.
RI = 1 – 2

González-Ortegón et al. 2015
Palaemon longirostris
There are only two concentrations apart from the control, and the spacing is about 19-fold.
No statistical significance has been reported by the authors for any of the effect types, but their chosen significance level is 0.01. It can be seen from table 1 that for "duration of development" there is statistical significance at the 0.05 level at 750 µg/l at 18°C and 20‰ salt, and at 24 °C and 32‰ salt. However, at 18°C and 20‰ the duration of development is about 20% lower than the control at 40µg/l and about 33% greater than the control at 750 µg/l.
At 24°C and 32‰ the duration of development is about 15% greater than in the control, while the duration at 40µg/l is equal to the control.
Estimation of a reliable EC_{10} does not seem possible, and the **NOEC = 40 µg/l.**

Joachim et al. 2021
Gasterosteus aculeatus, Dreissena polymorpha
Gasterosteus aculeatus: The results for this species are regarded as mesocosm results and are not included in the dataset of laboratory data.
Dreissena polymorpha: The mussels were caged and submerged into the mesocosm water, and this test is therefore not regarded as a real part of the mesocosm, but as a kind of laboratory test (*inter alia*, absence of possible trophic relationships). High mortality rates, effects on immunity, and high genotoxicity were found for encaged zebra mussels (Dreissena polymorpha) in all treatments.
The cumulative mortality in the control was acceptable after 2 months (ca. 7%) but rather high after 5 months exposure (ca. 30%). It has been indicated by the authors that there may be an issue with long-term encagement of mussels in a confined environment, concomitantly with increased temperatures (exposure was led from spring to autumn, therefore including summer months).
On the other hand, the correlation between mortality and DCF concentration is statistically significant ($r_s = 1$, $P = 0.05$; $r = 0.965$, $0.01 < P < 0.025$; $N = 4$ and P-values one-tailed), and it is the only test with a freshwater bivalve species.
The article gives a NOEC = 0.041 µg/l, but at the same time indicates in table 1 that the mortality is statistically significantly different from that in the control. Looking closer at table 1 the % mortality in the control is 29.7±9.6 (standard error), and mortality at 0.041 is 37.5±2.2 (SE). It is difficult to see how this could lead to statistical significance. In fact, it is difficult to see how any of the effects at the different concentrations could be significant; the % mortality at the highest tested concentration was 57,2±8.4. The number of replicates was 3, and the t-value for N= 3 is 4.303. To get the confidence interval the SE is multiplied by t.
It is, however, possible to derive an EC_{10} for mortality by regression:
Calculated **EC_{10} = 0.37 µg/l**

Kummerova et al 2016
Lemna minor

The growth data show a hormeses-like pattern with an increase of growth compared to the control at 0.1 and 10 µg/l, and a marked, and statistically significant, drop at the highest concentration (100 µg/l). This would normally lead to a NOEC = 10 µg/l.
On the other hand, the chlorophyll content does not show this pattern, and the drop in Chl content is statistically significant already at 0.1 µg/l. The NOEC based on Chl content therefore is NOEC < 0.1 µg/l.
In the current case the hormesis-pattern seen in the growth parameters might be interpreted as a reaction to diclofenac stress and, for example, an allocation of energy into survival, i.e., growth.
Based on the regression line the **EC_{10} = 1.7 µg/l.**

Lee et al. 2011
Daphnia magna, *Moina macrocopa*, *Oryzias latipes*
D. magna: NOEC = 8300 µg/l.
An EC_{10} can be calculated for young/female: **EC_{10} = 3217 µg/l**
M. macrocopa: NOEC = 16750 µg/l
An EC_{10} can be calculated for young/female: **EC_{10} = 2658 µg/l**
O.latipes: NOEC = 1000 µg/l.
An EC_{10} can be calculated for hatchability from the regression line given in figure 3 in the article: **EC_{10} = 7100 µg/l.**

Liu et al. 2017
Daphnia magna
mRNA expression, moulting, reproduction, mortality, and growth.
We do not regard the mRNA expressions as population relevant because it is very difficult to associate the effects seen with population relevant parameters such as reproduction, survival etc.
There was no clear dose-response in moulting, mortality and growth.
There was a clear decrease in the number of neonates in 1st production with increasing DCF concentration, rs = -1; P = 0.05, two-tailed; r = -0.979; P = 0.005.
An EC_{10} can be calculated **= 18 µg/l.**

Meden-Kunkel & Maletzki 2010
Desmodesmus subspicatus
Growth rate
The study is well documented, follows OECD 201 guideline, and fulfils all validity criteria, so is rated with RI 1.
The NOEC = 25000 µg/l. If possible, EC_{10} is preferred over NOEC.
The EC_{10} has been calculated by the authors by simple regression, and by way of the programme Tox Rat Pro XT. EC_{10} as been derived for both biomass and rate of increase. Rate of increase is the preferred parameter (according to guidance), and E_rC_{10} will be employed here.
E_rC_{10} = 52600 µg/l (calculated by the authors)
E_rC_{10} = 68200 µg/l (calculated with Tox Rat Pro XT).
E_rC_{10} = 52600 µg/l is chosen.

Memmert et al. 2013.
Danio rerio and *Oncorhynchus mykiss*
Rainbow trout:
There was a significant increase in growth, but the hatching rate and survival did not show a dose-response.
Zebrafish:

The authors regard the reduced growth (dry-weight?) in zebrafish as an artifact because the values are equal in the upper tail of the concentration series, and because of results from other studies. However, the overall correlation between dry-weight and concentration in the zebrafish study is highly significant, r_s = -0,941; P = 0.01; one-tailed; n=6, and when plotted with ln-transformed concentration values the graph actually looks reasonable.
Further, the correlation between length and concentration is as well statistically significant with r_s = -0.750; 0.025 < P < 0.05; one-tailed; n = 7, and also the survival from hatch to end of study is statistically significant with r_s = -0.714; P = 0.05; one-tailed; n=7.
The correlation between wet-weight and concentration was just not statistically significant.
EC_{10}s can be calculated for these zebrafish endpoints:
Dry weight: 8.6 µg/l
Length 33 µg/l
Survival hatch-end 485 µg/l
We would employ an **EC_{10} of 8.6 µg/l** for the Zebrafish.

Näslund et al. 2017

Gasterosteus aculeatus
Condition factor (a function of weight and length), mortality, jaw malformations.
A number of histopathological responses are recorded.
Condition factor: There was no clear dose-response.
Mortality: There is a statistically significant dose-response with r_s = 0.823, and r = 0.880; P < 0.001 (one-tailed in both cases, and N = 15). The NOEC for mortality is 102 µg/l. (If the mean concentration of the three nominal concentrations of 80 µg/l is used then NOEC = 82 µg/l). An **EC_{10} of 92 µg/l** can be calculated (regession) for mortality (survival).
Jaw malformations: The dose-response is statistically significant with r_s = 0.9; P = 0.05, and r = 0.939; 0.01 < P < 0.02. In both cases one-tailed and N = 5.
With such "jaw malformations" you would expect reduced food intake in wild fish, which would make it a population relevant endpoint. However, experts are actually a bit uncertain about to which extent the observations may be called "jaw malformations" as the measured parameters were only skin ulcerations of the jaw noted by macroscopic observations. On the contrary, other studies reporting "jaw malformation" from mandibular dysmorphism measured from 1/ radiological observation of the jaws to detect possible mandibular loss, 2/ Histochemical examination of the snout region, "histochemical localization of TRAP enzymatic activity in the fish mandible reflecting local osteoclastogenesis") (Yokota et al., 2018), but just lesions (sores). The authors also seem doubtful over the significance of it, hence the limited reporting in the paper.
An EC_{10} can be estimated (regression) at:
EC_{10} = 7.2 µg/l

Ribeiro et al. 2015

Danio rerio and *Paracentrotus lividus*
Danio rerio: The degree of effect on hatchability was statistically significant at the highest concentration, giving a NOEC of 1250 µg/l. The distribution of the data-points in the plot is such that derivation of an EC_{10} would be dubious. **NOEC = 1250 µg/l.**
Paracentrotus lividus: There were statistically significant dose-responses with both % normal larvae and larval length, though the larval length showed the strongest correlation. In both cases N = 5, and P-values are one-tailed.
EC_{10} values have been calculated with regression.
% normal larvae: r_s = -0,821; 0.05 < P <0.1. r = -0.812; 0.025 < P < 0.05. NOEC = 5 µg/l; EC_{10} = 5.4 µg/l.

However, another study (Scymaris 2020b) undertaken with the same organism and methodology but with twice as many data-points shows an increase in % 'normal' larvae with increasing DCF concentration (i.e., no adverse effect) up to concentrations close to 2000 µg/l. Ribeiro et al. (2015) used diclofenac base and DMSO to achieve the reported exposure concentrations in seawater. Scymaris (2020b) utilised diclofenac-Na which is soluble in seawater up to around 20 mg/l. Exposure concentrations were confirmed in Scymaris (2020b), but not measured in Ribeiro et al. (2015). There are also doubts over the control results in Ribeiro et al. (2015) for normal development which appears to suggest that only normal embryos were counted (0% abnormality, even in controls, is highly unlikely).
Overall, there are potential doubts regarding the abnormality endpoint from this study.
However, the larval length results appear more reliable (and this endpoint was not measured in Scymaris 2020d).
Larval length: r_s = -0.975; 0.025 < P < 0.05. r = -0.993; P = 0.0005. NOEC = 5 µg/l; **EC_{10} = 5.2 µg/l.**

Sarma et al. 2014
Plationus patulus and *Moina macrocopa*
P. patulus: The NOEC is 6250 µg/l. From the formula given in figure 3 the EC_{10} can be calculated.
EC_{10} = 1400 µg/l.
M. macrocopa: The NOEC is 12500 µg/l. From the formula given in figure 3 the EC_{10} can be calculated.
EC_{10} = 788 µg/l.

Schwarz et al 2017
Salmo trutta
Embryos and juveniles.
There were no dose-responses in the tests with embryos.
Juveniles: See also comments to Triebskorn 2017 below. Mortality, stress protein analysis, determination of lipid peroxides and histopathological analysis.
We do not regard the biomarkers as population relevant because it is very difficult to associate the effects seen with population relevant parameters such as reproduction, survival etc.
Survival of juveniles: There were statistically significant effects at 0.1, 100 and 200 µg/l, but not at 1 and 10 µg/l. The authors chose 10 µg/l as NOEC.
There is a statistically significant dose-response between survival and DCF concentration, r_s = -0,883, P = 0.05, one-tailed, N = 6. An EC_{10} can be derived by regression, EC_{10} = 3.5 µg/l
Concerning the behavioural effects (bite-marks on body and fins), a dose-response is observed, with statistically significant differences at the three highest does. It is equivocal if they are or are not population relevant. The determination of EC_{10} for bite-marks on the body was rather uncertain due to the distribution of the data-points in the plot, while the estimate of EC_{10} for bite-marks on fins is more straightforward. Estimated EC_{10} for bite-marks on fins was EC_{10} = 2.3 µg/l. This is fairly close to the estimated EC_{10} for survival.
EC_{10} = 3.5 µg/l is selected.

Scymaris 2020a
Lymnaea stagnalis
Reproduction. There seems to be a dose related decrease in the number of clutches per snail, with the smallest number at the highest DCF concentration, even though it is not statistically significant.

Although the NOEC is a greater-than this concentration, it has been included because it corresponds to 12% effect, which is close to 10%, and because it is the only value for this species and for gastropods.
NOEC = 1540 µg/l
We do not think it is possible to derive a meaningful EC_{10} or NOEC because the concentration drops drastically, and there was no renewal of the media during the test-period.

Stepanova et al. 2013
Cyprinus carpio
Mortality, larval development, histology, glutathione S-transferase, glutathione reductase, glutathione peroxidase, and thiobarbituric-acid-reactive-substances.
We do not regard the biomarkers as population relevant because it is very difficult to associate the effects seen with population relevant parameters such as reproduction, survival etc.
Mortality: The mortality at the highest concentration was statistically significantly greater than the mortality in the control, and the mortality NOEC is thus 1000 µg/l.
Larval development: The percentage of larvae reaching the juvenile stages was correlated with DCF concentration (r_s = -1, P = 0.05, two-tailed), and an EC_{10} could be derived.
EC_{10} = 674 µg/l

Tovar-Aguilar et al. 2019
Lecane papuana
Growth rate (5 days), hatching (3 days).
The article is in Spanish but seems to be reliable although details may have been missed. The article gives calculated EC_{10} values for the different endpoints.
Growth rate: EC_{10} = 734 µg/l
Hatch: **EC_{10} = 590 µg/l**

Triebskorn et al. 2017
Salmo trutta f. fario (embryos and juveniles), *Gammarus fossarum*, *Daphnia magna*
S.trutta: Embryos: development, survival, heart rate and body mass. Juveniles: mortality, behaviour, histology, biomarkers. *G. fossarum*: Reproduction. *D. magna*: Reproduction.
S.trutta: See also Schwarz 2017 above. There were two trials. The two trials are not fully comparable as the trouts in the first are younger than in the second. Unfortunately, the control mortality in the first trial was too high (46%), and the results from that part can only be indicative. We do not regard the endpoints on biomarkers and behaviour as relevant because they are difficult to interpret in relation to population effects (as also the guidance tells us).
There was no clear dose-response for length and weight of juvenile trouts, whilst mortality (survival) was statistically significantly correlated with DCF concentration.
The authors conclude on a NOEC = 10 µg/l, although the difference in effect between the control and the 0.1 µg/l group was statistically significant. The effects were not significant at 1 and 10 µg/l, but again significant at 100 and 200 µg/l. This picture is identical to that in Schwarz et al. 2017 but the data are somewhat different. Probably it is a question of uncertainties in reading the figures and maybe there could have been made small revisions from the report to the article.
The calculated EC_{10} = 4.3 µg/l.
Gammarus fossarum: A NOEC of 790 µg/l is given in the report for juveniles/female. A linear regression (with ln-transformed concentrations) on the part of the dataset where there is a decrease in the ratio gives an EC10 = 689 µg/l. This is in the same order of magnitude as the NOEC, and a linear regression is probably not appropriate in this case, so it is suggested to employ the NOEC.

NOEC = 790 µg/l
Daphnia magna:
Mortality (immobility): The mortality at the two highest concentrations were respectively 60% and 70%, and yet these effect values were not statistically significantly different from the control, where the mortality was lower than 20%. The report instead derived an EC_{10} = 3600 µg/l.
Offspring per surviving female: The reported NOEC = 1900 µg/l, and the EC_{10} = 3200 µg/l. The recommended endpoint is number of offspring per female employed in the study, not per surviving female (OECD 211, 2012). This is also how it is done with all other invertebrates. The EC10 should therefore in all probability be smaller, and the NOEC probably as well.
Time to hatch: The reported NOEC = <1900 µg/l. No EC_{10} was given, and as the test-data are not given it is not possible to calculate an EC_{10} from information in the report.
It is suggested to use the **NOEC = 1900 µg/l.**

<u>Vannini et al. 2018</u>
Azolla filiculoides (FW plant (fern)) and *Xanthoria parietina* (Lichen)
Data on the lichen are not included as it is terrestrial.
Azolla filiculoides: Four concentrations above the control were employed. The following endpoints were reported:
Photosynthetic efficiency, performance index, chlorophyll degradation and chlorophyll a and b content.
We regard the photosynthetic efficiency and the chlorophyll content as the most relevant endpoints. With both, there was only an increase in effect at the highest tested concentration. Chlorophyll content was the most sensitive factor, and as the effect at the highest tested concentration was statistically significant that concentration was equal to the LOEC. The NOEC therefore is equal to 10000 µg/l.
The NOEC, however, represents 121% of the control, and the EC_{10} will be greater. Interpolation between the datapoints of the two largest concentrations (ln-transformed) gives an EC_{10} = 23632 µg/l ≈ 24000 µg/l.
Although, the interpolation between two datapoints that are quite far apart is somewhat uncertain it is a better estimate than the NOEC, which represents less effect than that seen in the control.
We suggest employing the **EC_{10} = 24000 µg/**l.

<u>Weissmannová et al. 2018</u>
Desmodesmus subspicatus
Analytical measurement of the substance with actual concentrations reported.
Intrinsic growth-rate, **E_rC_{10} = 15540 µg/l.**

<u>Yokota et al. 2016</u>
Oryzias latipes
Reproduction
The NOEC is 25 µg/l. The effects seen at this concentration corresponds to about 7% effect. Interpolation between 25 g/l and 50 µg/l results in an EC10 = 26 µg/l, which is hardly different from 25 µg/l. It is suggested to keep the NOEC.
NOEC = 25 µg/l.

<u>Yokota 2017</u>
Oryzias latipes
Reproduction.

The LOEC for mean fertility per pair is 37 µg/l resulting in a NOEC = 7.1 µg/l. However, the effect seen at this concentration corresponds to about 1.5%.
The EC_{10} for swollen abdomen in females (because eggs are not laid) is estimated at **EC_{10} = 7.8 µg/l.**

10 Annex II Statistic details of the SSD approach

The data set of EC10 and NOEC data for Diclofenac is clustered in three with values below 40, between 590 and 1600 and 15000 (table 9.1, with different colouring of the tree clusters), which leads to three steps in the percentiles (figure 9.1). For reasons of simplification were the data sets of the values above 590 joined. A view of the violin- boxplot plot (figure 9.2) shows that the data is bimodal distributed. The question arises if the sample consists of two samples from two different populations. Therefore, the residuals of the modelled distribution functions (exemplary for loglogistic model) and the observations will be investigated.

Table 10.1:EC10/NOEC data of Diclofenac

Species	EC10/NOEC [µg/l]	Study
Dreissena polymorpha	0.25	Joachim et al. 2021
Lemna minor	1.7	Kummerova et al. 2016
Mytilus edulis	3.2	Ericson et al. 2010
Salmo trutta	3.5	Schwarz et al 2017
Oncorhynchus mykiss	5	Birzle 2015
Paraentrotus lividus	5.2	Ribeiro et al. 2015
Gasterosteus aculeatus	7.2	Naslund et al 2017
Oryzias latipes	7.8	Yokota 2017
Danio rerio	8.6	Memmert et al. 2013
Daphnia magna	18	Liu et al. 2017
Palaemon longirostris	40	González-Ortegón et al. 2015
Lecane papuana	590	Tovar-Aguilar 2019
Cyprinus carpio	674	Stepanova et al. 2013
Moina macropoda	788	Sarma et al. 2014
Gammarus fossarum	790	Triebskorn et al. 2017
Ceriodaphnia silvestrii	1000	de Oliveira et al. 2018
Plationus patulus	1400	Sarma et al. 2014
Lymnaea stagnalis	1540	Scymaris 2020a
Desmodesmus subspicatus	15540	Weissmannová et al. 2018
Azolla filiculoides	24000	Vannini et al. 2018
Dunaliella tertiolecta	25000	DeLorenzo & Fleming 2008

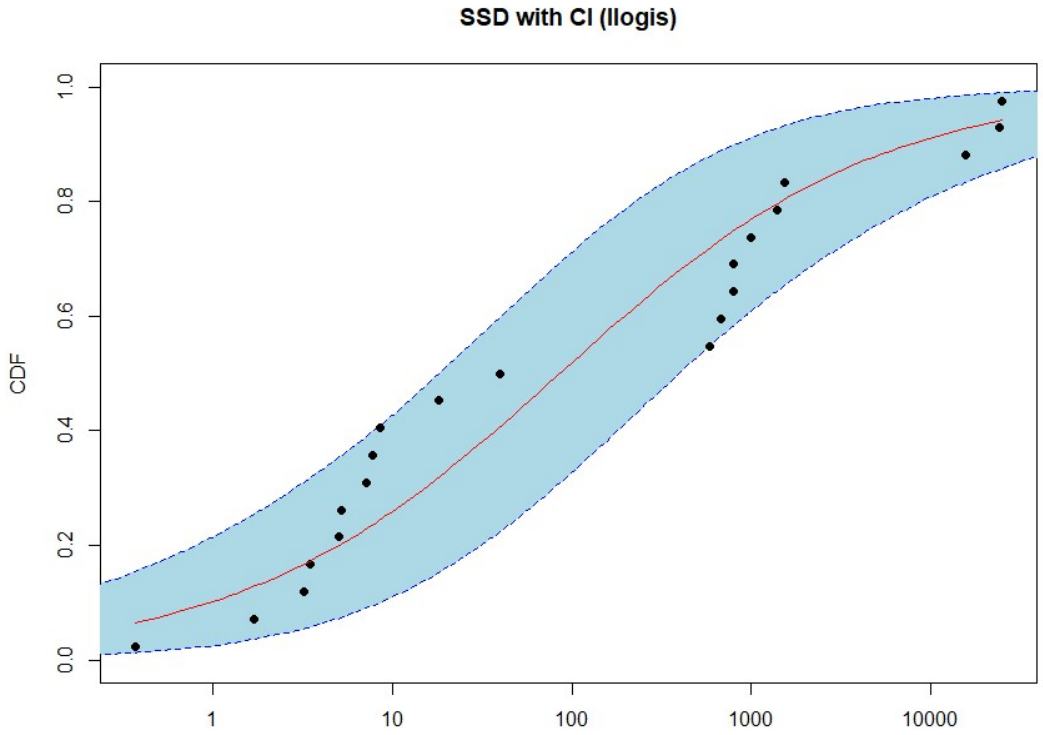

Figure 10.1:cumulative distribution of EC10/NOEC [µg/l], observed data and simulated loglogistic function with confidence intervals.

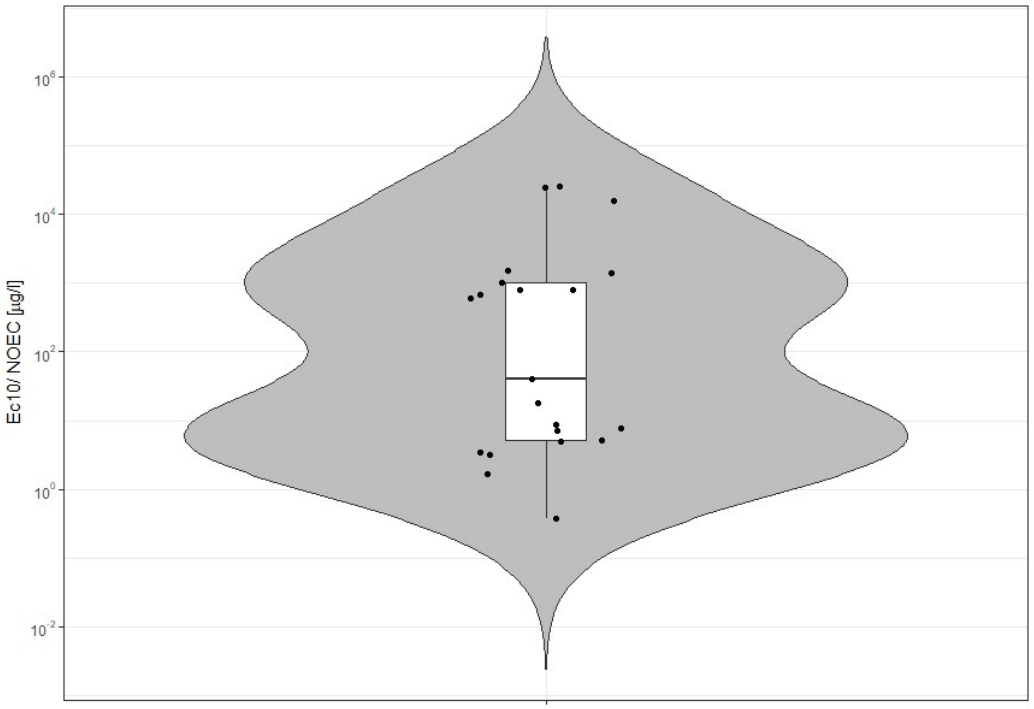

Figure 10.2: Violin – boxplot of the EC10/NOEC data of diclofenac

The fitted distribution function (figure 9.1) has a large wide range of the confidence intervals, which is a link for the high uncertainty of the model.

A view to the residuals (figure 9.3) shows, there can be two groups of residuals identified which underestimate and overestimate the observed values. Underestimation is linked to high EC10 values and overestimation to low.
The distributions of the two samples are different as the boxplots show (figure 9.3). A two-sided t-test is used to proof the hypotheses of the different distributions (pre-test of normal distribution and homoscedasticity were conducted if the t test criteria are fulfilled) and confirms a significant difference:

Two Sample t-test
t = -4.064, df = 19, p-value = 0.0006619

alternative hypothesis: true difference in means is not equal to 0.
95 percent confidence interval: -0.18135718 -0.05805642

sample estimates:
mean in group high mean in group low.
-0.06286658 0.05684022

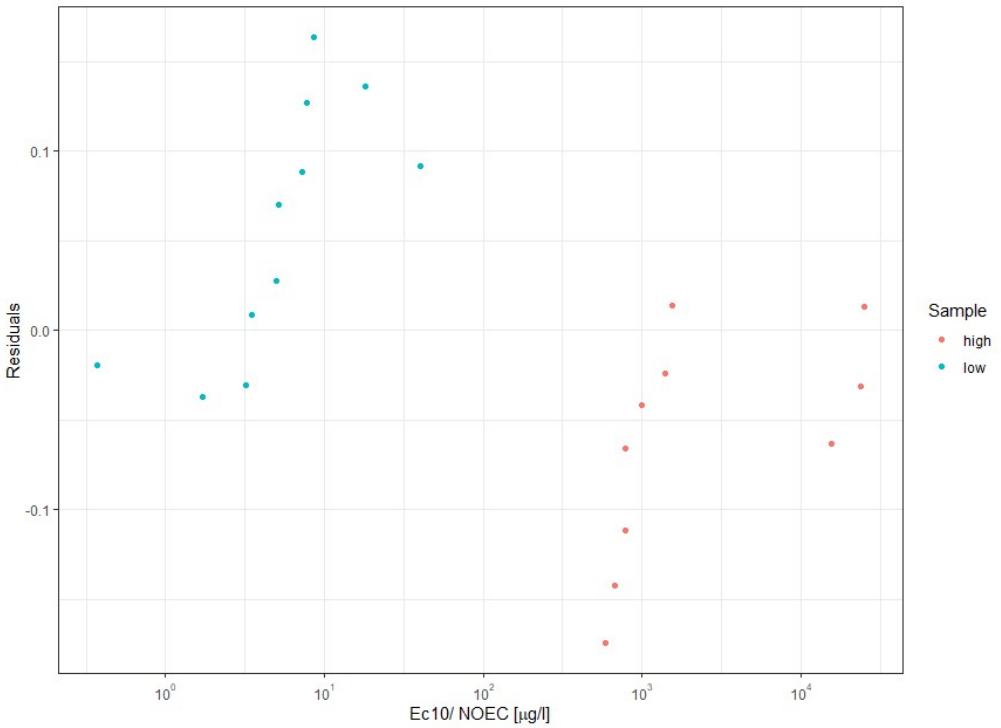

Figure 10.3: Residuals of the loglogistic model.

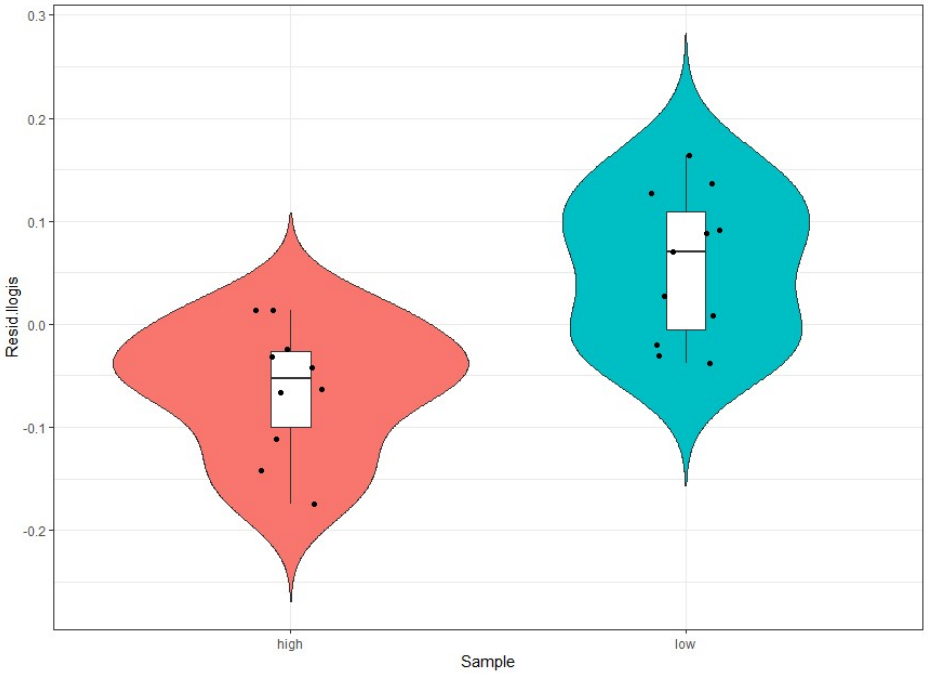

Figure 10.4: Violin- boxplot of the residuals of the loglogistic model and the observed data

If the data set is limited to the EC10 values below 40 a model with low confidence intervals (table 9.2, figure 9.5) and a more plausible residual structure can be estimated.

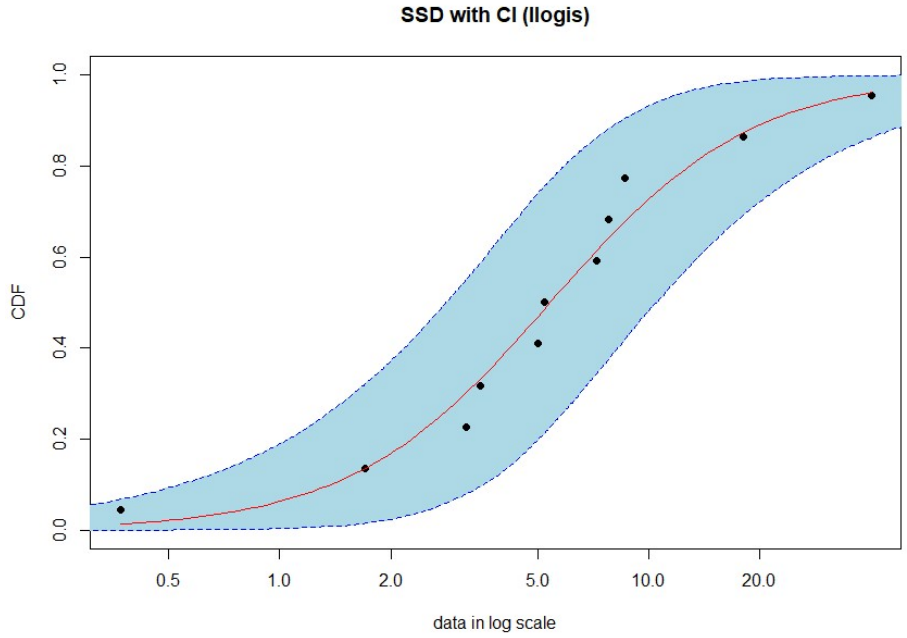

Figure 10.5: SSD with data set EC10 values below 40 µg/l

Table 10.2: Comparison of percentiles of the two data sets

	p=0.05	p=0.1	p=0.2	p=0.5
	all data			
Estimate	0.86	1.37	2.27	5.41
CI 2.50%	0.28	0.53	1.05	2.82
CI 97.50%	2.64	3.56	5.03	10.50
	data with EC10 < 40 µg/l			
Estimate	0.21	0.96	5.02	85.43
CI 2.50%	0.02	0.11	0.82	18.69
CI 97.50%	2.98	8.83	32.00	392.86

11 Annex III: Dose-response analysis of the most sensitive fish endpoint in a mesocosm study

(Joachim et al. 2021 – Ecotoxicology and Environmental Safety)

Mortality rate of founder fish was selected as the most sensitive endpoint. The data used for this analysis are reported in Table 11.1 and represented in Figure 11.1.

11.1.1 Data

Table 11.1: Mortality of founder fish at the end of the experiment. "Exposure conc." are expressed as actual mean measured concentrations (AECs) which are respectively 0.041, 0.44 and 3.82 µg/L for the 0.1, 1 and 10 µg/L treatments.

Replicate	Exposure conc. (µg/L)	Number Female (end of experiment)	Number Male (end of experiment)	Female mortality (%)	Male mortality (%)	Total female (start of experiment)	Total male (start of experiment)
3	0	6	5	60	50	15	10
8	0	9	6	40	40	15	10
12	0	9	5	40	50	15	10
6	0.041	6	7	60	30	15	10
7	0.041	5	8	66.67	20	15	10
9	0.041	6	5	60	50	15	10
2	0.44	6	5	60	50	15	10
5	0.44	3	9	80	10	15	10
11	0.44	6	5	60	50	15	10
1	3.82	1	4	93.33	60	15	10
4	3.82	0	0	100	100	15	10
10	3.82	0	0	100	100	15	10

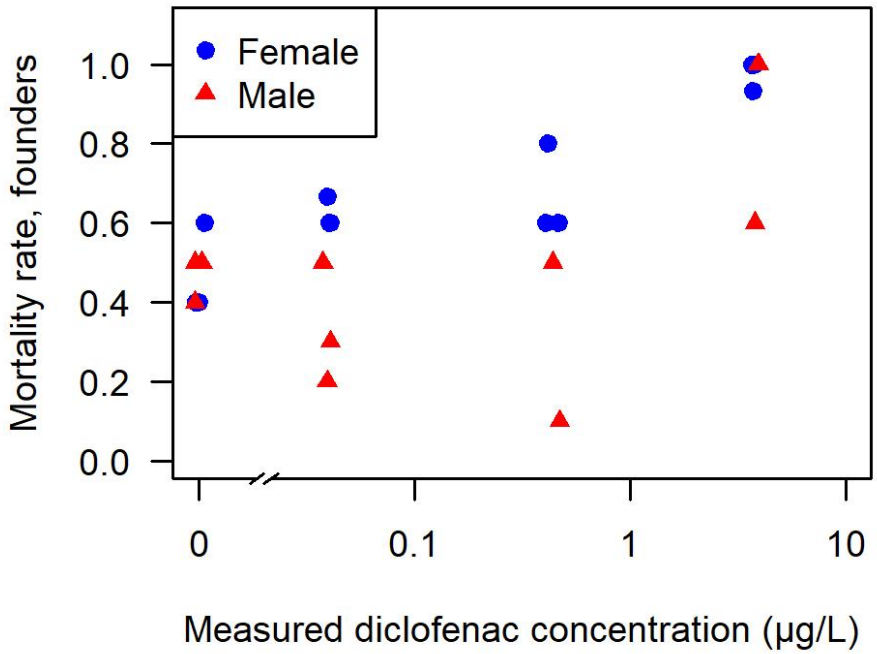

Figure 11.1: Mortality rates of founder male and female founder fish at end of the experiment for each treatment measured concentrations (expressed as actual mean measured concentrations (AECs) which are respectively 0.041, 0.44 and 3.82 µg/L for the 0.1, 1 and 10 µg/L treatments). The concentrations are jittered to allow all data points to be observable.

11.1.2 Differences in mortality rates between sex

According to Figure 11.1:, mortality rates appears to be lower for male founder fish compared to female founder fish. This was confirmed by a statistical analysis using the generalized linear model (GLM) using R version 3.6.1 (R Core Team, 2020) on the binomial data reported in Table 11.1:, to assess the effect of sex and concentration level on mortality. Mortality rates were significantly affected for both sex (p=0.040) and concentration (p=2.8e-05 at the highest concentration level). These results suggest that the dose-response relationship for mortality should be modelled separately for female and male founder fish.

```
Call:
glm(formula = cbind(value, total) ~ variable + as.factor(Concentration),
    family = binomial, data = data_melt)

Deviance Residuals:
    Min       1Q   Median       3Q      Max
-1.2787 -0.6260 -0.2342   0.2042   2.2894

Coefficients:
                              Estimate Std. Error z value Pr(>|z|)
(Intercept)                    -0.8232     0.2201  -3.740 0.000184 ***
Variable Number.Male            0.4673     0.2271   2.057 0.039657 *
as.factor(Concentration)0.041  -0.1010     0.2825  -0.358 0.720702
as.factor(Concentration)0.44   -0.1880     0.2868  -0.656 0.512014
as.factor(Concentration)3.82   -2.1070     0.5033  -4.187 2.83e-05 ***
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

(Dispersion parameter for binomial family taken to be 1)

    Null deviance: 49.635  on 23  degrees of freedom
Residual deviance: 16.384  on 19  degrees of freedom
AIC: 89.715
```

Dose-response modelling and EC10 determination

The dose-response relationship was modelled separately for female and male founder fish using the R package drc (Ritz et al. 2015). The logprobit model, or lognormal model, was selected because of the data is binomial rather than continuous. The upper asymptote, at high concentration levels, was set to 1. Three parameters were estimated: EC50, slope, and the baseline value.

Female founder fish

The modelled data is represented with prediction intervals in Figure 11.2. The lack-of-fit test comparing model fit to the model fit of an analysis of variance suggests that the logprobit dose-response model is acceptable (p=0.0584). The estimated slope is 1.01, the estimated baseline value is 0.543, and the estimated EC50 is 0.795 µg/L.

The estimated EC10 is 0.224 [0.0385; 1.30] µg/L.

Male founder fish

The modelled data is represented with prediction intervals in Figure 11.3. The lack-of-fit test comparing model fit to the model fit of an analysis of variance suggests that the logprobit dose-response model is acceptable (p=0.379). The estimated slope is 1.97, the estimated baseline value is 0.389, and the estimated EC50 is 2.57 µg/L.

The estimated EC10 is 1.34 [0.0241; 7445] µg/L.

The confidence interval for the EC10 is extremely large partly due to the fact that an increase in mortality was only observed at the highest concentration level and the uncertainty on the dose-response slope is also extremely high [-14.2; 18.2].

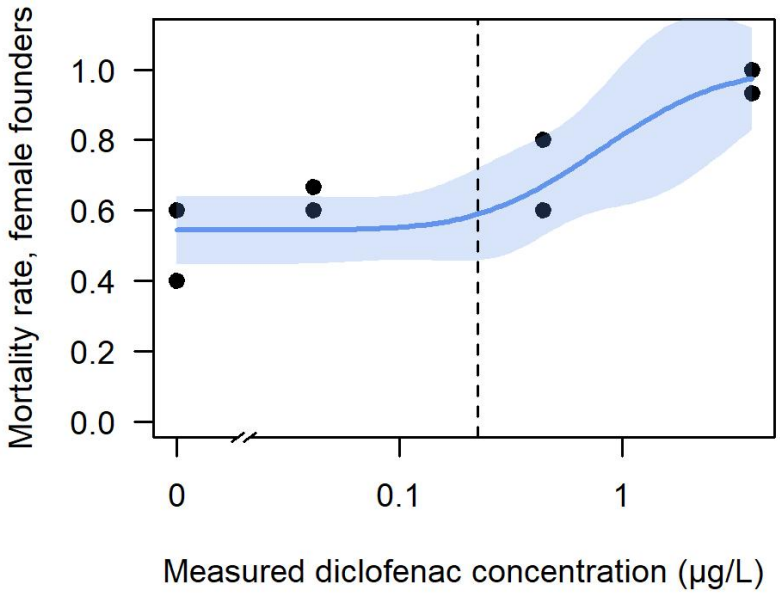

Figure 11.2: Modelled dose-response relationship for female founder fish with prediction intervals. "Measured diclofenac concentration" on the X axis are expressed as actual mean measured concentrations (AECs) which are respectively 0.041, 0.44 and 3.82 µg/L for the 0.1, 1 and 10 µg/L treatments. The dashed line represents the EC10**.**

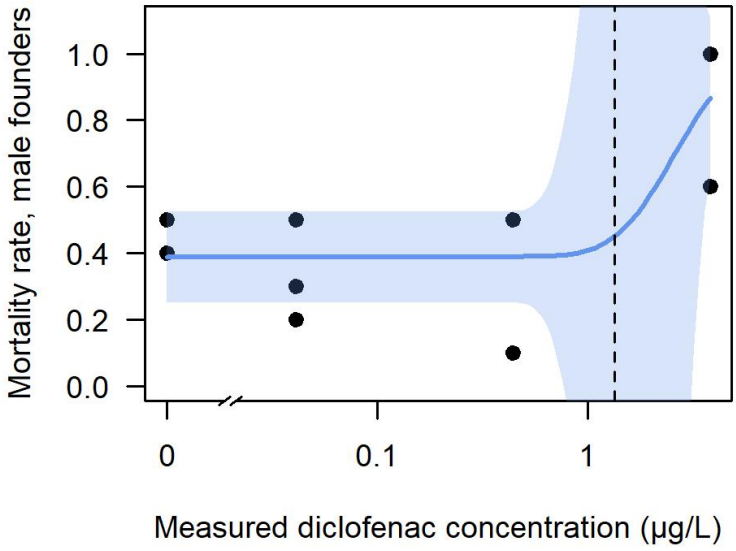

Figure 11.3: Modelled dose-response relationship for male founder fish with prediction intervals. "Measured diclofenac concentration" on the X axis are expressed as actual mean measured concentrations (AECs) which are respectively 0.041, 0.44 and 3.82 µg/L for the 0.1, 1 and 10 µg/L treatments. The dashed line represents the EC10.

12 Annex IV: Studies assessed but not used for deriving an EQS.

This list contains studies assessed for the deriving an EQS for diclofenac, but it was decided that these studies were not usable for various reasons. These could be e.g., failing the CRED criteria, wrong concentration range, no proper documentation, endpoints not relevant for EQS setting.

Ajima MNO, Ogo AO, Audu BS, Ugwoegbu KC. 2014. Chronic diclofenac (DCF) exposure alters both enzymatic and haematological profile of African catfish,*Clarias gariepinus*. Drug and Chemical Toxicology 01480545Ajima MNO, Kumar K, Poojary N, Pandey PK. 2021. Oxidative stress biomarkers, biochemical responses and Na+-K+-ATPase activities in Nile tilapia, *Oreochromis niloticus* exposed to diclofenac. Comparative Biochemistry and Physiology, Part C 240 108934

Alkimin GD, Daniel D, Dionisio R, Soares AMVM, Barata C, Nunes B. 2019. Effects of diclofenac and salicylic acid exposure on *Lemna minor*: Is time a factor? Environmental Research 177 108609

Alkimin GD, Soares AMVM, Barata C, Nunes B. 2020. Can salicylic acid modulate biochemical, physiological and population alterations in a macrophyte species under chemical stress by diclofenac. Science of The Total Environment 739 139715.

Bacsi I, B-Beres V, Kokai Z, Gonda S, Novak Z, Nagy SA, Vasas G. 2016. Effects of non-steroidal anti-inflammatory drugs on cyanobacteria and algae in laboratory strains and in natural algal assemblages. Environmental Pollution 212: 508-518

Balbi T, MontagnaT, Fabbri R, Carbone C, Franzellitti S, Fabbri E, Canesi L. Diclofenac affects early embryo development in the marine bivalve *Mytilus galloprovincialis*. Science of the Total Environment 642 601-609

Bickley LB, van Aerle R, Brown AR; Hargreaves A, Huby R, Cammack V, Jackson R, Santos EM, Tyler CR. 2017. Bioavailability and Kidney Responses to Diclofenac in the Fathead Minnow (*Pimephales promelas*). Environmental Science, Technology 51

Ceballos-Laita L, Calvo L, Bes MT, Fillat MF, Peleato ML. 2018. Effects of benzene and several pharmaceuticals on the growth and microcystin production in *Microcystis aeruginosa* PCC 7806. Limnetica, 34(1):237-246

Chen J-B, Gao H-W, Zhang Y-L, Zhang Y, Zhou X-F, Li C-Q, Gao H-P. 2014. Developmental Toxicity of Diclofenac and Elucidation of Gene Regulation in zebrafish (*Danio rerio*). Scientic Reports 4: 4841

Costa S, Coppola F, Pretti C, Intorre L, Meucci V, Soares AMVM, Freitas R, Solé M. 2020. The influence of climate change related factors on ther esponse of two clam species to diclofenac. Ecotoxicology and Environmental Safety 189 109899

Dietrich S, Ploessl F, Bracher F, Laforsch C. 2010. Single and combined toxicity of pharmaceuticals at environmentally relevant concentrations in *Daphnia magna* – A multigenerational study. Chemosphere 79 60-66

Duarte IA, Reis-Santos P, Novais SC, Rato LD, Lemos MFL, Freitas A, Vila Pouca AS, Barbosa J, Cabral HN, Fonseca VF. 2020. Depressed, hypertense and sore: Long-term effects of fluoxetine, propranolol and diclofenac exposure in a top predator fish. Science of the Total Environment, 712: https://doi.org/10.1016/j.scitotenv.2020.136564Eades and Waring. 2010. The effects of diclofenac on the physiology of the green shore crab *Carcinus maenas*. Marine Environmental Research 69 S46-S48

Efosa NJ, Kleiner W, Kloas W, Hoffmann F. 2017. Diclofenac can exhibit estrogenic modes of action in male *Xenopus laevis* and affects the hypothalamus-pituitary-gonad axis and mating vocalizations. Chemosphere 173 Groner F, Hohne C, Kleiner W, Kloas W. 2017.

Chronic diclofenac exposure affects gill integrity and pituitary gene expression and displays estrogenic activity in nile tilapia (*Oreochromis niloticus*). Chemosphere, 166:473-481

Fabbri R, Montagna M, Balbi T, Raffo E, Palumbo F, Canesi L. 2014. Adaptation of the bivalve embryotoxicity assay for the highthroughput screening of emerging contaminants in *Mytilus galloprovincialis*. Marine Environmental Research 99 1-8

Fekete-Kertész I, Kunglné-Nagy Z, Gruiz K, Magyar A, Farkas E, Molnár M. 2015. Assessing Toxicity of Organic Aquatic Micropollutants Based on the Total Chlorophyll Content of *Lemna minor* as a Sensitive Endpoint. Periodica PolytechnicaChemical Engineering 59(4), pp. 262-271

Ferrari B, Mons R, Vollat B, Fraysse B, Paxéus, N, Lo Giudice R, Pollio A and Garric J. 2004. Environmental risk assessment of six human pharmaceuticals: Are the current environmental risk assessment procedures sufficient for the protection of the aquatic environment? Environmental Toxicology and Chemistry 23, 1344-1354

Ferrari B, Paxeus N, Giudice R.L, Pollio A and Garric J. 2003. Ecotoxicological impact of pharmaceuticals found in treated wastewaters: study of carbamazepine, clofibric acid, and diclofenac. Ecotoxicology and environmental safety 55, 359-370

Fontes MK, Gusso-Chouerib PK, Maranhoa LA, de Souza Abessab DM, Mazurc WA, de Campos BG, Guimaraes LL, de Toledod MS, Lebree D, Marquese JR, Feliciof AA, Cesara A, Almeida EA, Pereira CDS. 2018. A tiered approach to assess effects of diclofenac on the brown mussel *Perna perna*: A contribution to characterize the hazard. Water Research 132 361-370

Fu Q, Fedrizzi D, Kosfeld V, Schlechtriem C, Ganz V, Derrer S, Rentsch D, Hollender J. 2020. Biotransformation changes bioaccumulation and toxicity of diclofenac in aquatic organisms. Environmental Science and Technology, 54 (7), 4400-4408. https://dx.doi.org/10.1021/acs.est.9b07127).

González-Ortegón E, Blascob J, Le Vay L, Giménez L. A multiple stressor approach to study the toxicity and sub-lethal effects of pharmaceutical compounds on the larval development of a marine invertebrate. Journal of Hazardous Materials 263P 233-238

Groner F, Hohne C, Kleiner W, Kloas W. 2017. Chronic diclofenac exposure affects gill integrity and pituitary gene expression and displays estrogenic activity in nile tilapia (*Oreochromis niloticus*). Chemosphere, 166:473-481

Guiloski IC, Piancini LDS, Dagostim AC, de Morais Calado SL, Fávaro LF, Boschena SL, Cestari MM, da Cunha C, Silva de Assis HC. 2017. Effects of environmentally relevant concentrations of the anti-inflammatory drug diclofenac in freshwater fish *Rhamdia quelen*. Ecotoxicology and Environmental Safety 139 291-300

Guyon A, Smith KF, Charry MP, Champeau O, Tremblay LA, 2018. Effects of chronic exposure to benzophenone and diclofenac on DNA methylation levels and reproductive success in a marine copepod. Journal of Xenobiotics Volume 8: 76-74Hoeger B, Kollner B, Dietrich DR, Hitzfeld B. 2005. Water-borne diclofenac affects kidney and gill integrity and selected immune parameters in brown trout (*Salmo trutta* f. fario). Aquatic toxicology 75 (1), 53-64

Hutchinson TH, Madden JC, Naidoo V, Walker CH. 2014 Comparative metabolism as a key driver of wildlife species sensitivity to human and veterinary pharmaceuticals. Phil. Trans. R. Soc. B 369: 20130583. http://dx.doi.org/10.1098/rstb.2013.0583

Kallio JM, Lahti M, Oikari A, Kronberg L. 2010. Metabolites of the aquatic pollutant diclofenac in fish bile. Environ Sci Tech., 44: 7213-7219.

Kermiche F, Berrebah H and Djebar MR. 2016. Toxicological effects of drugs (Diclofenac, Ibuprofen, mixture) and Hormesis on a non-target organism: *Paramecium* sp. Journal of Entomology and Zoology Studies 4(5): 187-191

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Liu J, Dan X, Lu G, Shen J, Wu D, Yan Z. 2018. Investigation of pharmaceutically active compounds in an urban receiving water: Occurrence, fate and environmental risk assessment. Ecotoxicology and Environmental Safety, 154:214-220.

Liu J, Lu G, Xie Z, Zhang Z, Li S, Yan Z. 2015. Occurrence, bioaccumulation and risk assessment of lipophilic pharmaceutically active compounds in the downstream rivers of sewage treatment plants. Science of the Total Environment, 511:54-62.

Lu G, Xie Z, Zhang Z. 2018. Effects of dissolved organic matter, feeding, and water flow on the bioconcentration of diclofenac in crucian carp (*Carassius auratus*). Environmental Science and Pollution Research, 25: 7776-7784.

Lubiana P, Prokkola JM, Nikinmaa M, Burmester T, Kanerva K, Götting M. 2016. The effects of the painkiller diclofenac and hypoxia on gene transcription and antioxidant system in the gills of three-spined stickleback. Comparative Biochemistry and Physiology, Part C 185-186 147-154

McRae NK, Glover CN, Burket SR, Brooks BW, Saw S. 2018. Acute exposure to an environmentally relevant concentration of diclofenac elicits oxidative stress in the culturally important galaxiid fish *Galaxias maculatus*. Environmental Toxicology and Chemistry, 37:224-235.

Mehinto AC, Hill EM, Tyler CR. 2010. Uptake and Biological Effects of Environmentally Relevant Concentrations of the Nonsteroidal Anti-inflammatory Pharmaceutical Diclofenac in Rainbow Trout (*Oncorhynchus mykiss*). Environmental Science and Technology 44: 2176-2182.

Mezzalini M, Gorbi S, Fattorini D, d'Errico G, Consolandi G, Milan M, Bargelloni L, Regoli F. 2018. Long-term exposure of *Mytilus galloprovincialis* to diclofenac, Ibuprofen and Ketoprofen: Insights into bioavailability, biomarkers and transcriptomic changes. Chemosphere, 198:238-248.

Mezzelani M, Gorbi S, Fattorini D, d'Errico G, Benedetti M, Milan M, Bargelloni L, Regoli F. 2016a. Transcriptional and cellular effects of Non-Steroidal Anti-Inflammatory Drugs (NSAIDs) in experimentally exposed mussels, *Mytilus galloprovincialis*. Aquatic Toxicology, 180:306-319.

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