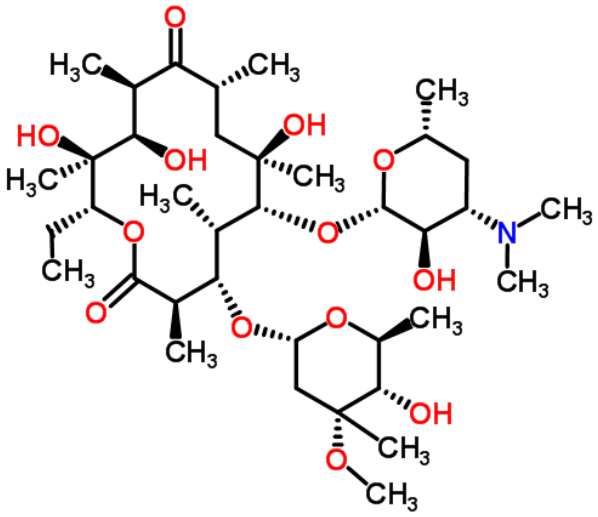
Miljøministeriet
Miljøstyrelsen

Fastsættelse af kvalitetskriterier for vandmiljøet

Erythromycin

CAS nr. 114-07-8



Vandkvalitetskriterium	$VKK_{ferskvand}$	0,5 µg/l
Vandkvalitetskriterium	$VKK_{saltvand}$	0,05 µg/l
Korttidsvandkvalitetskriterium	$KVKK_{ferskvand}$	1,0 µg/l
Korttidsvandkvalitetskriterium	$KVKK_{saltvand}$	0,1 µg/l
Sedimentkvalitetskriterium	$SKK_{ferskvand}$	47,7 µg/kg tørvægt (5% OC) 954 µg/kg tørvægt x f_{oc}
Sedimentkvalitetskriterium	$SKK_{saltvand}$	4,77 µg/kg tørvægt (5% OC) 95,4 µg/kg tørvægt x f_{oc}
Biota-kvalitetskriterium, sekundær forgiftning	$BKK_{sek.forgiftn.}$	14,7 mg/kg vådvægt fisk 4,1 mg/kg vådvægt musling
Biota-kvalitetskriterium, human konsum	HKK	120 mg/kg vådvægt

December 2022

Databladet er i april 2024 opdateret i forhold til at tydeliggøre ved hvilket organisk kulstof (OC) indhold sedimentkvalitetskriterierne er bestemt ved og enheden er rettet.

Dansk resumé og konklusioner

Erythromycin er et organisk stof, der tilhører gruppen af macrolider. Stoffet produceres naturligt af bakterien *Saccharopolyspora erythraea*, der tilhører slægten Actinomyces. Stoffet anvendes som et bredspektret antibiotikum overfor bakterielle infektioner i luftvejene, mave- og tarmsystemet, samt infektioner på huden og kønsdele. Stoffet anvendes tillige i veterinær-medicinen, og her ligeledes til behandling af bakterielle infektioner.

Stoffets fysisk-kemiske egenskaber, det fordeling imellem forskellige miljøer, det 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 (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].

Indledningsvis indeholder rapporten en sammenfatning af grundlag og viden om forekomsten af stoffet Erythromycin i relevante eksterne miljøer. Baseret på indrapporterede koncentrationer af Erythromycin i det eksterne miljø, viser den gennemførte screening følgende: de påviste og dokumenterede koncentrationer af stoffet 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 potentielt en lav risiko for at Erythromycin ikke kan overholde de tentative kriterier.

Tilsvarende screening af risiko for europæiske marine overfladevande kan ikke bedømmes, idet de tilvejebragte data fremstår opdelte og utilstrækkelige. Derfor konkluderes, 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 relevante indtag og konsum.

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[1] Joint Research Center (JRC) of the Commission of the European Union: Erythromycin – 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 Erythromycin (Publication date 1 March 2022), available on-line at: https://health.ec.europa.eu/publications/draft-environmental-quality-standards-priority-substances-under-water-framework-directive-0_en

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

Kvalitetskriterier er fastsat på baggrund af resultater, datakvalitet og bredde af de udførte undersøgelser i forhold til undersøgte akutte og kroniske effekter på specifikke organismer, trofiske niveauer og forskellige miljøer.

Erythromycin er undersøgt for toksikologiske og 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)[4] – R1: troværdig uden restriktioner; R2 – troværdig med restriktioner; R3 – ikke troværdige; R4 – ikke anvendelige. Alene studier med score R1/R2 er medtaget i udarbejdelsen af kvalitetskriterierne.

I det reducerede datamateriale af studier med høj kvalitet og troværdighed (R1/R2) for stoffet Erythromycin, findes der fortsat relevante og solide studier af såvel akutte som kroniske effekter på minimum 3 taksonomiske grupper i ferske miljøer. Data for de marine miljøer er begrænsede og derfor er det samlede datasæt anvendt til fastlæggelse af kvalitetskriterier for marint miljø baseret på en forudgående statistisk analyse af datasættet i henhold til fremgangsmåden fastsat i Europa-Kommissionens vejledning til fastsættelse af kvalitetskriterier i vandmiljøet (EC, 2018). Der mangler generelt studier af effekter overfor sedimentlevende organismer.

Erythromycin har en kendt påvirkning af specifikke biologiske processer, og der er på dette grundlag taget højde for specifikke arters følsomhed ved en statistisk tilgang til vurdering af datasættet, der omfatter en SSD-analyse (Sensitive Species Distribution). Denne statistiske tilgang til at fastlægge kortidsvandkvalitetskriteriet (KVKK) er suppleret med en deterministisk tilgang, og metoder for begge tilgange til datasættet er baseret på 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 omfatter for den deterministiske metode relevante studier af akutte effekter med mindst et studie fra hver af 3 trofiske niveauer (alger, krebsdyr og fisk). Datasættet rummer taksonomiske grupper såsom alger og cyanobakterier, der er specifikt sensitive overfor Erythromycin, og er vurderet tilstrækkeligt til alene af være baseret på en usikkerhedsfaktor på 10 for ferskvand. I rapporten er drøftet hvorvidt denne usikkerhedsfaktor kan nedsættes til 5 eller elimineres for saltvand som følge af datasættets bredde (større sikkerhed), men den tilknyttede ekspertgruppe for vurdering af antibiotika afviser en sådan ændring, da den tropiske marine krebsdyr *Penaeus vannamei* og marine kiselalge *Phaeodactylum tricornutum* ikke kan betragtes som ekstra taksonomiske grupper. For saltvand vil der jf. vejledningen være en supplerende usikkerhedsfaktor på 10, således at den samlede usikkerhedsfaktor for saltvand er 100.

[4] Klimisch, H. J., Andreae, M., and Tillmann, U. (1997). A systematic approach for evaluating the quality of experimental toxicological and ecotoxicological data. Regulatory toxicology and pharmacology, 25(1), 1-5.

Med udgangspunkt i laveste EC_{50} værdi på 10 µg/l for studier af udvikling af populationsdensitet (vækst inhibering) i kulturer af den marine alge *Tetraselmis suecica* kan der med afsæt i den deterministiske tilgang fastlægges følgende KVKK-værdier:

$KVKK_{ferskvand}$ = 10 µg/l / 10 = 1,0 µg/l
$KVKK_{saltvand}$ = 10 µg/l / 100 = 0,1 µg/l

Det samlede datasæt for akut toksicitet anvendt til den statistiske tilgang (SSD, Probabilistiske metode) er baseret på 22 tilgængelige værdier for 8 taksonomiske grupper, der omfatter både fersk- og saltvandsarter, og opfylder derved kravene i vejledningen (EC, 2018). Et mindre datasæt bestående af de 11 mest følsomme organismer er tillige vurderet med samme statistiske tilgang.

Dataanalysen frembringer ved statistisk analyse en 5% fraktil for farlighedskoncentration (HC_5) for stoffet Erythromycin, der for de 2 datasæt ligger på henholdsvis 5,23 µg/l og 2,67 µg/l. Der er ikke konstateret data i de 2 datasæt, der ligger under de statistisk fremkomne HC_5 værdier.
Med udgangspunkt i det fulde datasæt, og en usikkerhedsfaktor på 10 for ferskvand og 100 for saltvand, vil beregnede KVKK-værdier være:

$KVKK_{ferskvand}$ = 5,23 µg/l / 10 = 0,523 µg/l
$KVKK_{saltvand}$ = 5,23 µg/l / 100 = 0,0523 µg/l

Den videnskabelige komite SCHEER (SCHEER, 2022), noterer sig at der meget vel kan være tilstrækkeligt data til en statistisk tilgang til fastsættelse af kvalitetskriteriet, men bemærker at forskningscenteret (JRC, 2022) mener at datagrundlaget til en statistisk tilgang ikke er tilstrækkeligt. SCHEER anbefaler på dette grundlag, at den deterministiske tilgang, som anvendt ved ovenstående fastsættelse af korttidskvalitetskriterier (KVKK), fastholdes, således at KVKK-værdier er hhv. 1,0 µg/l (ferskvand) og 0,1 µg/l (saltvand).

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 omfatter for den deterministiske metode relevante studier af kroniske effekter med mindst et studie fra hver af 3 taksonomiske grupper, der repræsenterer 3 trofiske niveauer. På dette grundlag anvendes en usikkerhedsfaktor for ferskvand på 10. Der er ikke tilvejebragt studier der omfatter marine arter, og på dette grundlag anvendes en usikkerhedsfaktor for saltvand på 100. Begge i overensstemmelse med vejledningens anbefalinger (EC, 2018).

Datasættet for de kroniske effektniveauer er generelt mindre end for de akutte effektniveauer, og på dette grundlag er den statistiske metode fravalgt.

Med udgangspunkt i laveste EC_{10} værdi på 5 µg/l for studier af udvikling af populationsdensitet (vækst inhibering) i kulturer af Cyanobakterien *Anabaena sp.* kan der med afsæt i den deterministiske tilgang fastlægges følgende VKK-værdier:

$VKK_{ferskvand}$ = 5 µg/l / 10 = 0,5 µg/l
$VKK_{saltvand}$ = 5 µg/l / 100 = 0,05 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 dyrelivet mod sekundær forgiftning, såfremt der er evidens for, at et stof har potentiale for at kunne adsorbere til suspenderede stoffer og sediment. Erythromycin har en Koc værdi for organisk stof i jord på 1.877 l/kg (log Koc værdi på 3,27) og en log Kow værdi på 3,06, og opfylder derved krav om fastsættelse af kriterium for sediment ved at værdierne overskrider den udløsende værdi på 3.

Der er ikke tilvejebragt data fra undersøgelser af toksicitet for stoffet Erythromycin over for sediment arter, og der er derfor estimeret et kvalitetskriterium for sediment (SKK), der er baseret på anvendelse af den anbefalede metode om Ligevægts Fordeling (EqP). Beregningsmetoden anvender standardværdier og de udledte kvalitetskriterier for vand (VKK):

I et EU standard sediment med et 5 % organisk karbon indhold og ved anvendelse af en K_{oc} på 1.877 l/kg bestemmes fordelingskoefficienten mellem fast stof og vand i sediment, $Kp_{sed} = Foc_{sed}$ x K_{oc} = 0,05 x 1877 l/kg = 93,85[5] l/kg og fordelingskoefficienten mellem sediment og vand, $K_{sed\text{-}water}$ kan beregnes som følgende:

$K_{sed\text{-}water} = Fair_{sed}$ x $K_{air\text{-}water} + F_{water\text{-}sed} + F_{solid\text{-}sed}$ x $(Kp_{sed} / 1000)$ x RHO_{solid}
= 0 x 0,8 + 0,2 x (93,85 / 1000) x 2500
= 47,725 m^3/m^{-3}

Kvalitetskriterierne for sediment (SKK) kan bestemmes på baggrund af nedenstående formel: SKK = ($K_{sed\text{-}water}$ / RHO_{sed}) x VKK x 1000 og omsættes til tørvægt ved anvendelse af omregningsfaktoren på 2,6.

Det leder til følgende kvalitetskriterier for sediment (SKK):

$SKK_{ferskvand}$ = (47,725 / 1300) x 0,5 µg/l x 1000 x 2,6 = 47,7 µg/kg tørvægt (5% OC)
= 954 µg/kg tørvægt x f_{oc}
$SKK_{saltvand}$ = (47,725 / 1300) x 0,05 µg/l x 1000 x 2,6 = 4,77 µg/kg tørvægt (5% OC)
= 95,4 µg/kg tørvægt x f_{oc}

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.

For stoffet Erythromycin er der konstateret en eksperimentel Log K_{ow} værdi på 3,06 l/kg, og en feltbaseret Bio Akkumulations Faktor (BAF) på 4.492 l/kg for ferskvandsfisk. Begge oplysninger

[5] Beregningerne angivet i JRC-rapporten (2022) er udført ved anvendelse af en Koc-værdi på 570 l/kg, som er blevet rettet til 1.877 l/kg af Europa-Kommissionens videnskabelige komite for sundhed og miljø, SCHEER. Selve SKK-værdierne er afstemt i forhold til Koc-værdien på 1.877 l/kg.

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

Der er bestemt et forventet NOAEL-niveau (No Observable Adverse Effect Level) på 200 mg/kg kropsvægt for kaniner for stoffet Erythromycin ved indtag såvel akut som kronisk. Dette er baseret på et velunderbygget datagrundlag for bestemmelse af oral toksikologi, og effekter som påvirkning af udvikling og reproduktion for en lang række pattedyr. Beregningsgrundlaget i Method A i Europa-Kommissionens tekniske vejledning (EC, 2018) er anvendt:
Det daglige energibehov (DEE) bestemmes ved anvendelse af NOAEL-værdien på 200 mg/kg kropsvægt/dag for kaniner og en antaget kropsvægt på 2000 g for kaniner.

log DEE [kJ/d] = 0,8136 + 0,7149 x log (2000) = 3,1735
DEE = 1491

Den energinormaliseret føde koncentration kan bestemmes på baggrund af NOAEL, DEE og kropsvægten

$K_{energi\ normaliseret}$ [mg/kJ] = 200 mg/kg x (2 kg / 1491) = 0,2683 mg/kJ

Den energinormaliseret værdi skal konverteres til en koncentration i det kritiske fødeemne. For Erythromycin er det passende at bestemme $BKK_{sek.\ forgiftn.}$ for både fisk og musling. For muslinger anvendes et standard vandindhold på 92% og et energiindhold på 19 kJ/g_{tv}. For fisk anvendes et standard vandindhold på 74% og et energiindhold på 21 kJ/g_{tv}.

$K_{musling}$ [mg/kg_{vv}] = 0,2683 mg/kJ x 19000 kJ/kg x (1-0,92) = 408 mg/kg_{vv}

K_{fisk} [mg/kg_{vv}] = 0,2683 mg/kJ x 21000 kJ/kg x (1-0,74) = 1465 mg/kg_{vv}

Der anvendes en usikkerhedsfaktor på 100 baseret dels på anvendelse af et sub-akut studie (faktor 10) og dels på ekstrapolation til det eksterne miljø fra toksikologiske studier i laboratorier (faktor 10), som leder frem til følgende tentative kvalitetskriterier for biota:

$BKK_{sek.\ forgiftn.\ ferskvand}$ = 1465 mg/kg / 100 = 14,7 mg/kg vådvægt (fisk)
$BKK_{sek.\ forgiftn.\ ferskvand}$ = 408 mg/kg / 100 = 4,1 mg/kg vådvægt (muslinger)

Det er for det marine miljø konstateret, at selvom den marine fødekæde indeholder et led mere ved tilstedeværelse af top-prædatorer, så forventes Erythromycin som udgangspunkt at have en lav biomagnifikation over de trofiske niveauer (TMF < 1) i den marine fødekæde. På dette grundlag konkluderes, at en parallel standard for saltvand skal fastsættes til samme niveau som for ferskvand jf. den tekniske vejledning (EC, 2018).

$BKK_{sek.\ forgiftn.\ saltvand}$ = 14,7 mg/kg vådvægt (fisk)
$BKK_{sek.\ forgiftn.\ saltvand}$ = 4,1 mg/kg vådvægt (muslinger)

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 fastlæggelse af en tærskelværdi for humant

indtag som en NO(A)EL, oftest bestemt som et acceptabelt eller tolerabelt dagligt indtag eller referencedosis. På grundlag af en beregningsformel med standard human konsum af vandlevende organismer kan der bestemmes et kvalitetskriterium for biota til human konsum.

Indledningsvist fastslår rapporten, at stoffet Erythromycin ikke har egenskaber, som gør stoffet til et potentielt kræftfremkaldende, mutagent eller reproduktionstoksisk stof (CMR-vurdering).

Ved anvendelse af beregningsgrundlaget fastsat i Europa-Kommissionens tekniske vejledning (EC, 2018) er NOAEL på 100 mg/kg kropsvægt/dag bestemt for hunde omregnet til en grænseværdi for human sundhed (TL_{hh}):

TL_{hh} = 100 mg/kg kropsvægt/dag / 100 = 1 mg/kg kropsvægt/dag

Følgende kvalitetskriterium for human konsum af vandlevende organismer er beregnet:

HKK = (0,2 x 1 mg/kg kropsvægt/dag) / 0,00163 = 122,7 mg/kg biota vådvægt (afrundet til 120 mg/kg biota vådvægt)

Vandkvalitetskriterium baseret på $BKK_{sek.\ forgiftn.}$ og HKK
Der er beregnet et kvalitetskriterium for sekundær forgiftning af vandlevende organismer (biota) for beskyttelse af dyrelivet ($BKK_{sek.\ forgiftn.}$), og for samme vandlevende organismer er der beregnet et kvalitetskriterium for human konsum (HKK). Bestemmelserne i Europa-Kommissionens tekniske vejledning (EC, 2018) fastslår, at der derfor skal vurderes, hvilken af disse værdier der skal være afgørende for et kvalitetskriterium for biota.

Vurderingsgrundlaget er en konvertering af begge værdier til en sammenlignelig koncentration i vandsøjlen ved beregning baseret på tilvejebragte data om Bio Akkumulations Faktor (BAF). Med en BAF-værdi på 40 l/kg for muslinger svarer begge værdier (saltvand og ferskvand) for BKK til en koncentration af stoffet Erythromycin i vand på 0,106 mg/l. Med en BAF-værdi på 40 l/kg for muslinger svarer værdien for HKK til en koncentration af stoffet Erythromycin i vand på 3,07 mg/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.

Rapporten fastslår på dette grundlag, at kvalitetskriteriet for biota ($BKK_{sek.\ forgiftn.}$) skal fastholdes som et generelt beskyttelsesniveau for organismer højt i fødekæderne, herunder såvel vandlevende pattedyr som human konsum.

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

Kvalitetskriteriet for human konsum af drikkevand ($HKK_{Drikkevand}$) er fastsat i henhold til beregningsgrundlaget i Europa-Kommissionens tekniske vejledning (EC, 2018). Principielt er kriteriet fastsat på baggrund af toksikologiske studier af pattedyr og fastlæggelse af en tærskelværdi

for humant indtag som en NO(A)EL, oftest bestemt som et acceptabelt eller tolerabelt dagligt indtag eller referencedosis, og på grundlag af standard human konsum af drikkevand.

Ved anvendelse af beregningsgrundlaget fastsat i Europa-Kommissionens tekniske vejledning (EC, 2018), og en NOAEL på 100 mg/kg kropsvægt/dag bestemt for hunde, er der beregnet følgende kvalitetskriterium for human konsum af drikkevand:

$HKK_{Drikkevand}$ = (0,2 x 1 mg/kg kropsvægt/dag x 70 kg) / 2 L = 7 mg/l

Europa-Kommissionens videnskabelige komite for sundhed og miljø, SCHEER (SCHEER, 2022) støtter grundlag og metode for denne beregnede værdi, men pointerer tillige, at der for farmaceutiske stoffer bør søges en harmoniseret tilgang til fastsættelse af drikkevandskrav, der er baseret på en generel beskyttelse mod de sundhedsskadelige påvirkninger og en specifik beskyttelse mod de afledte effekter og risici ved en kronisk eksponering for disse kemikalier.

Indikativt kvalitetskriterium baseret på at hindre spredning af Antimikrobiel Resistens
Mikrobiel resistens overfor antibiotika (AMR) er globalt et alvorligt og stigende problem, der blev italesat af de Forenede Nationers Generalforsamling med vedtagelse af en deklaration om gennemførelse af fælles handlinger for at takle denne udfordring (UN, 2016)[6]. Udfordringen omfatter specifikt bekymringer om tiltagende forekomster af antibiotika resistente bakterier (AMB) og øget spredning af antibiotika resistente gener (AMG) imellem bakterier knyttet til mennesker, dyr og det eksterne miljø.

Fastsættelse af et kvalitetskriterium for at hindre spredning af Antimikrobiel Resistens i det eksterne miljø, sker på baggrund af et mål om videst muligt at hindre miljøforhold, som vil kunne skabe grundlag for en selektiv opformering af bakterier og genetisk materiale (AMB og AMG), der indeholder Antimikrobiel Resistens. Kvalitetskriteriet er indikativt, idet det faglige grundlag på nuværende tidspunkt fortsat skal modnes og kræver yderligere forskning og faglig indsigt.

Bengtsson-Palme og Larsson (2016)[7] har i et større studie om sikkerhed mod selektiv opformering af resistente bakterier, foreslået anvendelse af den mindste koncentration, der vurderes at kunne frembringe inhibering af mikrobiel vækst – Minimum Inhibitory Concentration (MIC). Til denne koncentrationsværdi tilføjes en usikkerhedsfaktor på 10 for at sikre, at et kvalitetskriterium til hindring af selektive miljøforhold med deraf følgende potentiel spredning af Antimikrobiel Resistens, er baseret på en stofkoncentration væsentligt under MIC-værdien.

I studiet er der frembragt data om MIC-værdier fra den offentlige database EUCAST etableret af den Europæiske Komité for Test af Antimikrobiel Følsomhed, og på grundlag heraf beregnet PNEC-MIC-værdier for en lang række antibiotiske stoffer. For stoffet Erythromycin er der tilvejebragt et datagrundlag for beregning af PNEC-MIC med en værdi på 1 µg/l.

Denne PNEC-MIC værdi for Antimikrobiel Resistens er højere end PNEC (0,5 µg/l) for økotoksikologiske effekter. Det pointeres dog, at den foreslåede PNEC-MIC ikke tager højde for tilstedeværelse af multiresistente bakterier eller kombinationseffekter afledt af flere samtidigt

[6] Forenede Nationer (UN, 2017): Deklaration vedtaget af FN's Generalforsamling den 22. september 2017. Tilgængelig online her: https://digitallibrary.un.org/record/842813
[7] Bengtsson-Palme, Johan og Larsson, D.G. Joakim: Concentrations of antibiotics predicted to select for resistant bacteria: Proposed limits for environmental regulation. Environment International 86 (2016).

tilstedeværende antibiotika, samt for miljøer med andre miljøfremmede stoffer, biocider og metaller, der også vil kunne bidrage til selektion af Antimikrobiel Resistens (AMR). Det anbefales at anvende den laveste af de to PNEC-værdier.

Fremgangsmåden understøttes og anbefales af den Internationale sammenslutning af Medicinalvareproducenter (IFPMA, 2022)[8].

Effekter af stoffets ionisering ved relevante pH værdier i det eksterne miljø
Stoffet Erythromycin er et ikke-ladet molekyle, der dog som en meget svag syre kan protolysere med en pKa værdi på 8,9. Stoffet forekommer derfor under miljørelevante forhold med pH værdier mellem 5 og 9, som et ikke-ladet stof.

Konklusion
Følgende kvalitetskriterier for vandmiljøet er udregnet for Erythromycin:

Korttidsvandkvalitetskriterium

$KVKK_{ferskvand}$	1,0 µg/l
$KVKK_{saltvand}$	0,1 µg/l

Vandkvalitetskriterium

$VKK_{ferskvand}$	0,5 µg/l
$VKK_{saltvand}$	0,05 µg/l

Sedimentkvalitetskriterium

$SKK_{ferskvand}$	47,7 µg/kg tørvægt (5% OC) 954 µg/kg tørvægt x f_{oc}
$SKK_{saltvand}$	4,77 µg/kg tørvægt (5% OC) 95,4 µg/kg tørvægt x f_{oc}

Biotakvalitetskriterium, sekundær forgiftning

$BKK_{sek.forgiftn.}$	14,7 mg/kg vådvægt fisk
$BKK_{sek.forgiftn.}$	4,1 mg/kg vådvægt musling

Biotakvalitetskriterium, human konsum

HKK	120 mg/kg biota vådvægt

[8] Tell, J. et al.: Science-based Targets for Antibiotics in Receiving Waters from Pharmaceutical Manufacturing Operations. Integrated Environmental Assessment and Management – Vol. 15, no. 3, pp. 312-319 (2019)

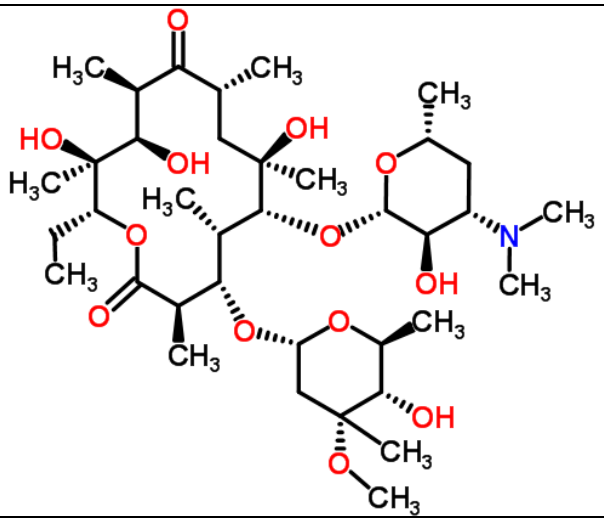
ERYTHROMYCIN

Changes on the dossier after SCHEER final opinion:
Following the final SCHEER opinion published on 1st March 2022 (SCHEER, 2022)[9] the dossier has been updated by the JRC in the sections 7.1 "Acute aquatic ecotoxicity", 7.2. "Chronic aquatic ecotoxicity", 7.3. "Sediment ecotoxicity", 7.5. "Secondary poisoning and Section 7.6. "Human health".
The SCHEER endorsed the MAC-QS values (1.0 µg/L for freshwater and 0.1 µg/L for marine water) derived by deterministic approach. In agreement with the SCHEER opinion, these MAC-QS values are proposed due to some uncertainties in the probabilistic approach.
The deterministic derived AA-$QS_{fw,eco}$ of 0.5 µg/L and an AA-$QS_{sw,eco}$ of 0.05 µg/L are endorsed by the SCHEER. The JRC has included for clarification in section 7.2., the rationale for not performing the derivation of the AA-QS by probabilistic approach.
The SCHEER required the calculations of the sediment EQS, to protect benthic organisms, to be reviewed as they currently do not use the appropriate AA-QS values in the Section 7.3. The QS for sediments have been recalculated using the proper AA-QS values and an experimental Koc value has been selected for the derivation.
In the section 7.5., the SCHEER supported the $QS_{Biota,secpois,fw}$ in fish of 15 mg/kg_{ww} and 4.1 mg/kg_{ww} for bivalves for freshwater. However, according to the SCHEER opinion (SCHEER, 2022) a QS to protect marine organisms from secondary poisoning should be provided. The JRC proposes a $QS_{biota,sec\ pois}$ for marine water based on the same $QS_{biota,sec\ pois}$ for freshwater since erythromycin is not expected to biomagnify in small birds or mammals within marine food chains.
To protect human health, the $QS_{biota\ hh\ food}$ of 120 mg/kg and provisional drinking water $QS_{dw,hh}$ of 7 mg/L were supported by the SCHEER. The back-calculation to water has been amended using the reliable BAF value of bivalves in Section 7.6, resulted in $QS_{water,\ biota}$ of 3.07 mg/L.

1 Chemical identity

Common name	Erythromycin
Chemical name (IUPAC)	(3R,4S,5S,6R,7R,9R,11R,12R,13S,14R)-6-{[(2S,3R,4S,6R)-4-(Dimethylamino)-3-hydroxy-6-methyltetrahydro-2H-pyran-2-yl]oxy}-14-ethyl-7,12,13-trihydroxy-4-{[(2R,4R,5S,6S)-5-hydroxy-4-methoxy-4,6-dimethyltetrahydro-2H-pyran-2-yl]oxy}-3,5,7,9,11,13-hexamethyloxacyclotetradecane-2,10-dione
Synonym(s)	--
Chemical class (when available/relevant)	macrolide antibiotic
CAS number	114-07-8

[9] SCHEER final opinion on Erythromycin (Publication date 1 March 2022), available on-line at: https://health.ec.europa.eu/publications/draft-environmental-quality-standards-priority-substances-under-water-framework-directive-0_en

EU number	204-040-1
Molecular formula	C37H67NO13
Molecular structure	
Molecular weight ($g \cdot mol^{-1}$)	733.94

2 Existing evaluations and Regulatory information

Annex I EQS Dir. (2013/39/EU)	Not Included
Existing Substances Reg. (793/93/EC)	Not applicable
Plant Protection Products (PPP) (EC No 1107/2009, repealing Directive 91/414/EEC)	Not included
Biocides (EU No. 528/2012, repealing Directive 98/8/CE)	Not included
PBT substances	Not included
Substances of Very High Concern (1907/2006/EC)	Not included
POPs (Stockholm convention)	Not included
Other relevant chemical regulation (veterinary products, medicament, ...)	Approved Pharmaceutical
Endocrine disrupter	Not investigated
Regulation (EC) No 1272/2008 (Classification and Labelling Regulation)	No harmonised classification on erythromycin is available.

3 Proposed Quality Standards (QS)

3.1 Environmental Quality Standard (EQS)

QS for freshwater is the "critical QS" for derivation of an Environmental Quality Standard

	Value	Comments
Proposed AA-EQS for [freshwater] [µg·L^{-1}] Corresponding AA-EQS in [marine water] [µg·L^{-1}]	0.5 0.05	**See Section 7.2 and 7.4.**
Proposed MAC-EQS for [freshwater] [µg·L^{-1}] Proposed MAC-EQS for [marine waters] [µg·L^{-1}]	1 0.1	**See Section 7.1 and 7.4.**

3.2 Specific Quality Standard (QS)

Protection objective	Unit	Value	Comments
Predators (secondary poisoning)	[mg·kg^{-1}biota $_{ww}$]	**Freshwater:** 14.7 mg.$kg^{-1}_{biota\ ww}$ (fish) 4.1 mg.$kg^{-1}_{biota\ ww}$ (bivalves) **Marine water:** 14.7 mg.$kg^{-1}_{biota\ ww}$ (fish) 4.1 mg.$kg^{-1}_{biota\ ww}$ (bivalves)	**See section 7.5**
	[mg·L^{-1}]	0.10 mg.L^{-1} (bivalves)	
Benthic community (freshwater)	[µg.kg^{-1} $_{dw}$]	47.7	**See section 7.3**
Benthic community (marine)	[µg.kg^{-1} $_{dw}$]	4.77	
Human health via consumption of fishery products	[µg·kg^{-1}biota $_{ww}$]	120000	**See section 7.6**
	[µg·L^{-1}]	3070	
Human health via consumption of water	[µg·L^{-1}]	7000	

4 Major uses

Erythromycin is an organic substance produced by *Saccharopolyspora erythraea* (formerly known as *Streptomyces erythraeus*) used as an antibiotic which belongs to the macrolide group. It binds to the 50S ribosomal subunits of susceptible bacteria, resulting in inhibition of bacterial protein synthesis and translation.[10]

Erythromycin is a broad-spectrum antibiotic widely used in human and veterinary medicine for treating several bacterial infections against pathogenic both Gram-positive and Gram-negative bacteria. It is used in the clinical treatment of respiratory, skin, gastrointestinal and genital infections.[11] Erythromycin is used in veterinary medicine for the treatment of clinical and subclinical mastitis in lactating cows, for the treatment of infectious diseases due to erythromycin-sensitive bacteria in cattle, sheep, swine and poultry, and for the treatment of chronic respiratory diseases due to mycoplasma in poultry, mainly as the base, thiocyanate ester and stearate salt. The most often recommended doses (as erythromycin base) range from 5 to 20 mg/kg bw/day for bovines including lactating cows, pigs and sheep, for 3 to 5 days by intramuscular route and 20 mg/kg bw/day via drinking water for broiler chickens and laying hens.[12]

As a medicinal product, erythromycin is currently authorised in the following European Member States (MS) and European Free Trade Association (EFTA) countries: Austria, Belgium, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Iceland, Ireland, Italy, Lithuania, Luxembourg, Netherlands, Norway, Poland, Portugal, Romania, Slovakia, Spain, and Sweden.[13]

[10] Drugbank [accessed 23 March 2021]: https://go.drugbank.com/drugs/DB00199

[11] Farzam K, Nessel TA, Quick J. Erythromycin. [Updated 2020 Nov 27]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2021 Jan-. Available from: https://www.ncbi.nlm.nih.gov/books/NBK532249/

[12] The European Agency for the Evaluation of Medicinal Products (EMA), Veterinary Medicines and Information Technology Unit. Erythromycin-erythromycin thiocyante-erythromycin stereate: Summary report. EMEA/MRL/720/99-FINAL, January 2000. Available from: https://www.ema.europa.eu/en/documents/mrl-report/erythromycin-summary-report-2-committee-veterinary-medicinal-products_en.pdf

[13] List of nationally authorised medicinal products available online at: https://www.ema.europa.eu/en/documents/psusa/erythromycin-list-nationally-authorised-medicinal-products-psusa/00001257/201903_en.pdf (Accessed on April 2021)

5 Environmental Behaviour

5.1 Environmental distribution

		Master reference
Water solubility ($mg \cdot L^{-1}$)	2000	Drugbank (In Carvalho et al., 2015);
	1.4	Klasmeier et al. 2011 (In UBA, 2014)
	0.5	Kümmerer 2003 (In UBA, 2014)
Volatilisation	Volatilisation of erythromycin from soil or water is not expected due to the low vapour pressure and Henry's Law constant.	UBA (2014)
Vapour pressure (Pa)	3.04×10^{-25} (estimated)	ChemIDPlus
	2.83×10^{-23} Pa at 25°C (calculated)	US EPA 2012a (In UBA, 2014)
	<0.000001 Pa	Kümmerer 2003 (In UBA, 2014)
Henry's Law constant ($Pa.m^3.mol^{-1}$)	5.49×10^{-23} (calculated)	US EPA 2012a (In UBA, 2014)
Adsorption	The criteria triggering an assessment for sediment effects are met, since both the $LogK_{oc}$ and $LogK_{ow}$ exceed the trigger value of 3. Therefore, the sediment toxicity assessment should be performed.	
Organic carbon – water partition coefficient (K_{OC})	Soil K_{oc} = 570 $L \cdot kg^{-1}$	PubChem
	Soil K_{oc} = 1877 $L \cdot kg^{-1}$ (experimental)	**Barron et al. 2009 (In UBA, 2014)**
	Sludge K_{oc} = 616 $L \cdot kg^{-1}$	Barron et al. 2009 (In UBA, 2014)
	K_{oc} = 25 – 570 $L \cdot kg^{-1}$ (calculated)	US EPA 2012a (In UBA, 2014)
Bioaccumulation	Based on the $LogK_{ow}$ value, which slightly exceeds the trigger of 3, the secondary poisoning assessment should be performed.	
Octanol-water partition coefficient (Log Kow)	3.06 (experimental)	US EPA 2012a (McFarland et al. 1997, UBA, 2014); ChemIDPlus
	1.14. – 1.65 (calculated)	US EPA 2012b (In UBA, 2014)

	0.65 – 2.5 (diverse sources)	KIWA 2000 (In UBA, 2014)
BCF (measured)	48.5	NORMAN, 2014 (In Carvalho et al., 2015)
BCF (calculated)	9.3 – 49	US EPA, 2012a (In UBA, 2014)
	The consensus prediction for this chemical is considered unreliable since only one prediction can be made.	US EPA, 2012b (In UBA, 2014)
BAF	4492 L/kg for fish, field-based in China, river Haihe	Gao et al., 2012 (in UBA, 2014)
	11- 54 L/kg for mussels (ribbed horsemussel *Geukensia demissa*), field-based in USA, San Francisco Bay	Klosterhaus et al., 2013 (in UBA, 2014)
	Phytoplankton 8.7; Zooplankton 162; Snail (*Bellamya* sp.) 4.4; Bivalve (*Corbiculidae*) 32; Common carp (*Cyprinus carpio*) 32; Lake anchovy (*Coilia ectenes*) 3.8; Crucian carp (*Carassius auratus*) 32; Yellow catfish (*Pelteobagrus fulvidraco*) 103. Field-based in China, Taihu Lake.	Xie Z. et al., 2015

5.2 Abiotic and Biotic degradations

		Master reference
Hydrolysis	Cannot be estimated	US EPA 2012a (In UBA, 2014)
Photolysis	Reduced atmospheric oxidation due to sorption to airborne particulates	US EPA 2012a (In UBA, 2014)
	Hydroxyl radicals reaction half-life = 19 Min (calculated)	
Biodegradation	Not readily biodegradable	US EPA 2012a (In UBA, 2014); NORMAN, 2014 (In Carvalho et al., 2015)
	Not degradable, 7 % biodegradation	LANUV NRW 2007 (In UBA, 2014)
	Very high level of concern regarding persistence (based on estimated biodegradation (US EPA 2009))	Ortiz de Garcia et al. 2013 (In UBA, 2014)

	Half-life during manure storage = 41±1 days, half-life in soil = 20±1.2 days.	Schlüsener 2005 (In UBA, 2014)
Dissipation in water/sediment	In freshwater under aerobic and anaerobic conditions the estimated half-life DT_{50} was 16.12 ~ 17.63 days without sediment and 6.80 ~ 13.83 days with sediment. In sea water under aerobic and anaerobic conditions, the estimated DT_{50} was 28.88 ~ 37.87 days without sediment and 11.11 ~ 11.38 days with sediment.	Kwon (2016)
Metabolites	Dehydrato-erythromycin (or erythromycin-H2O)	UBA (2014)
	N-Demethylerythromycin	Senta et al., 2017

6 Measured environmental concentrations

6.1 Freshwater

Note: This section has been revised and updated after the final adoption of erythromycin QS values by the SCHEER committee in the plenary meeting on 1 March 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), and assuming that the PNEC is equal to the freshwater AA-EQS=0.5 µg/L..

6.1.1 Data availability and data scenarios

To update the information on exposure in the erythromycin's dossier, the JRC has used disaggregated monitoring data existing at the beginning of current prioritisation exercise, which started in 2014 (Carvalho et al., 2016), and also recent data (after 2014) which were officially reported to the EEA (Watch List and WISE) by the EU Member States (MS). The collected disaggregated raw data for measured environmental concentrations (MECs) in inland surface water are summarised in Table 6.1.1 showing the source, dataset and corresponding periods of monitoring. A short description of each of the referred datasets is provided thereafter below.

Table 6.1.1: Sources, dataset and available disaggregated raw monitoring data for measured environmental concentrations (MECs) in 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)	3748 samples from 296 sites in 2 MS (2006 – 2014)
EEA, Watch List (2019)	5413 samples from 487 sites in 25 MS (2014 - 2019)
EEA, WISE (2020)	4724 samples from 413 sites in 25 MS (2008 – 2019; not monitored in each year)
Additional data received or retrieved after the 18th meeting of WFD CIS WG Chemicals (held in October 2020)	EEA, WISE (2022): 2873 samples from 506 sites in 20 MS (2020 – 2021)

Note: The additional monitoring data were considered separately in the risk assessment analysis.

The Prioritisation dataset (Carvalho et al., 2016; https://circabc.europa.eu/w/browse/52c8d8d3-906c-48b5-a75e-53013702b20a) includes data collected at the beginning of the second prioritisation exercise 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 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 dataset, managed by the EEA, has been received in November 2020 (information about WISE data could be found on https://www.eea.europa.eu/data-and-maps/data/waterbase-water-quality-icm-1). Recently, the JRC has retrieved data from WISE database also for the period 2020-2021.

Further, the JRC acknowledged the point raised by the stakeholders that despite the constant improving of sensitivity of analytical techniques, any set of measured environmental concentrations (MECs) 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 MEC, 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 Limits of Quantification (LOQ) of applied analytical methods are adequate in relation 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 analysis (Table 6.1.2).

Table 6.1.2. 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 (i.e. >LOQ)
Scenario 2 (Sc2)	**All monitoring samples** (quantified and non-quantified). When the substitution approach is feasible, the non-quantified samples in Sc2 are set equal to half of LOQ as described in Directive 2009/90/EC. Other substitutions are also possible (for example substitution at LOQ).
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 in the prioritisation exercise 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 non-quantified samples are set to half LOQ[14] in both Scenario 2 (Sc2) and Scenario 3 (Sc3). However, Sc2 comprises all monitoring records, which could lead to non-confirmed exceedances when ½LOQ>PNEC, while Sc3 takes into account quantified monitoring samples and non-quantified samples only when ½ LOQ ≤ 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)**.** The information for Sc1 and Sc2 scenarios is also presented for completeness.

Then, the records from the datasets, shown in Table 6.1.1, have been combined in a single dataset (called thereafter COMBI dataset), however, the additional data from WISE 2022 (EEA) were considered separately. Besides, should be noted that duplicated records are possible between the individual datasets. The duplicates, particularly between Watch List and WISE datasets, have been found and eliminated from the COMBI dataset which is used later 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 6.1.3 for Sc1 and Sc2 data scenarios (the information for Sc3 is given after the data quality check). Furthermore, the detailed statistics per country for Sc2 (and also for Sc3) is provided in **a complementary Excel file entitled MEC_Erythromycin_dossier** (including the number of sites, number samples, fraction from all

[14] 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″.

samples, number of quantified samples, info about LOQ values, statistics of MEC, etc.). It evidenced that two MS are overrepresented in the combined dataset holding together about 87.2% of all samples (MS#06 contributed with 48%, while MS#07 with 39.2%).

Table 6.1.3. Available disaggregated data for measured environmental concentrations (MECs) across EU MS (jointly data from all countries after the elimination of duplicated records; for the period 2006 – 2019 in the combined dataset (COMBI dataset) for Sc1 and Sc2 data scenarios (the information for Sc3 is given after the data quality check).

Scenario	Member States (MS)	Sites	Samples	Quantified samples (% of all samples)
Sc1	18	375	1552	100
Sc2	25	815	10111	15.4

6.1.2 Quality of data

The quality of measured environmental concentrations (MEC) 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 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 sensitivity of the applied analytical methods (LOQ-PNEC criterion), union representativeness of data and uncertainty (bias) related to non-quantified (censored) concentrations.

For instance, considering the data from all MS together, Figure 6.1.1 shows the range of LOQs of non-quantified samples per country while Figure 6.1.2 informs how many non-quantified samples fulfilled the LOQ-PNEC condition (½ LOQ≤PNEC) in each of the MS. It was found that all MS monitored with sufficiently sensitive analytical methods and the amount of available monitoring data is satisfactory. The detailed information about the LOQ values per MS for non-quantified samples in Sc2 dataset is provided in the accompanying Excel file.

After the LOQ-PNEC check the decisive Sc3 data scenario is developed considering PNEC=0.5 µg/L. In fact, since the good quality of monitoring data, the Sc3 is equal to Sc2. The basic information for Sc3 scenario is presented in Table 6.1.4. 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.

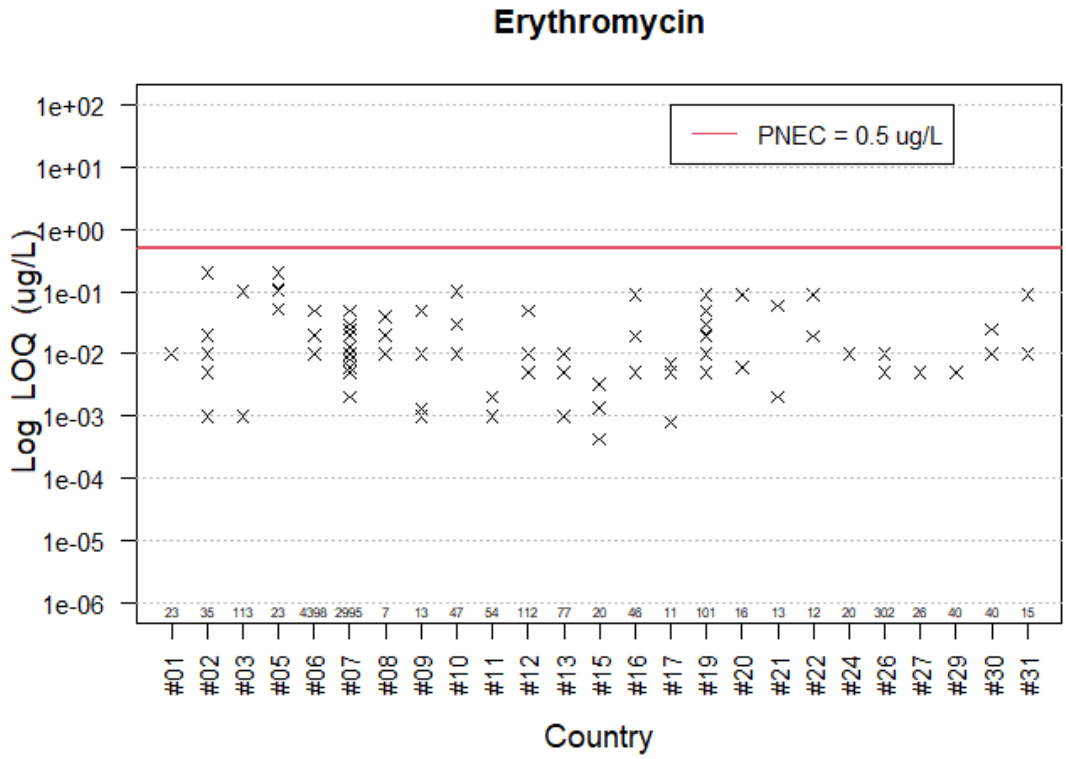


Figure 6.1.1: Range of LOQs for non-quantified samples in Sc2 scenario of combined dataset per country. The lowermost line of the figure shows the overall number of non-quantified samples in each reporting MS. For confidentiality the countries' names are coded. The red line indicates the PNEC value.

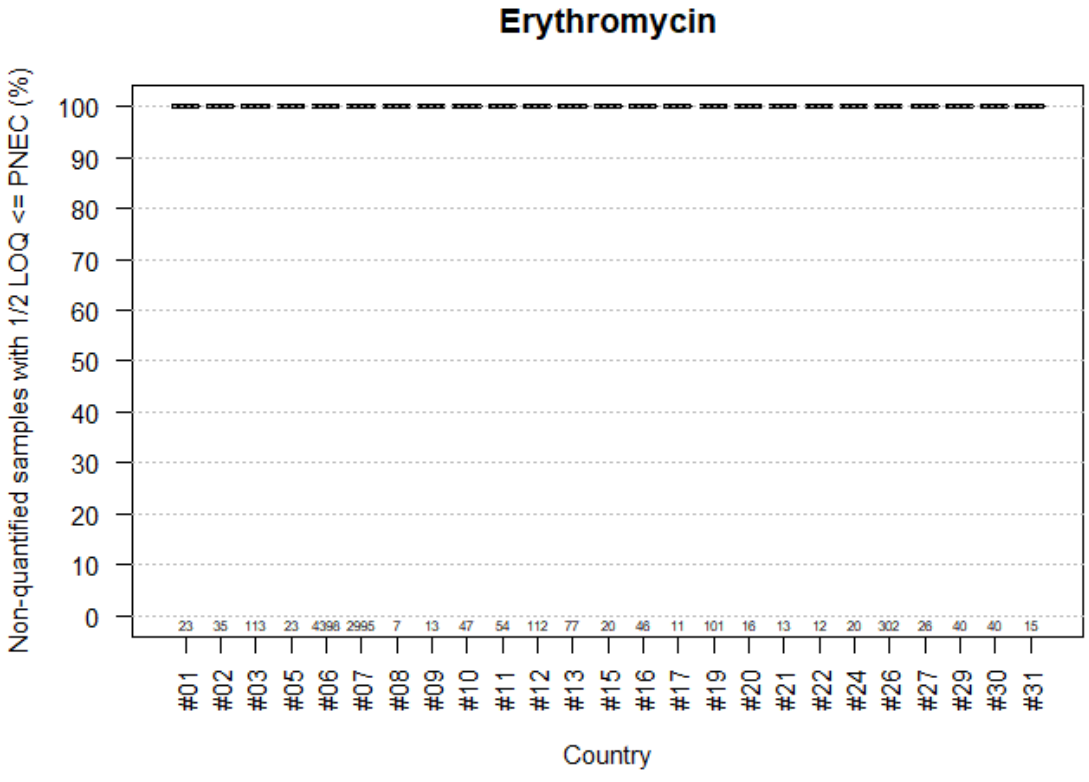


Figure 6.1.2: Number of non-quantified samples fulfilled LOQ-PNEC condition (½ LOQ≤PNEC) as percentage from reported non-quantified samples per country in Sc2 scenario of the combined dataset. The lowermost line of the figure shows the overall number of non-quantified samples in each reporting MS. For confidentiality the countries' names are coded.

Table 6.1.4: Available data for the measured environmental concentrations (MEC) across EU MS (jointly data from all countries after the elimination of duplicated records) for the period 2006 – 2019 in Sc3 of the combined dataset (PNEC=0.5 µg/L).

Scenario	Member States (MS)	Sites	Samples	Quantified samples (% of all samples)
Sc3	25	815	10111	15.4

Then, plots of histogram (Figure 6.1.3) and cumulative frequency (Figure 6.1.4) have been prepared for measured concentrations (data from all MS together) in Sc3 of the combined dataset. About 36.6% of all samples are non-quantified records have LOQ=0.01 µg/L which explains the high amount of 0.005 µg/L concentrations (Figure 6.1.3). The cumulative frequency (Figure 6.1.4) is compared to a log-normal distribution with the same mean and standard deviation as for monitoring dataset. It was found that the empirical distribution could be approached approximately by the log-normal one.

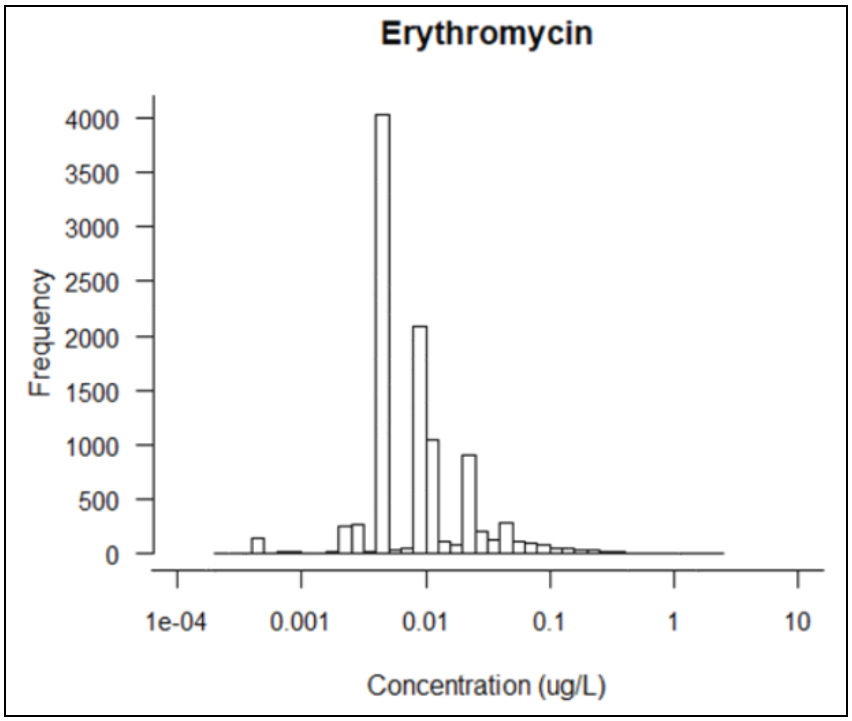


Figure 6.1.3: Histogram of concentrations (data from all MS together) for Sc3 scenario of the combined dataset. About 36.6% of all samples are non-quantified records have LOQ=0.01 µg/L which explains the high amount of 0.005 µg/L concentrations

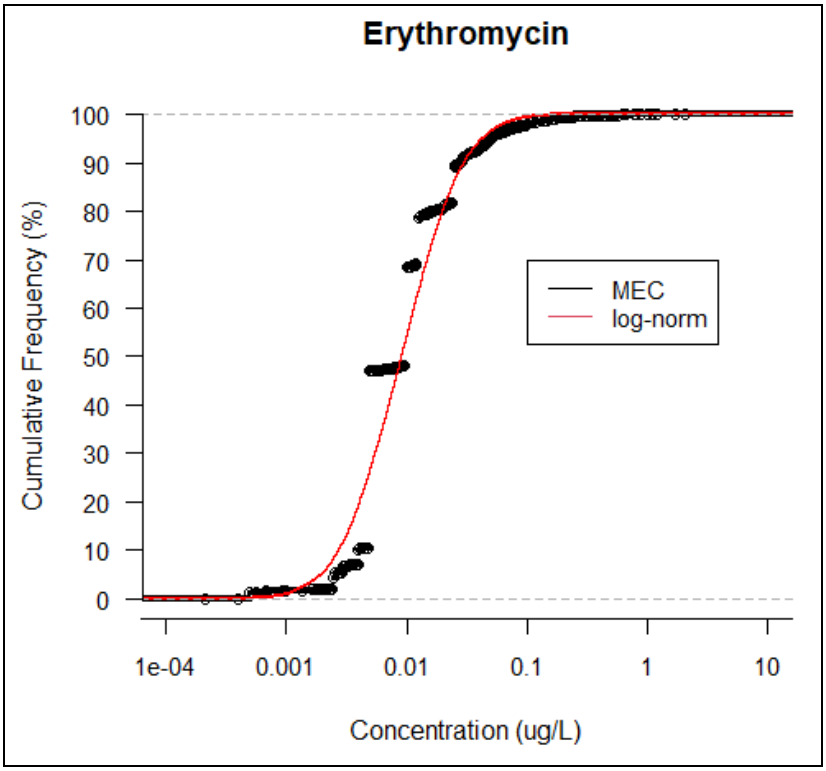


Figure 6.1.4: Cumulative frequency of concentrations (data from all MS together) for Sc3 scenario of the combined dataset. The red line represents a cumulative frequency of log-normal distribution with the same mean and standard deviation as for monitoring dataset.

6.1.3 Summary statistics of measured concentrations

The summary (descriptive) statistics of measured environmental concentrations (MECs) 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 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 6.1.5 (the underlying data cover a period 2006-2019). 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 the lower bound (1% of LOQ) and common substitution (50% of LOQ) while the median and higher percentiles (≥ P90) are similar to the upper bound of replacement (99% of LOQ).
According to ProUCL 5.1 tool, the assessed variance in Sc3 by KM method is about 2.8*10^{-3} µg/L. The 95% upper confidence limit (95% UCL) of mean concentration, estimated by KM, is 0.0122 µg/L through bootstrapping and 0.0137 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 0.0997 µg/L by KM approach assuming normal distribution and higher, 0.242 µg/L, according Chebyshev method (ProUCL 5.1).

Table 6.1.5: Summary statistics of measured environmental concentrations (µg/L) 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	4.20E-04	4.20E-06	2.10E-04	4.16E-04
Mean	1.14E-02	9.57E-03	1.71E-02	2.47E-02
StDev	5.29E-02	5.30E-02	5.30E-02	5.27E-02
Median	0.015	0.0002	0.01	0.015
P90	0.05	0.023	0.028	0.0495
P95	0.06	0.0468	0.05	0.06
P99	0.16	0.16	0.16	0.16
Max	2.1	2.1	2.1	2.1

In addition for a sake of completeness, Table 6.1.6 compares summary statistics of measured environmental concentrations for Sc3 scenario (jointly data from all MS) estimated by Kaplan-Meier 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).

Finally, Table 6.1.7 analyses summary statistics when all MS are presented in the Sc3 dataset versus the hypothetical scenario of excluding the most data-rich countries (MS#06 and MS#07). These statistics are estimated by Kaplan-Meier method for dataset containing censored data (ProUCL 5.1 tool). Increased statistical estimates were obtained for higher percentiles (≥P90) of MECs but a lowering of the mean concentration when the overrepresented MS (#06 and #07) were excluded from the combined dataset. Furthermore, the table provides descriptive statistics of measured concentrations considering the additional monitoring data from 20 reporting MS during the period 2020-2021 (WISE 2022), which were found by Kaplan-Meier method of the ProUCL 5.1 tool. Comparing to the combined dataset, the additional data showed a lowering of the mean concentration, percentiles of MECs and max concentration.

Table 6.1.6. Summary statistics of measured environmental concentrations for Sc3 scenario (jointly data from all MS) estimated by Kaplan-Meier method for dataset containing censored data (ProUCL 5.1 tool of the US EPA) in comparison to 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).

Concentration (µg/L)	Scenario Sc1	Scenario Sc2	Scenario Sc3 KM method (ProUCL 5.1)
Min	5.60E-04	2.10E-04	4.20E-04
Mean	6.13E-02	1.71E-02	1.14E-02
StDev	1.23E-01	5.23E-02	5.29E-02
Median	0.031	0.01	0.015
P90	0.12	0.028	0.05
P95	0.19	0.05	0.06
P99	0.535	0.16	0.16
Max	2.1	2.1	2.1

Table 6.1.7: Comparison of summary statistics for measured environmental concentrations when all MS are presented in the Sc3 dataset and the hypothetical scenario of excluding the most data-rich country. The table provides also a descriptive statistics of measured concentrations considering the additional monitoring data from 20 reporting MS during the period 2020-2021 (WISE 2022). The statistics are estimated by Kaplan-Meier method for dataset containing censored data (ProUCL 5.1 tool).

Concentration (µg/L)	All countries presented in Sc3 of the combined dataset	Scenario "the most data-rich MS excluded from Sc3" (without #06 and #07)	Only additional data from WISE for the period 2020-2021 (Sc3 scenario)
Min	4.20E-04	4.20E-04	5.00E-05
Mean	1.14E-02	9.83E-03	3.73E-03
StDev	5.29E-02	8.36E-02	2.53E-02
Median	0.015	0.01	0.01
P90	0.05	0.09	0.035
P95	0.06	0.1	0.0484
P99	0.16	0.2	0.1
Max	2.1	2.1	0.9

6.1.4 Temporal trend

The temporal trend of erythromycin is verified in the period 2006-2019 according to annual variability of 95th percentiles (P95) of MECs estimated by Kaplan-Meier nonparametric method of ProUCL 5.1 tool of the US EPA (https://www.epa.gov/land-research/proucl-software).
Considering data from all MS together (see Figure 6.1.5), onwards 2011, the annual 95th percentiles of MECs showed generally a gradual increasing trend up to 0.1 µg/L with some yearly variability and oscillations. However, the P95 remain always below the PNEC value of 0.5 µg/L.
No substantial change of temporal pattern of P95 of MECs was observed if the most data-abundant MS (#06 and #07) were eliminated from the combined dataset Sc3. Under this scenario, in the recent years the P95 are stabilised at about 0.1 µg/L.
The additional monitoring data for 20 MS from WISE 2022 dataset (see Table 6.1.1) showed a decrease of the annual P95, respectively, to 0.049 µg/L in 2020 and 0.045 µg/L in 2021.

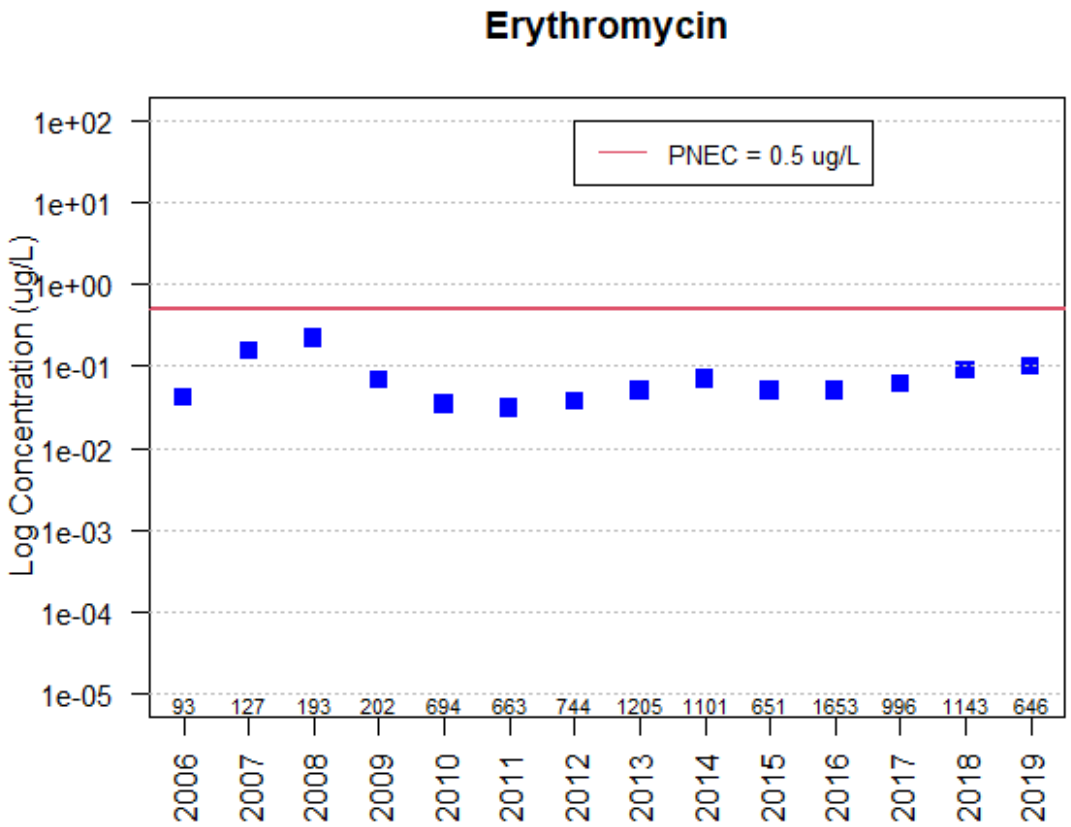


Figure 6.1.5: Plot for 95th percentiles of measured environmental concentrations per year for Sc3 scenario of the combined dataset considering **data from all MS**. Onwards 2011, the annual 95th percentiles of MECs showed generally a gradual increasing trend up to 0.1 µg/L with some yearly variability and oscillations but the P95 remain always below the PNEC value of 0.5 µg/L.

6.1.5 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 and MAC-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=0.5 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 6.1.8).

The Risk Quotient RQ(P95) is estimated by the 95th percentile (P95) of measured concentrations considering the data 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 6.1.5) are 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 6.1.8: Risk assessment screening results. The evaluation is based on measured environmental concentrations in Sc3 scenario of the combined dataset and PNEC=0.5 µg/L**.** The Risk Quotient RQ(P95) is calculated with 95th percentile (P95) of measured concentrations considering together the data from all MS. The P95 is estimated by Kaplan-Meier nonparametric method for dataset containing censored data (ProUCL 5.1 tool of the US EPA). The STE (Spatial, Temporal and Extent of PNEC exceedances) is assessment tool developed by the JRC (the table shows also the Spatial, Temporal and Extent of PNEC exceedance factors of the STE score). A given country is specified "Exceeding MS" if the 95th percentile of its measured concentrations is higher than the PNEC value.

Scenario	**RQ(P95)**	Fspat	Ftemp	Fext	**STE score**	**Exceeding MS** (% from total)	Total number of reporting MS
Sc3	0.12	0.0003	0.19	0.0	0.19	1 (4%)	25

The performed risk screening indicated a low risk for inland surface waters at EU level because the overall RQ(P95)=0.12, viz. it is lower than one, and only 1 MS out of the 25 reporting countries in Sc3 showed exceedances (about 4% from all MS).
Notes:

1. The low concern for freshwaters in EU is confirmed also if the most data-abundant MS (#06 and #07) were excluded from the combined dataset (Sc3 scenario) because the corresponding P95=0.1 µg/L did not exceed the PNEC=0.5 µg/L (see Table 6.1.7), and respectively RQ(P95)=0.2 .
2. The available latest data for exposure from WISE 2022 (see Tables 6.1.1 and 6.1.7) likewise confirmed that erythromycin is posing a low risk in the recent years (2020-2021) since RQ(P95)=0.097.

Compliance check

The compliance check, which is a core part of the developed risk assessment, was performed according to the EQS Directive[15] (amended by the Directive 2013/39/EU). The compliance is based on MECs in Sc3 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 and when the maximum concentrations (or 99th percentile[16] of concentrations) in reporting MS do not exceed the MAC-EQS. In the compliance analysis the non-quantified concentrations in the Sc3 dataset were assumed to be equal to a half of LOQs[17] i.e. the substitution approach, adopted by the Directives 2009/90/EC and 2013/39/EU, was applied.
At first, Figure 6.1.6 visualises a boxplot of annual average concentrations at monitoring sites (Sc3 data scenario) for the time period 2006-2019 comparing to the freshwater AA-EQS=0.5 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 6.1.9. The analysis showed for the recent years only occasional annual exceedances at monitoring sites (2 exceeding sites in 2017-2018).
Furthermore, according to the available latest data for exposure in 20 MS from WISE 2022 (see Table 6.1.1) none of the monitoring sites showed exceeding annual mean concentrations during the period 2020-2021.
Therefore, the above observations confirm no failure of compliance in regard to the freshwater AA-EQS.
Finally, regarding the compliance with the freshwater MAC-EQS=1 µg/L, the 99th percentiles of MECs from individual MS per year (Sc3 scenario of the combined dataset) were compared with the MAC-EQS. The results are presented in Table 6.1.10. In the time-period up to 2019, only one MS

[15] 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''.
[16] Directive 2008/105/EC Annex I Part B Paragraph 2 states that "In accordance with Section 1.3.4 of Annex V to Directive 2000/60/EC, Member States may introduce statistical methods, such as a percentile calculation, to ensure an acceptable level of confidence and precision for determining compliance with the MAC-EQS".
[17] 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".

showed P99 exceeding the freshwater MAC-EQS in 2017-2018. According to the additional recent data for exposure in 20 MS from WISE 2022, no MAC-EQS exceedances happened in 2020-2021. All these allow concluding no failure of compliance in regard to the freshwater MAC-EQS.

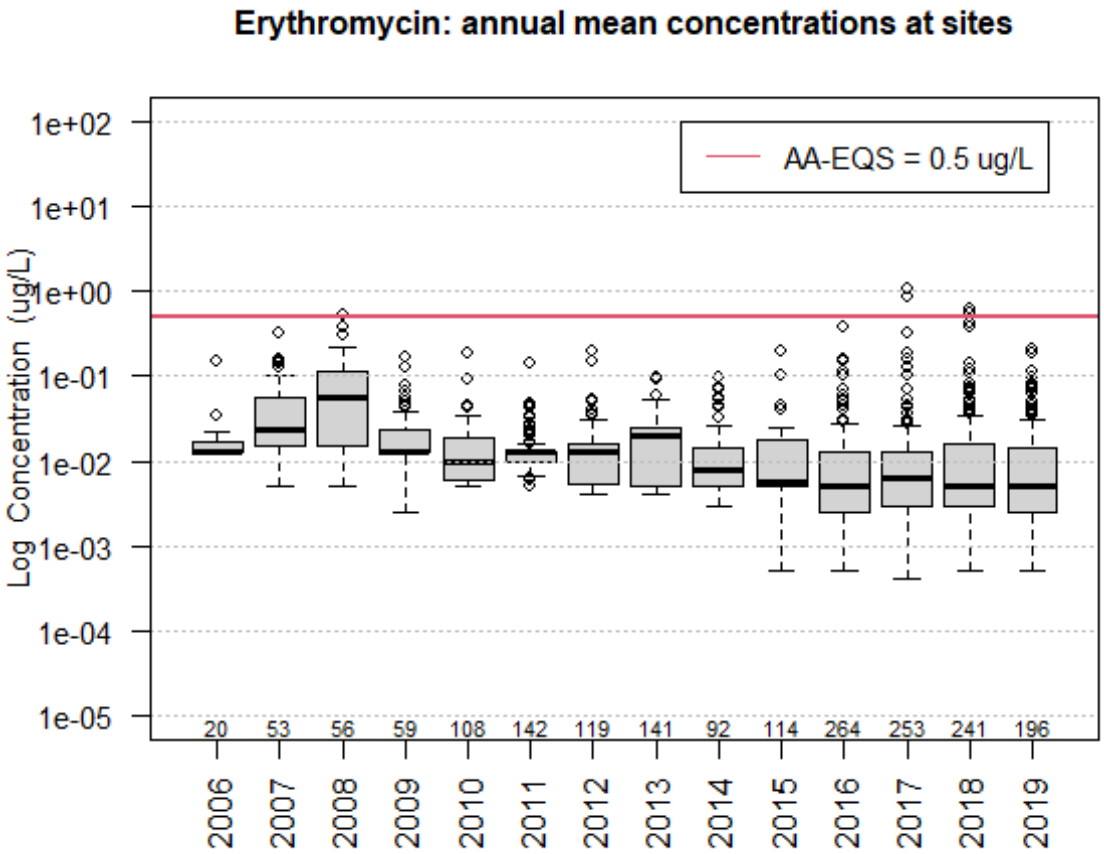


Figure 6.1.6: Boxplot of annual average values of measured concentrations at monitoring sites in Sc3 scenario for the time period 2006-2019. In this analysis the non-quantified concentrations are assumed to be equal to a half of LOQ (Directives 2009/90/EC and 2013/39/EU). The lowermost line of the figure gives the overall number of monitoring sites in each year. The red line indicates the freshwater AA-EQS.

Table 6.1.9: 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 (Directives 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	2	20	0	0
2007	1	53	0	0
2008	3	56	1	1.79
2009	1	59	0	0

2010	2	108	0	0
2011	2	142	0	0
2012	3	119	0	0
2013	2	141	0	0
2014	2	92	0	0
2015	10	114	0	0
2016	24	264	0	0
2017	23	253	2	0.79
2018	21	241	2	0.83
2019	21	196	0	0

Table 6.1.10: Number of reporting MS in Sc3 scenario of the combined dataset which 99th percentiles of MECs exceeded annually the freshwater MAC-EQS (given also as a percentage from the total number of reporting MS for each year). 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).

Year	Number of reporting MS	Number of exceeding MS	% of exceeding MS from all
2006	2	0	0
2007	1	0	0
2008	3	0	0
2009	1	0	0
2010	2	0	0
2011	2	0	0
2012	3	0	0
2013	2	0	0
2014	2	0	0
2015	10	0	0
2016	24	0	0
2017	23	1	4.35
2018	21	1	4.76
2019	21	0	0

Conclusion:
The performed risk screening and the observed no failures of compliance in regard to the freshwater AA-EQS and MAC-EQS, estimated through the monitoring data for exposure described in this dossier, showed that Erythromycin poses a low risk in the EU inland surface waters.

6.2 Coastal/Transitional water

This section is not fully developed because currently there are available a small amount of disaggregated monitoring data for the compartment of coastal/transitional water.

The available raw data from the EEA (Watch List and WISE database) are described in Table 6.2.1. The 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 6.2.2.

Table 6.2.1: Sources and available disaggregated raw monitoring data for measured environmental concentrations in coastal/transitional water compartment.

Source/Dataset	Available disaggregated raw data
EEA, Watch List (2019)	38 samples (36.8% quantified) from 14 sites in 6 MS for the period 2015-2019.
EEA, WISE (2020)	19 samples (only one quantified) from 12 sites in 5 MS for the period 2019-2020

Table 6.2.2: Available raw data for the measured environmental concentrations from several MS (after the elimination of duplicated records) for the period 2015 – 2020 in the combined dataset for Sc2 scenario (coastal/transitional water).

Scenario	Member States (MS)	Sites	Samples	Quantified samples (% of all)
Sc2	7	21	54	25.9

Regarding the quality of available monitoring data in Sc2 scenario, the range of LOQs of non-quantified samples is from 0.0008 µg/L to 0.05 µg/L, thus none of the non-quantified samples is taken with LOQs higher than the marine water AA-EQS (0.05 µg/L). However, the total amount of data is scarce for making a reliable risk assessment, but for a sake of completeness, the descriptive statistic of measured concentrations was estimated. The results are presented in Table 6.2.3. In statistical analysis the non-quantified concentrations are assumed to be equal to a half of LOQs.

Table 6.2.3: 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.

Concentration (µg/L)	Min	Mean	StDev	Median	P90	P95	P99	Max
	$2.4*10^{-4}$	0.007	0.0152	0.0029	0.012	0.0217	0.079	0.08

7 Effects and Quality Standards

Literature data were collected from the reports of Carvalho et al. (2015) and UBA (2014), and studies were not further re-assessed for their reliability in the present dossier. A data search was performed at the beginning of 2021, in order to identify any relevant ecotoxicological study on erythromycin published among 2015-2021. Six potentially relevant studies were assessed for their reliability by the JRC using the in-house developed JRC Literature Evaluation Tool (LET) based on the CRED evaluation method (Moermond et al., 2016). Studies were assessed for their relevance and reliability, and the classes assigned (R1-4) matched those of Klimisch et al. (1997) with R1-Reliable without restrictions, R2-Reliable with restrictions, R3-Not reliable, and R4-Not assignable. The acute and chronic ecotoxicity data of erythromycin for freshwater and marine water organisms are reported in the tables below. Studies which are shown in grey cannot be used directly for EQS derivation according to the EQS Technical Guidance (EC, 2018), but should be mentioned as additional information. Values in ">" and "<", even if they are valid, cannot be used directly for the EQS derivation (shown in grey), but serve as additional information as well. Key data which are shown in bold were selected for EQS derivation. A single endpoint per species was selected, based on the lowest relevant endpoint observed.

7.1 Acute aquatic ecotoxicity

The key acute ecotoxicity data of erythromycin for freshwater and marine water organisms are reported in the table below.

ACUTE EFFECTS			Master reference
Algae & aquatic plants ($\mu g \cdot L^{-1}$)	**Freshwater**	**Algae, *Pseudokirchneriella subcapitata* / 72h EC_{50}: 20 (growth rate) Reliability evaluation: 1-2 [a]**	**Isidori et al. 2005 (In Carvalho et al., 2015)**
		Algae, *Pseudokirchneriella subcapitata* / 72h EC_{50}: 36.6 (biomass) Reliability evaluation: 1-2 [a]	Eguchi et al. 2004 (In Carvalho et al., 2015)
		Algae, *Pseudokirchneriella subcapitata* / 72h EC_{50}: 38 (yield) Reliability evaluation: 1	Machado and Soares (2019)
		Algae, *Pseudokirchneriella subcapitata* / 72h EC_{50}: 350 (growth, chlorophyll fluorescence) Reliability evaluation: 1-2 [a]	Gonzaléz-Pleiter et al. 2013 (In Carvalho et al., 2015)
		Algae, *Chlorella vulgaris* / 72h EC_{50}: 33800 (biomass) Reliability evaluation: 1-2 [a]	Eguchi et al. 2004 (In Carvalho et al., 2015)
		Algae, *Chlorella vulgaris* / 96h EC_{50}: 85.7 (algal cell growth) Reliability evaluation: 2	**Wang, G (2019)**
		Algae, *Chlamydomonas reinhardtii* / 72h EC_{50}: 360 (population growth: cellular density) Reliability evaluation: 2	**Sendra et al. (2018b)**
		Aquatic plant, *Lemna minor* / 7 days EC_{50}: 5620 (frond number) Reliability evaluation: 1-2 [a]	**Pomati et al. 2004 (In Carvalho et al., 2015)**
		Cyanobacteria, *Anabaena cylindrica* (strain NIES-19) / 144h EC_{50}: 35 (biomass) Reliability evaluation: 3-4 [b]	Ando et al. 2007 (In Carvalho et al., 2015)
		Cyanobacteria, *Anabaena flos-aquae* / 72h EC_{50}: 140 (yield) Reliability evaluation: 1-2 [a]	Förster et al. 2013 (In Carvalho et al., 2015)
		Cyanobacteria, *Anabaena flos-aquae* / 72h EC_{50}: 348 (growth rate) Reliability evaluation: 1-2 [a]	**Förster et al. 2013 (In Carvalho et al., 2015)**
		Cyanobacteria, *Anabaena flos-aquae* (strain ATCC 29413) / 144h EC_{50}: 270 (biomass) Reliability evaluation: 3-4 [b]	Ando et al. 2007 (In Carvalho et al., 2015)
		Cyanobacteria, *Anabaena sp.* / 72h EC_{50}: 22 (growth: inhibition of constitutive luminescence) Reliability evaluation: 1-2 [a]	**Gonzaléz-Pleiter et al. 2013 (In Carvalho et al., 2015)**

		Cyanobacteria, *Anabaena variabilis* (strain NIES-23) / 144h EC_{50}: 430 (biomass) Reliability evaluation: 3.4 [b]	Ando et al. 2007 (In Carvalho et al., 2015)
		Cyanobacteria, *Microcystis aeruginosa* (strain NIES-44) / 144h EC_{50}: 23 (biomass) Reliability evaluation: 3-4 [b]	Ando et al. 2007 (In Carvalho et al., 2015)
		Cyanobacteria, *Microcystis wesenbergii* (strain NIES-107) / 144h EC_{50}: 23 (biomass) Reliability evaluation: 3-4 [b]	Ando et al. 2007 (In Carvalho et al., 2015)
		Cyanobacteria, *Nostoc sp.* (strain PCC 7120) / 144h EC_{50}: 200 (biomass) Reliability evaluation: 3-4 [b]	Ando et al. 2007 (In Carvalho et al., 2015)
		Cyanobacteria, *Synechococcus leopoldensis* (strain IAM-M6) / 144h EC_{50}: 160 (biomass) Reliability evaluation: 3-4 [b]	Ando et al. 2007 (In Carvalho et al., 2015)
		Cyanobacteria, *Synechococcus sp.* (strain PCC 7002) / 144h EC_{50}: 230 (biomass) Reliability evaluation: 3-4 [b]	Ando et al. 2007 (In Carvalho et al., 2015)
		Cyanobacteria, *Microcystis aeruginosa* (strain FACHB-905) / 96 h **EC_{50}: 22.97 (growth)** **Reliability evaluation: 1**	**Wu et al. (2020)**
	Marine	**Algae, *Dunaliella tertiolecta* / 96 h** **EC_{50}: 5750 (yield)** **Reliability evaluation: 1**	**Machado and Soares (2019)**
		Algae, *Tetraselmis suecica* / 72 h **EC_{50}: 10 (cell population density, growth inhibition)** **Reliability evaluation: 2**	**Sendra et al. (2018)**
		Diatoms, *Phaeodactylum tricornutum* / 72h EC_{50}: <100 (growth: cell density) Reliability evaluation: 2	Sendra et al. (2018)
		Diatoms, *Cylindrotheca closterium* / 72h EC_{50}: <100 (growth: cell density) Reliability evaluation: 2	Sendra et al. (2018)
		Diatoms, *Chaetoceros gracilis* / 72h EC_{50}: <100 (growth: cell density) Reliability evaluation: 2	Sendra et al. (2018)

		Diatoms, *Phaeodactylum tricornutum* / 72h EC_{50}: 1310 (population growth, cell density) Reliability evaluation: 2	**Sendra et al. (2018b)**
Invertebrates ($\mu g \cdot L^{-1}$)	**Freshwater**	Rotifer, *Brachionus calyciflorus* / 24h LC_{50}: 27530 (mortality) Reliability evaluation: 1-2 [a]	Isidori et al. 2005 (In Carvalho et al., 2015)
		Rotifer, *Brachionus calyciflorus* / 48h EC_{50}: 940 (mortality) Reliability evaluation: 1-2 [a]	**Isidori et al. 2005 (In Carvalho et al., 2015)**
		Crustacean, *Ceriodaphnia dubia* / 48h EC_{50}: 10230 (immobilisation) Reliability evaluation: 1-2 [a]	**Isidori et al. 2005 (In Carvalho et al., 2015)**
		Crustacean, *Daphnia magna* / 24h EC_{50}: 22450 (immobilisation) Reliability evaluation: 1-2 [a]	**Isidori et al. 2005 (In Carvalho et al., 2015)**
		Crustacean, *Daphnia magna* / 48h EC_{50}: 207800 (immobilisation) Reliability evaluation: 1-2 [a]	Ji et al. 2012 (In Carvalho et al., 2015)
		Crustacean, *Moina macrocopa* / 48h EC_{50}: 135500 (immobilisation) Reliability evaluation: 1-2 [a]	**Ji et al. 2012 (In Carvalho et al., 2015)**
		Crustacean, *Thamnocephalus platyurus* / 24h EC_{50}: 17680 (immobilisation) Reliability evaluation: 1-2 [a]	**Isidori et al. 2005 (In Carvalho et al., 2015)**
	Marine	**Crustacean, *Penaeus vannamei* / 48h EC_{50}: 22.7 (immobilisation) Reliability evaluation: 1-2 [a]**	**Williams et al. 1992 (In Carvalho et al., 2015)**
	Sediment	No data	
Fish ($\mu g \cdot L^{-1}$)	**Freshwater**	***Morone saxatilis* / 96h LC_{50}: 349000 (mortality) Reliability evaluation: 1-2 [a]**	**Bills et al. 1993 (In Carvalho et al., 2015)**
		Danio rerio / 96h LC_{50}: >1000000 (mortality) Reliability evaluation: 1-2 [a]	Isidori et al. 2005 (In Carvalho et al., 2015)
		***Pimephales promelas* / 96h LC_{50}: 61000 (mortality) Reliability evaluation: 1-2 [a]**	**Sanderson et al. 2003 (In Carvalho et al., 2015)**
		***Oreochromis niloticus* (juveniles) / 96h LC_{50}: 242.7 (mortality) Reliability evaluation: 2**	**El-Nahhal and El-Dahdouh (2015)**
	Marine	No data	
	Sediment	No data	

Other taxonomic groups (µg·L^{-1})	Bacteria, marine species: *Vibrio fischeri* / 30 Min. EC_{50}: >100000 Reliability evaluation: 2	Isidori et al. 2005 (In UBA Report, 2014)
	Bacteria, *Pseudomonas putida* / 16h. EC_{50}: 54498 Reliability evaluation: 2	**Alexy, 2003 (In UBA Report, 2014)**
	Bacteria, *Enterococcus faecalis* / 6h. EC_{50}: 1866 Reliability evaluation: 2	**Alexy, 2003 (In UBA Report, 2014)**
	Insects, *Culex pipiens* (larvae) / 48h LC_{50}: 60.2 (mortality) Reliability evaluation: 2	**El-Nahhal and El-Dahdouh (2015)**

a. These studies were also found in the UBA report (2014) where they were assessed and considered as reliable.
b. The study of Ando et al. (2007) was considered as reliable in Carvalho et al. (2015). Due to a contrasting reliability evaluation of this paper noted in Le Page et al. (2017), Ando et al. (2007) was re-assessed in the present dossier, and it was considered as not reliable (no chemical analysis performed, only one dose tested, no replicates used).

7.1.1 Derivation of a MAC-QS for the freshwater pelagic community (MAC-QS$_{fw, eco}$)

Due to the limited toxicity data available for marine water species (only four reliable acute toxicity data), freshwater and marine water data were combined for QS derivation without statistical analysis, according to the EQS Technical Guidance (EC, 2018).

Deterministic approach.

For the MAC-QS freshwater derivation, there is at least one short-term L(E)C_{50} from each of three trophic levels of the base set. The mode of action of erythromycin consists in the inhibition of bacterial protein synthesis primarily by binding to the 23S rRNA molecule in the 50S ribosomal subunit (Farzam et al., 2020). The target organisms are prokariotic organisms, however not only bacteria, but also cyanobacteria that have structures similar to those of Gram-negative bacteria, and the taxonomic group of algae are included as well due to the presence of prokaryotic-like ribosomes in their chloroplasts and mitochondria (Machado and Soares, 2019; Wu et al., 2020). Among different species from the acute toxicity dataset, the taxonomic group of algae and cyanobacteria appeared to be much more sensitive to erythromycin compared to the other taxonomic groups (see Figure 7.1 below), and bacteria are present in the dataset. In line with the EQS-guidance (EC, 2018), an AF of 10 was applied to the lowest (72-h) EC_{50} of 10 µg/L for the endpoint of cell population density (growth inhibition) measured for the marine algae *Tetraselmis suecica* (Sendra et al., 2018)[18] resulting in a **MAC-QS$_{fw,eco}$ of 1,0 µg/L**. The endpoint growth was calculated from the cell density of the cultures measured with a flow cytometer after 72 hours of exposure to erythromycin following OECD guidelines. Furthermore, the concentration-response curves were prepared as variation of growth in percentage (y-axis) against erythromycin concentration in mg/L (x-axis) and used to determine the EC_{50} values

Probabilistic approach

[18] In the first revision of the present draft EQS dossier in 2021, experts of the subgroup on macrolides found that the lowest bound EC_{50} of 10 µg/L sufficiently covers the LOECs of 100 µg/L. I It was suggested that the author should be contacted to get the raw experimental data with the aim of calculate the growth inhibition rate (ErC_{50}) value. However, the JRC pointed out that EC_{50} value of 10 µg/L from Sendra et al. (2018) for the algae species *Tetraselmis suecica* because the endpoint growth was calculated following OECD guidelines.

According to the EQS Technical Guidance (EC, 2018), for substances for which the specific mode of action and/or the most sensitive taxa are known, the species sensitivity distribution (SSD) approach should be performed in the following cases: a) The entire dataset (at least 10 L(E)C_{50} values from different species covering at least 8 taxonomic groups); b) Only those taxa that are expected to be particularly sensitive (at least 10 data points from the most sensitive group). According to EC (2018), the dataset for an SSD should contain preferably more than 15, but at least 10 L(E)C_{50} values, from different species covering at least 8 taxonomic groups. In the acute dataset of erythromycin the following taxa are included: (1) fish (i.e., *Pimephales promelas*); (2) a second family in the phylum Chordata (as fish species *Morone saxatilis* and *Oreochromis niloticus*); (3) a crustacean (5 species included in the dataset such as *Daphnia magna*); (4) an insect (represented by the mosquito species *Culex pipiens*); (5) a phylum other than Arthropoda or Chordata (Rotifera, *Brachionus calyciflorus*); (6) an order of insect or any phylum not already represented (the marine diatom species, *Phaeodactylum tricornutum*); (7) algae or cyanobacteria (five species of algae and three species of cyanobacteria are included); (8) higher plants (*Lemna minor*). Therefore, the SSD approach could be applied to the entire acute toxicity dataset.
The figure 7.1 illustrates the variation in sensitivity among the species of acute toxicity data of erythromycin to freshwater and marine water organisms. It is noted that most sensitive taxonomic group are algae and cyanobacteria.
As indicated above, among different species from the acute toxicity dataset, the taxonomic group of algae and cyanobacteria appeared to be much more sensitive to erythromycin compared to the other taxonomic groups (Figure 7.1). It is noted, however, that also the larvae of the crustacean species *Penaeus vannamei* and the insect larvae *Culex pipiens* showed high sensitivity to erythromycin. Calma et al. (2018) investigated possible mechanisms which could explain the disrupting effects of erythromycin on the life cycle of *Aedes aegypti*, another culicidae species. The authors discussed that erythromycin might be responsible for affecting nutrient availability, and hormonal regulation, but also for increasing resistance in commensal bacteria, which are needed for stimulation of egg eclosion in *A. aegypti*. However, the molecular mechanisms of such effect still warrant further investigations (Calma et al., 2018). No further explanations of this high sensitivity to erythromycin could be specifically found for *P. vannamei* and *Culex pipiens*.

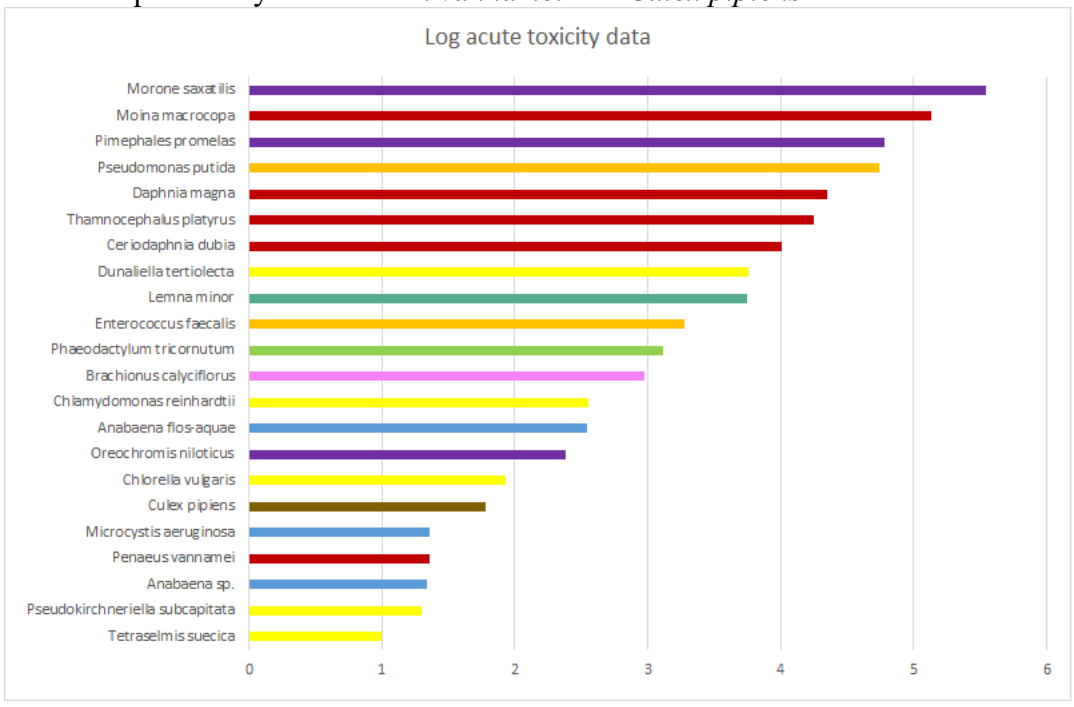


Figure 7.1. Representation of acute toxicity data of erythromycin to freshwater and marine water organisms. Acute LC_{50} or EC_{50} values are plotted on the X-axis and are shown on a log-scale.

In the present evaluation SSD based on the entire dataset was performed as well the SSD analysis with only algae, cyanobacteria, bacteria and diatoms species (hereafter cited as "algae taxa group"), which are expected to be particularly sensitive. In total, the specific SSD based on algae consisted of 11 data points. Whereas, the SSD based on the entire dataset consists of twenty-two data points (as reported in Table 7.1) and it also includes the most sensitive species groups to the mode of action of the antibiotic erythromycin.

Table 7.1. Selected acute toxicity data of erythromycin used in the probabilistic approach.

Taxonomic group	Species	Duration	Effect measured and endpoint	Value (µg/L)	Reference
Freshwater					
Algae	*Pseudokirchneriella subcapitata*	72 h	algal growth, EC_{50}	20	Isidori et al., (2005)#
Algae	*Chlorella vulgaris*	96 h	algal cell growth, EC_{50}	85.7	Wang et al. (2019)
Algae	*Chlamydomonas reinhardtii*	72 h	population growth (cellular density), EC_{50}	360	Sendra et al. (2018b)
Cyanobacteria	*Anabaena sp.*	72 h	growth inhibition of constitutive luminescence, EC_{50}	22	Gonzaléz-Pleiter et al. (2013)#
Cyanobacteria	*Anabaena flos-aquae*	72 h	growth rate, EC_{50}	348	Förster et al. (2013)#
Cyanobacteria	*Microcystis aeruginosa*	96 h	growth (inhibition rate), EC_{50}	22.97	Wu et al 2020
Bacteria	*Enterococcus faecalis*	6 h	EC_{50}	1866	Alexy 2003 In UBA, 2014
Bacteria	*Pseudomonas putida*	16 h	EC_{50}	54498	Alexy 2003 In UBA, 2014
Crustacean, Cladocera	*Ceriodaphnia dubia*	48 h	immobilisation, EC_{50}	10230	Isidori et al., (2005)#
Crustacean, Anostraca	*Thamnocephalus platyurus*	24 h	immobilisation, EC_{50}	17680	Isidori et al., (2005)#
Crustacean, Cladocera	*Daphnia magna*	24 h	immobilisation, EC_{50}	22450	Isidori et al., (2005)#
Crustacean, Diplostraca	*Moina macrocopa*	48 h	immobilisation, EC_{50}	135500	Ji et al. (2012)#
Insect, Diptera	*Culex pipiens*	48 h	mortality, LC_{50}	60.2	El-Nahhal and El-Dahdouh (2015)
Rotifer	*Brachionus calyciflorus*	48 h	mortality, EC_{50}	940	Isidori et al. (2005)#
Aquatic plant	*Lemna minor*	7 days	frond number, EC_{50}	5620	Pomati et al. (2004)#
Fish	*Oreochromis niloticus*	96 h	mortality, LC_{50}	242.7	El-Nahhal and El-Dahdouh (2015)
Fish	*Pimephales promelas*	96 h	mortality, LC_{50}	61000	Sanderson et al. (2003)#
Fish	*Morone saxatilis*	96 h	mortality, LC_{50}	349000	Bills et al. (1993)#
Marine water					
Algae	*Tetraselmis suecica*	72 h	cell population density (growth inhibition), EC_{50}	10	Sendra et al. (2018)
Algae	*Dunaliella tertiolecta*	96 h	algal growth (yield), EC_{50}	5750	Machado and Soares (2019)
Diatom	*Phaeodactylum tricornutum*	72 h	population growth (cellular density), EC_{50}	1310	Sendra et al. (2018b)
Crustacean, Decapoda	*Penaeus vannamei*	48 h	immobilisation, EC_{50}	22.7	Williams et al. (1992)#

#Cited in Carvalho et al. (2015).

In the present evaluation, species sensitivity distributions for specific algae taxonomic group (algae, cyanobacteria, diatoms and bacteria species) and for the complete acute toxicity dataset were

compared. Figure 7.2.a shows the SSD graph ((log)-normal function) of the twenty-two acute toxicity data for erythromycin, where an HC_5 value of 5.23 µg/L (95% CL 0.58– 24.23 µg/L) was obtained. The log-normal distribution (Figure 7.2.b.) based on the eleven acute toxicity data of the algae taxa group for erythromycin, gave a lower HC_5 value of 2.67 µg/L (95% CL 0.12-16.11 µg/L). A narrower confidence interval was noted for the HC_5 estimate based on algae species rather than for the HC_5 value based on the entire dataset.
No acute toxicity data among the available dataset fall below the derived HC_5 values.

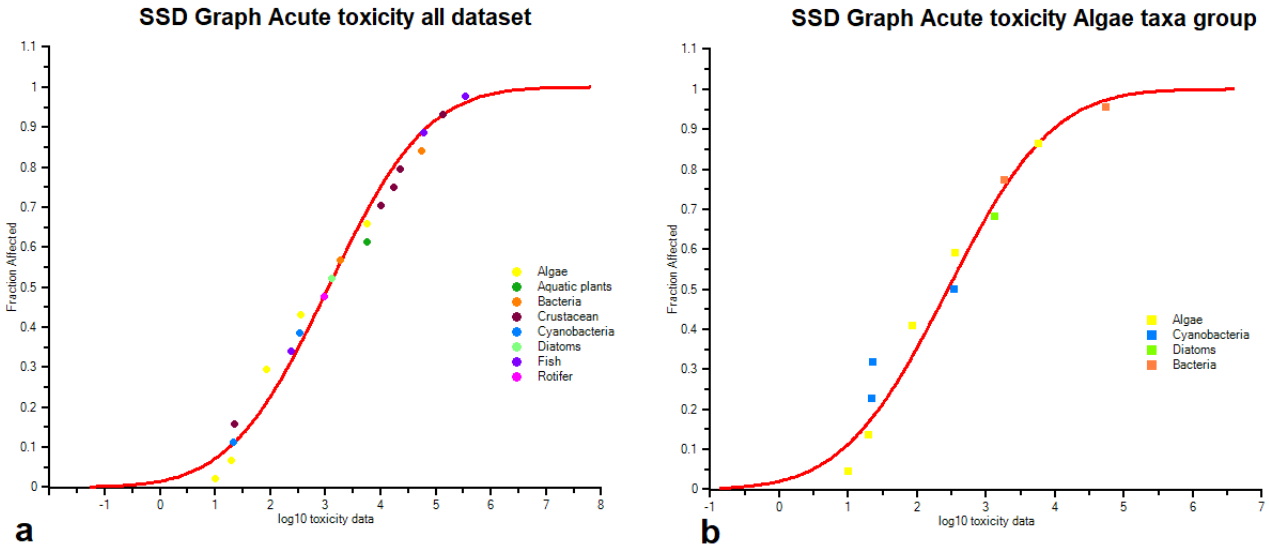


Figure 7.2. Species sensitivity distribution of the acute erythromycin toxicity data representing: a) all aquatic organisms, b) algae taxa group (software ETX 2.3 by RIVM). The red line represents the fitted (log)-normal distribution to the data (RIVM, 2004).

The statistical uncertainties around the 5th percentile estimate (i.e. the HC_5), were subsequently tested on normality using statistical criteria by Kolmogorov-Smirnov, Anderson-Darling and Cramer von Mises tests, and visual goodness-of-fit techniques. In both SSD evaluations, the normal distribution was accepted by all tests at the P= 0.1 level, and a good fitting of the curve to the data was observed, in particular for the SSD curve fitted to the entire acute toxicity dataset.
The distribution of the data was shown below in the frequency distribution histograms (Figure 7.3.a. for all aquatic organisms and 7.3.b. for algae taxa group).

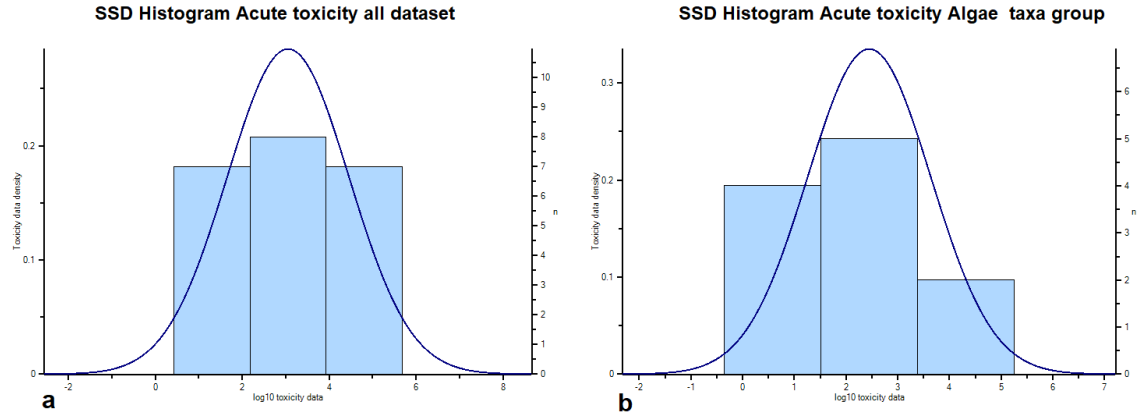


Figure 7.3. Frequency distribution of the acute erythromycin toxicity data for a) all aquatic organisms, and b) for algae taxa group (software ETX 2.3 by RIVM).

Figure 7.3a shows that aquatic organisms' toxicity data are normally distributed, with most values falling towards the centre. While, an asymmetrical distribution of the algae data was noted in the frequency distribution histogram as shown in Figure 7.3.b.
Overall, despite the larger confidence interval around the HC_5 estimate, the HC_5 value of 5.23 µg/L derived from the SSD based on the entire acute toxicity dataset was taken forward in the assessment, considering the better fitting of the curve to the data, and the symmetrical distribution of the data considered for the normal distribution.
In order to select the assessment factor (AF), field or mesocosm studies could be used to inform the size of the AF applied to an HC_5 resulted from an SSD or to QS derived using the AF method. However, no field and mesocosm studies were available on erythromycin. It should be also considered for selecting the AF that the dataset covers the most sensitive species groups in view of the mode of action of erythromycin.
According to the EQS Technical Guidance (EC, 2018), the median estimate of the **HC_5** value of **5.23 µg/L** was used as the basis of the quality standard. An AF is therefore needed to extrapolate to the MAC-$QS_{fw, eco}$. This AF should normally be 10, unless other lines of evidence or criteria suggest that a higher or lower one is appropriate. Based on the above assessment, an AF of 10 was deemed as adequate. Therefore, the HC_5 was divided by an AF 10, giving an **SSD-based MAC-$QS_{fw, eco}$** is **0.523 µg/L.**
In the first revision of the present draft EQS dossier in 2021, experts of the subgroup on macrolides found that it is not realistic at all that the SSD-based MAC-QS (0.523) is similar to the deterministic AA-QS (0.5). Because the MAC-QS represents the acute no effect level, the best option for derivation of the MAC would be to establish an SSD with acute L(E)C_{10} or NOEC values, this would deliver an HC_5 at the acute, no effect level. Comparing the acute HC_5-LC_{10} with the HC_5-LC_{50} gives insight into the AF to be applied to the latter. However, the JRC pointed out that there is no sufficient data to perform the SSD with acute L(E)C_{10} or NOEC values. Based on the uncertainties in the SSD analysis, the MAC-$QS_{fw,eco}$ of 1 µg/L based on the AF approach could be proposed as critical MAC value in the present EQS dossier.
The view of the SCHEER opinion (2022) was that, whilst there could well be enough data for the probabilistic approach, they note the JRC is of the opinion that there are not enough data. On balance, the SCHEER can agree with the Commission on a preference for the **MAC-$QS_{fw,eco}$ of 1 µg/L** based on some uncertainties in the probabilistic approach.

7.1.2 Derivation of a MAC-QS for the marine water pelagic community (MAC-$QS_{sw, eco}$)

Deterministic approach.
For the marine water MAC-QS derivation, the available dataset contains at least one short-term L(E)C_{50} from each of three trophic levels of the base set (fish, crustaceans and algae) plus two or more short-term L(E)C_{50}s from additional specific saltwater taxonomic groups, and potentially sensitive taxa are included in the dataset.
According to the EQS Technical Guidance (EC, 2018), when additional information on the sensitivity of specific saltwater taxonomic groups is available, the additional assessment factor of 10 can be lowered to 5 (one additional marine taxonomic group) or 1 (two or more additional marine taxonomic groups). Marine species from taxa other than algae, crustaceans and fish include: macrophyta, mollusca, rotifers, hydroids, annelida, and echinoderms. In addition, marine organisms that belong to the taxa algae, crustaceans or fish but have a different life form or feeding strategy

than the representatives in the freshwater toxicity dataset can be considered as additional marine taxonomic groups and may allow a reduction of the AF (EC, 2018).
In the acute toxicity dataset of erythromycin, the marine diatoms *Phaeodactylum tricornutum*, the marine crustacean *Penaeus vannamei* and the marine algae species are available in the dataset. Regarding the life form, it is noted that the marine green algae *Tetraselmis suecica* and *Dunaliella tertiolecta* are two motile flagellated algae species, such as the freshwater green algae *Chlamydomonas reinhardtii*. Therefore, the two marine green algae species could not be considered as additional marine taxonomic groups. The species *P. tricornutum* is a polymorphic and raphidic diatom that exists in three different forms depending on environmental conditions, i.e. oval, fusiform or triradiate. The oval form is preferentially benthic while the fusiform and triradiate types are more frequent as planktonic (Tesson et al., 2009). The raphe system also gives the ability to move longer and faster, relative to their body size (Kociolek et al., 2015). Based on these different characteristics from the available freshwater algae, it is believed that this diatom species could be considered as an additional marine taxonomic group (other than algae, crustaceans and fish taxa). *Penaeus vannamei* (Class Malacostraca, order Decapoda)[19] is a tropical marine crustacean with a diet based on phytoplankton and zooplankton. The larval stages of *P. vannamei* are planktonic and do not swim, so the larvae are carried towards the shore by tidal currents. Adults of *P. vannamei* are instead benthic species, and feeding is based on benthic detritus, worms, bivalves and other crustaceans[20]. The freshwater crustaceans *Thamnocephalus platyurus* (Class Branchiopoda, Order Anostraca) and *Ceriodaphnia dubia*, *Daphnia magna* and *Moina macrocopa* (Class Branchiopoda, order Diplostraca, suborder Cladocera)[7] larvae are swimmers and are mostly pelagic[21]. Those crustaceans are suspension filter feeders and their diet are based on bacteria, detritus and algae[22,23]. However, as it was pointed out by experts of the subgroup on antibiotics during the revision of erythromycin's dossier in 2021, these morphological differences were not considered sufficient to consider these species as additional taxonomic groups. Therefore, the crustacean marine *P. vannamei* and the marine diatoms *Phaeodactylum tricornutum* cannot be accepted as an additional marine taxonomic groups, thus justifying the selection of an additional AF of 10 to the minimum AF of 10. Therefore, the application of an AF of 100 to the lowest EC_{50} (72h) of 10 µg/L for the endpoint of cell population density (growth inhibition) in the marine algae species *Tetraselmis suecica* (Sendra et al., 2018) resulted resulting in **MAC-$QS_{sw,eco}$ of 0.1 µg/L.**

Probabilistic approach
For the **marine water MAC-EQS derivation**, when the datasets for freshwater and saltwater are combined, an additional AF of 10 is used on top of the default of 10 to deal with residual uncertainty, resulting in a total AF of 100 (EC, 2018). However, when one typically marine taxonomic group is present in the dataset, this additional AF can be reduced to 5, and when two typically marine taxonomic groups are present, no additional assessment factor is necessary (EC,

[19] Retrieved [April, 2021], from the Integrated Taxonomic Information System (ITIS) on-line database, www.itis.gov. https://doi.org/10.5066/F7KH0KBKttps://www.itis.gov/
[20] FAO 2010-2021. Fisheries and Aquaculture Department-Fisheries Division (NFI). In: *FAO Fisheries Division* [online]. Rome. Updated. [Cited April 2021]. Available online at: http://www.fao.org/fishery/culturedspecies/Penaeus_vannamei/en#tcNA0078
[21] Ebert D. Ecology, Epidemiology, and Evolution of Parasitism in Daphnia [Internet]. Bethesda (MD): National Center for Biotechnology Information (US); 2005. Chapter 2, Introduction to Daphnia Biology. Available from: https://www.ncbi.nlm.nih.gov/books/NBK2042/ (accessed on April, 2021).
[22] Wyoming Game & Fish Department Available from: https://wgfd.wyo.gov/WGFD/media/content/PDF/Habitat/SWAP/Crustaceans/Beavertail-Fairly-Shrimp.pdf (accessed on April, 2021).
[23] FAO FISHERIES TECHNICAL PAPER 361, Manual on the Production and Use of Live Food for Aquaculture: Manual on the Production and Use of Live Food for Aquaculture. Retrieved on-line from: http://www.fao.org/3/w3732e/w3732e0x.htm (accessed on April, 2021).

2018). According to the EQS Technical Guidance (EC, 2018), marine toxicity test data can be accepted as additional marine taxonomic groups if they belong to taxonomic groups other than algae, crustaceans and fish, and/or having a different life form or feeding strategy.

As explained above, based on comments of the experts' subgroup on erythromycin, the marine crustacean *Penaeus vannamei,* the marine diatom *Phaeodactylum tricornutum,* and the marine algae species *Tetraselmis suecica* and *Dunaliella tertiolecta*, were not considered as additional marine taxonomic groups, and thus an additional AF of 10 should be applied. Therefore, the application of an AF of 100 to the HC_5 value of 5.23 µg/L (calculated in the section 7.1.1.) resulted in a **MAC-$QS_{sw,eco}$** derivation is **0.0523 µg/L.**
As argued in the derivation of MAC-QS freshwater, and in accordance with the SCHEER opinion (2022), the JRC proposed a preference for the MAC-$QS_{sw,eco}$ of 0.1 µg/L derived by deterministic approach based on some uncertainties in the probabilistic approach.

7.2 Chronic aquatic ecotoxicity

The key chronic ecotoxicity data of erythromycin for freshwater and marine water organisms are reported in the table below.

CHRONIC EFFECTS			**Master reference**
Algae & aquatic plants ($\mu g \cdot L^{-1}$)	**Freshwater**	**Algae, *Pseudokirchneriella subcapitata* / 72h** **NOEC: 10.3 (biomass)** **Reliability evaluation: 1-2** [a]	**Eguchi et al. 2004 (In Carvalho et al., 2015)**
		Algae, *Pseudokirchneriella subcapitata* / 72h EC_{10}: 36 (growth: chlorophyll fluorescence) Reliability evaluation: 1-2 [a]	Gonzaléz-Pleiter et al. 2013 (In Carvalho et al., 2015)
		Algae, Pseudokirchneriella subcapitata / 72h EC_{10}: 5 (yield) Reliability evaluation: 1	Machado and Soares (2019)
		Algae, *Chlorella vulgaris* / 72h **NOEC: 12500 (biomass)** **Reliability evaluation: 1-2** [a]	**Eguchi et al. 2004 (In Carvalho et al., 2015)**
		Cyanobacteria, *Synechococcus leopoldensis* (strain IAM-M6) / 144h NOEC: 2 (biomass) Reliability evaluation: 3-4 [b]	Ando et al. 2007 (In Carvalho et al., 2015)
		Cyanobacteria, *Microcystis wesenbergii* (strain NIES-107) / 144h NOEC: 4.7 (biomass) Reliability evaluation: 3-4 [b]	Ando et al. 2007 (In Carvalho et al., 2015)
		Cyanobacteria, *Anabaena variabilis* (strain NIES-23) / 144h NOEC: 47 (biomass) Reliability evaluation: 3-4 [b]	Ando et al. 2007 (In Carvalho et al., 2015)
		Cyanobacteria, *Nostoc* sp. (strain PCC 7120) / 144h NOEC: 100 (biomass) Reliability evaluation: 3-4 [b]	Ando et al. 2007 (In Carvalho et al., 2015)
		Cyanobacteria, *Synechococcus* sp. (strain PCC 7002) / 144h	Ando et al. 2007 (In Carvalho et al., 2015)

		NOEC: 7.8 (biomass) Reliability evaluation: 3-4 [b]	
		Cyanobacteria, *Microcystis aeruginosa* (strain NIES-44) / 144h NOEC: 10 (biomass) Reliability evaluation: 3-4 [b]	Ando et al. 2007 (In Carvalho et al., 2015)
		Cyanobacteria, *Anabaena flos-aquae* (strain ATCC 29413) / 144h NOEC: 47 (biomass) Reliability evaluation: 3-4 [b]	Ando et al. 2007 (In Carvalho et al., 2015)
		Cyanobacteria, *Anabaena cylindrica* (strain NIES-19) / 144h NOEC: 3.1 (biomass) Reliability evaluation: 3-4 [b]	Ando et al. 2007 (In Carvalho et al., 2015)
		Cyanobacteria, *Anabaena flos-aquae* / 72h NOEC: 30 (yield, growth rate) Reliability evaluation: 1-2 [a]	**Förster et al. 2013 (In Carvalho et al., 2015)**
		Cyanobacteria, *Anabaena flos-aquae* / 72h LOEC: 90 (yield, growth rate) Reliability evaluation: 1-2 [a]	Förster et al. 2013 (In Carvalho et al., 2015)
		Cyanobacteria, *Anabaena* sp. / 72h EC_{10}: 5 (growth: inhibition of constitutive luminescence) Reliability evaluation: 1-2 [a]	**Gonzaléz-Pleiter et al. 2013 (In Carvalho et al., 2015)**
		Cyanobacteria, *Synechocystis* sp. / 5 days NOEC: 10 (growth) Reliability evaluation: 1-2 [a]	**Pomati et al. 2004 (In Carvalho et al., 2015)**
		Aquatic plant, *Lemna minor* / 7days NOEC: 10 (frond number) Reliability evaluation: 1-2 [a]	**Pomati et al. 2004 (In Carvalho et al., 2015)**
	Marine	**Algae, *Dunaliella tertiolecta* / 96 h EC_{10}: 1880 (yield) Reliability evaluation: 1**	**Machado and Soares (2019)**
Invertebrates ($\mu g \cdot L^{-1}$)	**Freshwater**	Crustacean, *Ceriodaphnia dubia* / 7 days EC_{50}: 220 (population growth) Reliability evaluation: 1-2 [a]	Isidori et al. 2005 (In Carvalho et al., 2015)
		Crustacean, *Moina macrocopa* / 7 days NOEC: 50000 (survival reproduction)	**Ji et al. 2012 (In Carvalho et al., 2015)**

		Reliability evaluation: 1-2 [a]	
		Crustacean, *Daphnia magna* / 21 days **NOEC: 248 (reproduction)** **Reliability evaluation: 1-2 [a]**	**Meinertz et al. 2010 (In Carvalho et al., 2015)**
		Crustacean, *Daphnia magna* / 21 days NOEC: 33300 (survival) Reliability evaluation: 1-2 [a]	Ji et al. 2012 (In Carvalho et al., 2015)
		Crustacean, *Daphnia magna* / 21 days NOEC: 11100 (reproduction growth) Reliability evaluation: 1-2 [a]	Ji et al. 2012 (In Carvalho et al., 2015)
	Marine	No data	
Fish ($\mu g \cdot L^{-1}$)	**Freshwater**	***Oryzias latipes* / 100 days** **NOEC: 10000 (adult survival growth)** **Reliability evaluation: 1-2 [a]**	**Ji et al. 2012 (In Carvalho et al., 2015)**
		Oryzias latipes / 10 days NOEC: 1000000 (hatchability, time to hatch) Reliability evaluation: 1-2 [a]	Ji et al. 2012 (In Carvalho et al., 2015)
		Oryzias latipes / 40 days NOEC: 100000 (juvenile survival) Reliability evaluation: 1-2 [a]	Ji et al. 2012 (In Carvalho et al., 2015)
		Oryzias latipes / 40 days NOEC: 1000000 (juvenile growth) Reliability evaluation: 1-2 [a]	Ji et al. 2012 (In Carvalho et al., 2015)
	Marine	No data	
Other taxonomic groups ($\mu g \cdot L^{-1}$)		**Bacteria, *Pseudomonas putida* / 16h.** **EC_{10}: 10718** **Reliability evaluation: 2**	**Alexy, 2003 (In UBA, 2014)**
		Bacteria, *Enterococcus faecalis* / 6h. **EC_{10}: 562** **Reliability evaluation: 2**	**Alexy, 2003 (In UBA, 2014)**

a. These studies references were also found in the NORMAN (2014) and in the UBA report (2014) where the reliability of the studies have been assessed and considered reliable.
b. The study of Ando et al. (2007) was considered as reliable in Carvalho et al. (2015). Due to a contrasting reliability evaluation of this paper noted in Le Page et al. (2017), Ando et al. (2007) was re-assessed in the present dossier, and it was considered as not reliable (no chemical analysis performed, only one dose tested, no replicates used).

7.2.1 Derivation of AA-QS for the freshwater pelagic community ($AA\text{-}QS_{fw, eco}$)

Deterministic approach

Chronic ecotoxicity data are available for at least three species (normally fish, aquatic invertebrates and algae) representing three trophic levels. Therefore, an AF of 10 (Table 3 in EC, 2018) can be

applied to the lowest EC_{10} (72 h) of 5 µg/L for the endpoint of growth in the Cyanobacteria species *Anabaena sp.* (Gonzaléz-Pleiter et al., 2013), resulting in an **AA-$QS_{fw,eco}$ of 0.5 µg/L**.

Probabilistic approach

According to the EQS Technical Guidance (EC, 2018), for substances for which the specific mode of action and/or the most sensitive taxa are known, the species sensitivity distribution (SSD) approach should be performed in the following cases: a) The entire dataset (at least 10 chronic values from different species covering at least 8 taxonomic groups); b) Only those taxa that are expected to be particularly sensitive (at least 10 data points from the most sensitive group).
As mentioned in the section 7.1.1., algae, cyanobacteria, diatoms and bacteria species could be consider as sensitive group to erythromycin. According EC (2018) the HC_5 may be estimated using only data from the most sensitive group, provided that the minimum number of 10 data points is present. In the present assessment, no sufficient reliable data from the most sensitive group (algae, cyanobacteria and bacteria) was found to be available in the chronic dataset of erythromycin (only eight data points), therefore the probabilistic approach could not be performed for the AA-QS derivation.
According to EQS TGD (EC, 2018), for the entire the dataset, the SSD should contain preferably more than 15, but at least 10 L(E)C_{50} values, from different species covering at least 8 major taxonomic groups. For estimating a $QS_{fw, eco}$, the following taxa would normally need to be represented: (1) fish (*Oryzias latipes*); (2) a second family in the phylum Chordata (not included; there is only 1 species of fish); (3) a crustacean (2 species included in the dataset such as *Daphnia magna*); (4) an insect (not included); (5) a phylum other than Arthropoda or Chordata (not included); (6) an order of insect or any phylum not already represented (2 species of bacteria); (7) algae or cyanobacteria (there are 3 algae and 3 cyanobacteria species); (8) higher plants (aquatic plant *Lemna minor*). In the combined chronic dataset of erythromycin insects, additional fish species and a phylum other than Arthropoda or Chordata are not represented. Therefore, no species sensitivity distribution could be derived for the chronic ecotoxicity dataset based on erythromycin due to the insufficient data points and taxonomic groups available to construct a specific and a general SSD, respectively.

7.2.2 Derivation of AA-QS for the marine water pelagic community (AA-$QS_{sw, eco}$)

Deterministic approach

Long-term results from three freshwater species representing three trophic levels (Table 4 in EC, 2018) are available in the chronic ecotoxicity dataset, but no ecotoxicity data are available on specific marine species. Therefore, an AF of 100 was chosen. The selected AF applied to the lowest EC_{10} (72 h) of 5 µg/L for the endpoint of growth measured for the Cyanobacteria *Anabaena sp.* (Gonzaléz-Pleiter et al., 2013), resulted in an **AA-$QS_{sw,eco}$ of 0.05 µg/L**.

Probabilistic approach

As mentioned for the section above, the probabilistic approach could not be performed for the AA-QS derivation due to the few data available in the chronic toxicity dataset.

7.3 Sediment ecotoxicity

Based on the experimental and estimated Koc values (section 5.1.), erythromycin is expected to adsorb to suspended solids and sediment. Hence, the sediment toxicity assessment should be performed. No sediment toxicity data are available for erythromycin. Therefore, the Equilibrium Partitioning (EqP) method can be used to estimate the $QS_{sediment}$ (EC, 2018), based on the following equations and input data (Table 7.2).

According to the EQS Technical Guidance (EC, 2018), experimentally determined values for Koc are preferable. Therefore, the Koc of 1877 L/kg (Barron et al. 2009 cited in UBA, 2014) was selected for the derivation.

$$Kp_{sed} = Foc_{sed} \cdot K_{oc} \qquad \text{Equation 1}$$

$$K_{air-water} = \frac{H}{R \cdot TEMP} \qquad \text{Equation 2}$$

$$K_{sed-water} = Fair_{sed} \cdot K_{air-water} + Fwater_{sed} + Fsolid_{sed} \cdot \frac{Kp_{sed}}{1000} \cdot RHO_{solid} \qquad \text{Equation 3}$$

$$QS_{sediment,EqP,ww} = \frac{K_{sed-water}}{RHO_{sed}} \cdot QS_{fw,eco} \cdot 1000 \qquad \text{Equation 4}$$

$$CONV_{sed} = \frac{RHO_{sed}}{Fsolid_{sed} \cdot RHO_{solid}} \qquad \text{Equation 5}$$

$$QS_{sediment,EqP,dw} = CONV_{sed} \cdot QS_{sediment,EqP,ww} \qquad \text{Equation 6}$$

Table 7.2. List of input and estimated parameters used in the EqP method for calculation of the QS for sediment.

Parameter	Description	Value	Source
K_{oc}	partition coefficient between organic carbon and water	1877 $L \cdot kg^{-1}$	Barron et al. 2009 (In UBA, 2014) (see section 5.1.)
Foc_{sed}	weight fraction of organic carbon in sediment	0.05 $kg \cdot kg^{-1}$	Default value (EC, 2018)
Kp_{sed}	partition coefficient solid-water in sediment	28.5 $L \cdot kg^{-1}$	Equation 1
H	Henry's law constant	5.49E-23 $Pa \cdot m^3 \cdot mol^{-1}$	Oekotoxzentrum (2015)
R	gas constant	8.314 $Pa \cdot m^3 mol^{-1} \cdot K^{-1}$	Default value (EC, 2018)
TEMP	environmental temperature	285 K	Default value (EC, 2018)

Parameter	Description	Value	Source
$K_{air\text{-}water}$	air-water partition coefficient	2.31695E-26 $m^3 \cdot m^{-3}$	Equation 2
$Fair_{sed}$	fraction air in sediment	0 $m^3 \cdot m^{-3}$	Default value (EC, 2018)
$Fwater_{sed}$	fraction water in sediment	0.8 $m^3 \cdot m^{-3}$	Default value (EC, 2018)
$Fsolid_{sed}$	fraction solids in sediment	0.2	Default value (EC, 2018)
RHO_{solid}	density of the solid phase	2500 $kg_{solid} \cdot m_{solid}^{-3}$	Default value (EC, 2018)
$K_{sed\text{-}water}$	partition coefficient between sediment and water	15.05 $m^3 \cdot m^{-3}$	Equation 3
FRESHWATER			
$QS_{fw,eco}$	quality standard for direct ecotoxicity on freshwater aquatic organisms	5E-04$mg \cdot L^{-1}$	In this dossier (see section 7.2.1)
$QS_{sed,EqPww}$	wet weight quality standard for sediment based on equilibrium partitioning	0.002315385 $mg \cdot kg_{ww}^{-1}$	Equation 4
*RHO*sed	bulk density of wet sediment	1300 $kg_{ww} \cdot m^{-3}$	Default value (EC, 2018)
$CONV_{sed}$	conversion factor for sediment concentration wet-dry weight sediment	2.6 $kg_{ww} \cdot kg_{dw}^{-1}$	Equation 5
$QS_{sedEqp,dw}$	dry weight quality standard for sediment based on equilibrium partitioning	0.00602 $mg \cdot kg_{dw}^{-1}$	Equation 6
MARINE WATER			
$QS_{sw,eco}$	quality standard for direct ecotoxicity on marine aquatic organisms	5E-05 $mg \cdot L^{-1}$	In this dossier (see section 7.2.2.)
$QS_{sed,EqPww}$	wet weight quality standard for sediment based on equilibrium partitioning	0.000231538 $mg \cdot kg_{ww}^{-1}$	Equation 4
*RHO*sed	bulk density of wet sediment	1300 $kg_{ww} \cdot m^{-3}$	Default value (EC, 2018)
$CONV_{sed}$	conversion factor for sediment concentration wet-dry weight sediment	2.6 $kg_{ww} \cdot kg_{dw}^{-1}$	Equation 5
$QS_{sedEqp,dw}$	dry weight quality standard for sediment based on equilibrium partitioning	0.000602 $mg \cdot kg_{dw}^{-1}$	Equation 6

The derived standard QS for sediment resulted in a **$QS_{sedEqPdw}$ freshwater of 47.7 µg/kg_{dw}** and **$QS_{sedEqPdw}$ saltwater of 4.77 µg/kg_{dw}**.
Based on the Log Kow values, erythromycin was not considered as a highly lipophilic substance, and therefore the additional AF of 10 was not applied to the $QS_{sediment}$ (EC, 2018).

7.4 Tentative QS_{water}

The following table shows the tentative QS_{water} calculated for erythromycin in the current dossier.

Table 7.3. Tentative QS for erythromycin.

Tentative QS_{water}	Relevant study for derivation of QS	Assessment factor	Tentative QS
$MAC_{freshwater, eco}$	**AF approach, *Tetraselmis suecica* /72 h EC_{50}: 10 µg/L (cell population density, growth inhibition)**	**10**	**1 µg·L^{-1}**
	SSD approach, 22 acute toxicity data HC_5: 5.23 (95% CL 0.58– 24.23) µg/L	10	0.523 µg·L^{-1}
$MAC_{marine\ water, eco}$	**AF approach, *Tetraselmis suecica* /72 h EC_{50} : 10 µg/L (cell population density, growth inhibition)**	**100**	**0.1 µg·L^{-1}**
	SSD approach, 22 acute toxicity data HC_5: 5.23 (95% CL 0.58– 24.23) µg/L	100	0.0523 µg·L^{-1}
$AA\text{-}QS_{freshwater, eco}$	***Anabaena* sp. / 72 h EC_{10}: 5 µg/L (growth)**	**10**	**0.5 µg·L^{-1}**
$AA\text{-}QS_{marine\ water, eco}$		**100**	**0.05 µg·L^{-1}**
$AA\text{-}QS_{freshwater, sed}$	**EqP**	**-**	**47.7 µg·kg_{dw}^{-1}**
$AA\text{-}QS_{marine\ water, sed}$	**EqP**	**-**	**4.77 µg·kg_{dw}^{-1}**

7.5 Secondary poisoning

According to the EQS Technical Guidance (EC, 2018), the biota standard to protect wildlife from secondary poisoning ($QS_{biota, sec pois, fw}$) should be derived when there is evidence of bioaccumulation potential of the substance.

The potential for bioaccumulation of erythromycin is indicated by an experimental value LogKow of 3.06 (US EPA, 2012a), that slightly exceeds the trigger value of 3, and by a field-derived BAF-value for freshwater fish of 4492 L/kg (Gao et al., 2012) (see table 7.2). Therefore, the criteria triggering an assessment for secondary poisoning are met.

The available toxicity data for mammals are presented in the table below.

Secondary poisoning of top predators		**Master reference**
Mammalian oral toxicity ($mg \cdot kg^{-1}_{bw} \cdot d^{-1}$)	Rat / Oral / acute / mortality LD_{50} : 9272	Pfizer, 2007
	Mouse / Oral / acute / mortality LD_{50} : 2929	Pfizer, 2007
	Rat / intraperitoneal / acute / mortality LD_{50} : 374	EMA (2000a,b)
	Guinea pig / intraperitoneal / acute / mortality LD_{50} : 413	EMA (2000a,b)
	Rat / Oral / acute / mortality LD_{50} : 4600	Muñoz et al 2010 (In UBA, 2014)
	Various laboratory animals (mice, rats, hamsters, guinea pigs, rabbits and dogs)/ oral / acute LD_{50}: >300	EMA (2000a)
	Rat / Oral / reproduction and development / Teratogenic, Embryo, Fetal Development LOAEL : 6000	Pfizer, 2007
	Mouse / Oral / reproduction and development / Teratogenic, Embryo, Fetal Development LOAEL : 12000	Pfizer, 2007
	Rat / Oral diet (in gum arabic) / reproduction / Teratogenicity / No teratogen effects NOAEL : ≥ 2000	EMA (2000b)
	Rat / Oral / chronic/ repeated dose/ 13 weeks / no compound related adverse effects were reported NOAEL : ≥ 370	EMA (2000b)
	Rabbit/ Oral / chronic / repeated dose / 31 days / (salt not stated) / no compound related adverse effects were reported **NOAEL : ≥ 200**	EMA (2000b)
	Dog / Oral / chronic / repeated dose / 3 months / no compound related adverse effects were reported NOAEL : ≥ 100	EMA (2000b)
	Monkey / Oral / chronic / repeated dose / 64	EMA (2000b)

	days / (salt not stated) / no compound related adverse effects were reported NOAEL : ≥ 75	
	Mouse / Oral / carcinogenic study / 2 years / erythromycin stearate / no carcinogenic effects NOAEL : ≥ 1400 mg/kg bw	EMA (2000b)
	Rat / Oral / carcinogenic study / 2 years / erythromycin ethylsuccinate / no carcinogenic effects NOAEL : ≥ 500 mg/kg bw	EMA (2000b)
Avian oral toxicity	Not available	

Note: An assessment of the studies is not possible as original publications and dossiers are not available.

The lowest toxicity value in the dataset was the NOAEL value of 75 $mg \cdot kg^{-1}_{bw} \cdot d^{-1}$ for monkey following an exposure duration of 64 days (EMA, 2000b), but the erythromycin salt tested was not stated. Given this lack of information, it was preferred to choose the lowest value that follows in the data set. Therefore, the chronic toxicity NOAEL value of 100 $mg \cdot kg^{-1}bw \cdot d^{-1}$ for dog following a repeated dose exposure with a longer exposure duration of 3 months could be selected as critical study. However, in the first revision of the draft EQS dossier on the macrolide erythromycin in 2021, experts of the subgroup found that the subacute study with rabbits, with an assessment factor of 100, will end up below the study with dogs, and should therefore be selected as critical endpoint. Thus, the NOAEL value of 200 $mg \cdot kg^{-1}_{bw} \cdot d^{-1}$ was selected for calculation of the QS_{biota}. Lastly, it should be noted that no compound related adverse health effects were found neither in the repeated dose toxicity study initially considered as key study, nor in the additional studies investigating repeated dose toxicity, reproduction and developmental toxicity, and carcinogenicity. The available BAF values are listed in Table 7.5.

Table 7.5. BAF values reported for erythromycin.

Species	**BAF [L/kg]**	**Exposure**	**Further information**	**Reference**
Phytoplankton (mainly *Chlorophyta, Bacillariophyta* and *Cyanophyta*)	8.7 dry weight	Field Concentration of erythromycin in water: Min: n.d. Max: 10.4 ng/L Mean: 3.58 ng/L Median: 3.89 ng/L	Taihu Lake, China. BAF whole body. The sampling campaign was performed in May 2013	Xie Z. et al., 2015
Zooplankton (mainly *Copepoda, Cladocera,* and Rotifers)	162 dry weight			
Mussel (*Anodonta*)	n.d.			
Snail (*Bellamya* sp.),	4.4 dry weight			
Bivalve (*Corbiculidae*)	32 dry weight			
Common carp (*Cyprinus carpio*)	32 dry weight			
Lake anchovy (*Coilia ectenes*)	3.8 dry weight			

Species	BAF [L/kg]	Exposure	Further information	Reference
Crucian carp (*Carassius auratus*)	32 dry weight			
White shrimp (*Exopalaemon modestus*)	n.d.			
Yellow catfish (*Pelteobagrus fulvidraco*)	103 dry weight			
Zoobenthos [mussel (*Anodonta*), snail (*Bellamya* sp.) and bivalve (*Corbiculidae*)]	447 (7.8 -3511) wet weight	Field Concentration of erythromycin in water: Min: n.d. Max: 15 ng/L Median: 1.5 ng/L Frequency 78%	Taihu Lake, China. BAF whole body. The sampling campaign was performed in December 2014.	Xie Z. et al., 2017
Fish species [silver carp (*Hypophtha lmichthys molitrix*), common carp (*Cyprinus carpio*), crucian carp (*Carassius auratus*), lake anchovy (*Coilia ectenes*), whitebait (*Reganisalanx brachyrostralis*), redfin culter (*Cultrichthys erythropterus*) and yellow catfish (*Pelteobagrus fulvidraco*)]	Muscle 442 (320-967); gills 226 (35-1341); brain 363 (49-1696); liver 728 (155-2029) [wet weight]			
Fish, bluntsnout bream (*Megalobrama amblycephala*)	44.8 (n=1)	Field Average concentration of erythromycin in water 19.2 ng/L BAF [dry weight]	Baiyangdian Lake. Sampling was performed twice in August 2008 and October 2010	Li et al., 2012
Fish, crucian carp (*Carassius auratus*)	29.4 (n=7)			
Fish, common carp (*Cyprinus carpio*)	34.1 (n=1)			
Fish, silver carp (*Hypophthalmichthys molitrix*)	34.5 (n=2)			
Fish, yellow catfish *(Pelteobagrus fluvidraco*)	43.9 (n=1)			
Fish, parva loach (*Misgurnus anguillicaudatus*)	43.8 (n=3)			
Fish, *topmouth gudgeon (Pseudorasbora parva)*	25.8 (n=2)			
Crustacean, lobster (*Palinuridae*)	54.8 (n=1)			
Crustacean, crab (*Eriocheir sinensis*)	11.5 (n=2)			
Reptile, turtle (*Pelodiscus sinensis*)	54.3 (n=1)			

Species	BAF [L/kg]	Exposure	Further information	Reference
Crustacean, shrimp (*Macrobrachium nipponense*)	17.1 (n=2)			
Fish *Carassius auratus*	4492 (n=4) dry weight	Field Concentration of erythromycin in water (Haihe River, mainstream): Min: 3.1 ng/L Max: 10.3 ng/L Mean: 6.5 ng/L Median: 5.6 ng/L	Haihe River, China. BAF whole body. The sampling campaign was performed in September 2010	Gao et al., 2012
Mussels (ribbed horsemussel, *Geukensia demissa*)	11- 54 (mean 40 ± SD 20) wet weight	Field-based Concentration of erythromycin in water: Min: 1.0 ng/L Max: 12.1 ng/L Median: 2.4 ng/L	USA, San Francisco Bay The sampling campaign was performed in December of 2009 and January 2010 (five nearshore sites)	Klosterhaus et al., 2013

n.d.: not detected.

For the derivation of the $QS_{biota,\ sec\ pois,\ fw}$, the Method A of the EQS Technical Guidance was followed (EC, 2018) to the selected toxicological endpoint NOAEL value of 200 mg/kg bw/d in rabbits.

For normalisation of the erythromycin concentration in food to energy content with method A, the daily energy expenditure (DEE; kJ/d) can be estimated with equation 7 assuming a conservative low body weight of 2 kg (2000 g) for rabbits.

$$\log DEE\left[\frac{KJ}{d}\right] = 0.8136 + 0.7149 \cdot \log bw[g] \qquad \textbf{Equation 7}$$

The diet concentration on an energy basis (mg/kJ) for erythromycin can now be calculated with toxicological endpoint expressed as daily dose (200 mg/kg bw/d) and the body weight (bw; 2 kg), using equation 8.

$$C_{energy\ normalised}\ [\mathrm{mg/kJ}] = dose \cdot \frac{bw}{DEE} \qquad \textbf{Equation 8}$$

This results in an energy content normalised concentration of erythromycin of **0.268 mg/kJ**.
To derive risk limits for secondary poisoning, the energy normalised should be converted into threshold concentrations in the prey that is considered as the critical food item in the food chain. However, as it was pointed out by experts of the subgroup on macrolides during the revision of the dossier on erythromycin in 2021, it seems more appropriate to derive the $QS_{sec\ pois}$ for both the QS_{biota} for fish and mussels.

In order to convert the derived endpoint to the concentration in the critical food item, the following formula is used:

$$C_{food\ item}\ [\mathrm{mg/kg_{ww}}] = C_{energy\ normalised}\ [\mathrm{mg/kJ}] \cdot Energycontent_{fooditem,dw} \cdot (1 - moisturefraction_{fooditem})$$ Eq. 9

The standard moisture content and energy content of fish are 74% and 21 kJ/g_{dw}, respectively (see Table 7 in EC, 2018). Based on equation 9, the concentration in the critical food item is determined to be **1465 mg/kg_{ww}** (fish). For invertebrates (bivalves) the standard moisture is 92% and energy content 19kJ/g_{dw} (Table 7 in EC, 2018), therefore the $C_{food\ item}$ for fish resulted in **408 mg/kg_{ww}** (bivalves)**.**

To extrapolate to the required protection level of the ecosystem, the $QS_{biota,\ secpois}$ will be derived by applying an assessment factor of 100 to the lowest value selected (AF 10 from Table 9, NOEC subacute study for mammals; and AF 10 from Table 10, lowest chronic value, in EC, 2018).

$$QS_{biota,sec\,pois,fw}\ [\mathrm{mg/Kg}] = \frac{Lowest\ chronic\ value}{AF}$$ Equation 10

The application of an AF of 100 to the lowest credible chronic datum resulted in a **$QS_{Biota,\ sec\ pois,\ fw}$ in fish** of **14.7 mg/kg_{ww}** and **4.1 mg/kg_{ww} for bivalves**

The biota standard should be converted into a water column concentration standard for comparison with other water column standards. Assuming a steady state distribution between water and organism, the water standard $QS_{water,\ biota}$ can be calculated from the selected BAF value, as follows:

$$QS_{water,biota}\ [\mu\mathrm{g/L}] = \frac{QS_{biota}\ [\mu\mathrm{g/Kg}]}{BAF[L/Kg]}$$ Equation 11

Considering the biota standard of 14.6 mg/kg_{ww} for fish, the corresponding water standard ($QS_{water,\ biota}$) with the selected BAF value for fish of 4492 L/kg_{dw} (equal to 1168 L/kg_{ww}) (Gao et al., 2012) was calculated to be 0.0125 mg/L. However, in the first revision of the draft EQS dossier on the macrolide erythromycin in 2021, experts of the subgroup assessed the BAF value of 4492 L/kg_{dw} as not reliable, due to uncertainties in the study of Gao et al., 2012. In more details, experts pointed out that the calculation of the BAF value was unclear, erythromycin was detected only in 4 samples of fish, only data from one sampling site was used, and water samples were only collected in one year, but over a spatial scale of more than 100 km. As a conclusion, this BAF value for fish from the study by Gao et al. (2012) was not used for the water standard derivation. Also the study of Xie et al. (2015) was considered as not assignable, since the sampling sites in Lake Taihu, which is one of the largest freshwater lakes in China, had a quite distant emplacements, so that biota and water samples might not originate from the same location. The follow-up study Xie et al. (2017) had similar issues as Xie et al. (2015). Therefore, no reliable BAF values were available to derive the $QS_{water,biota}$ for fish.
For bivalves, the water standard calculated with the BAF value for mussels of 40 L/kg_{ww} from the study Klosterhaus et al. (2013) resulted in a **$QS_{water,\ biota}$ of 0.10 mg/L (bivalves).**

For the **marine environment**, an additional step is required considering that the marine food chain also includes top predators eating fish-eating birds and mammals. According to the EQS Technical

Guidance (EC, 2018), if the marine water TMF (lipid) is below 0.8, the risk limit should be calculated for bivalves.
A TMF > 1 indicates biomagnification through the food web or from prey-to-predator; otherwise, trophic dilution is suggested. Zhang et al. (2020) provided a TMF of 0.02 for dehydrated erythromycin in the coral reef fishes from the South China Sea. Authors observed that log-transformed wet-weight-based concentrations of dehydrated erythromycin in both offshore and coastal fishes were decreased significantly with increasing trophic levels ($p < 0.05$). These results demonstrated that dehydrated erythromycin undergoes trophic dilution in the food web of coral reef fishes (Zhang et al., 2020).
As suggested during the revision of the draft dossier by the expert's subgroup, a separate $QS_{biota,sec\ pois}$ for marine water is probably not necessary as erythromycin does likely not biomagnify in small birds or mammals, and no data are available to perform calculations. Therefore, the same $QS_{sec\ pois}$ values derived for freshwater were proposed for marine water **$QS_{biota,sec\ pois,sw}$** of **4.1 mg/kg_{ww} for bivalves** and **14.7 mg/kg_{ww} for fish**.
For the back calculation to water, using the BAF value of 40 L/Kg for marine mussels (Klosterhaus et al., 2013) the **$QS_{water,\ biota}$ for bivalves** resulted to be **0.10 mg/L**.

Tentative QS_{biota}	**Relevant study for derivation of QS**	**Assessment Factor**	**Tentative QS**
Biota	Rabbit/ Oral / chronic / repeated dose / 31 days **NOAEL : ≥ 200 mg.kg^{-1}_{bw}.d^{-1}**	AF = 100	**Freshwater:** **14.7** mg.$kg^{-1}_{biota\ ww}$ (for fish) **4.1** mg.$kg^{-1}_{biota\ ww}$ (for bivalves) corresponding in water to **0.10** mg.L^{-1} (bivalves) **Marine water:** **14.7** mg.$kg^{-1}_{biota\ ww}$ (for fish) **4.1** mg.$kg^{-1}_{biota\ ww}$ (for bivalves) corresponding in water to **0.10** mg.L^{-1} (bivalves)

7.6 Human health

Human health via consumption of fishery products

Secondary poisoning of top predators		**Master reference**
Mammalian oral toxicity ($mg \cdot kg^{-1}_{bw} \cdot d^{-1}$)	Rat / Oral / acute / mortality LD_{50} : 9272	Pfizer, 2007
	Mouse / Oral / acute / mortality LD_{50} : 2929	Pfizer, 2007
	Rat / intraperitoneal / acute / mortality LD_{50} : 374	EMA (2000a,b)
	Guinea pig / intraperitoneal / acute / mortality LD_{50} : 413	EMA (2000a,b)
	Rat / Oral / acute / mortality LD_{50} : 4600	Muñoz et al 2010 (In UBA, 2014)
	Various laboratory animals (mice, rats, hamsters, guinea pigs, rabbits and dogs)/ oral / acute LD_{50}: >300	EMA (2000a)
	Rat / Oral / reproduction and development / Teratogenic, Embryo, Fetal Development LOAEL : 6000	Pfizer, 2007
	Mouse / Oral / reproduction and development / Teratogenic, Embryo, Fetal Development LOAEL : 12000	Pfizer, 2007
	Rat / Oral diet (in gum arabic) / reproduction / Teratogenicity / No teratogen effects NOAEL : ≥ 2000	EMA (2000b)
	Rat / Oral / chronic/ repeated dose/ 13 weeks / no compound related adverse effects were reported NOAEL : ≥ 370	EMA (2000b)
	Rabbit/ Oral / chronic / repeated dose / 31 days / (salt not stated) / no compound related adverse effects were reported NOAEL : ≥ 200	EMA (2000b)
	Dog / Oral / chronic / repeated dose / 3 months / no compound related adverse effects were reported **NOAEL : ≥ 100**	EMA (2000b)
	Monkey / Oral / chronic / repeated dose / 64 days / (salt not stated) / no compound related adverse effects were reported NOAEL : ≥ 75	EMA (2000b)
	Mouse / Oral / carcinogenic study / 2 years / erythromycin stearate / no carcinogenic	EMA (2000b)

	effects NOAEL : ≥ 1400 mg/kg bw	
	Rat / Oral / carcinogenic study / 2 years / erythromycin ethylsuccinate / no carcinogenic effects NOAEL : ≥ 500 mg/kg bw	EMA (2000b)
CMR	No evidence of CMR properties	EMA (2000a)

Note: An assessment of the studies is not possible as original publications and dossiers are not available.

The $QS_{biota,\ hh\ food}$ is intended to protect humans against adverse health effects from consuming contaminated fishery products. Hence, the derivation of a biota standard for human health is triggered on the basis of the hazardous properties of a substance. Based on the data reported in the table above, erythromycin is neither mutagenic, carcinogenic nor toxic for reproduction (EMA, 2000a,b). However, the human health assessment was still conducted based on several notified classifications of the substance as H302, "Harmful if swallowed"[24], under the Classification and Labelling system (Reg. No. 1272/2008/EC).
The $QS_{biota,\ hh\ food}$ is calculated based on the threshold level, human health (TL_{hh}) that represents the Oral Reference Doses (RfD), Acceptable Daily Intake (ADI), Tolerable Daily Intake (TDI). The following table (Table 7.6) lists the available microbiological and pharmacological ADI values found for erythromycin. No toxicological ADI were retrieved for this substance.

Table 7.6. ADI values reported for erythromycin.

Human health via consumption of fishery products		**Master reference**
Mammalian oral toxicity	Microbiological ADI: 5 µg/kg bw Indirect drinking water exposure to Dehydrato-erythromycin, calculation based on the therapeutic dose, the lifetime of erythromycin and the daily ingestion of 2 L of water using the "worst-case" predictions for surface water concentrations with the additional assumption of no drug removal during drinking water treatment.	Webb et al. 2003 (In UBA 2014)
	Pharmacological ADI: 40 µg/kg/day Based on the therapeutic/pharmacologic dose of erythromycin in adults that was used as the point of departure (POD) with uncertainty factors for the ADI derivation. POD is the lowest single therapeutic dose in adults of 250 mg/day or 3.6 mg/kg/day taken four times per day. An AF of 90 was applied to the POD.	Schwab et al. 2005 (In UBA 2014)
	Microbiological ADI: 15 mg/person (60 kg)/day Laboratory study using a model of a human intestine and toxicity in the human colonic microbiota.	Carman et al. 2005 (In UBA 2014)

[24] Available online at: https://echa.europa.eu/it/information-on-chemicals/cl-inventory-database/-/discli/details/51542

According to the EQS Technical Guidance (EC, 2018), the $QS_{biota,\ hh,\ food}$ should be derived based on the following equation (EC, 2018):

$$QS_{biota,hh\ food}\ [\mu g/\ kg_{biota}] = \frac{0.2 \cdot TL_{hh}}{0.00163} \qquad \text{Equation 12}$$

where the threshold level human health, TL_{hh}, should be the acceptable daily intake (ADI) or tolerable daily intake (TDI), if available, a reference dose (RfD), or a benchmark dose. The basis for the human-toxicological threshold levels is in principle a NO(A)EL from a mammalian toxicity study, which is useful if established threshold levels are not available (EC, 2018). In the present assessment, only microbiological and pharmacological ADI were available. Therefore, the TL_{hh} was calculated from the $NOAEL_{min}$ (the lowest no observed adverse effect level value from a review of mammalian toxicology data) of 100 mg/kg bw/day in dogs using equation 13:

$$TL_{hh} = \frac{NOAEL_{min}}{100} \qquad \text{Equation 13}$$

The $QS_{biota,\ hh,\ food}$ (expressed as µg/kg biota) was then calculated with the equation 12, resulting in a **$QS_{biota,\ hh\ food}$** of **122.7 mg/kg** (rounded to 120 mg/kg)**.**

The biota standard should be converted into a water column concentration standard for comparison with other water column standards as:

$$QS_{water,hh\ food}\ [\mu g/L] = \frac{QS_{hh,food}\ [\mu g/Kg]}{BAF[L/Kg]} \qquad \text{Equation 14}$$

For the back calculation to water, using the BAF value of 40 L/Kg for marine mussels (Klosterhaus et al., 2013) the **$QS_{water,\ biota}$ for bivalves** resulted to be **3.07 mg/L**.

According to TGD EQS (EC, 2018) once a $QS_{biota,hh\ food}$ has been estimated, it is needed to decide whether secondary poisoning of wildlife ($QS_{biota,\ secpois}$) or for protection of human health ($QS_{biota,\ hh\ food}$) should 'drive' the biota standard. To do this, the $QS_{biota,\ hh\ food}$ should be compared with the $QS_{biota,\ secpois}$ converted into a water column concentration. In the current dossier, the resulting $QS_{biota,hh\ food}$ (106 µg/L) was higher than the $QS_{water,\ biota}$ (3.365µg/L). Therefore, the $QS_{biota,\ secpois}$ will be taken forward as the EQS_{biota}.

Tentative $QS_{biota,\ hh}$	**Relevant study for derivation of $QS_{biota,\ hh}$**	**Assessment Factor**	**Tentative $QS_{biota,\ hh}$**
Human health	NOAEL of 100 mg/kg bw/day in dogs	-	**122.7 mg/kg_{bw}** corresponding in water to 3.07 mg·L^{-1} (bivalves)

Human health via consumption of drinking water
According to the EQS Technical Guidance (EC, 2018), if neither an EU drinking water standard nor WHO guideline value is available, the risk to human health arising from substances in drinking water is calculated according to equation 15. Such situation occurs to erythromycin.

$$QS_{dw,hh}\ [\mu g/\ L] = \frac{0.2 \cdot TL_{hh} \cdot bw}{Uptake_{dw}}$$ Equation 15

Where the human body weight (bw) and the daily uptake of drinking water ($uptake_{dw}$) are assumed to be of 70 kg and 2 litres, respectively (EC, 2018). As for the $QS_{biota,\ hh\ food}$, the TL_{hh} value was instead derived from equation 12 using the selected NOAEL of 100 mg/kg bw/day in dogs, and a fraction of 0.2 of the TL_{hh} is allocated to the intake of the substance via drinking water (EC, 2018). This results in a provisional drinking water **$QS_{dw,\ hh}$** of **7 mg/L** for erythromycin.
The SCHEER supports this drinking water standard. Nevertheless, the SCHEER also considers that in order to protect human health, a harmonised approach based on drinking water limit should be sought for pharmaceuticals, in order to mitigate the risks from chronic exposure to these chemicals (SCHEER, 2022).

Human health via consumption of drinking water		**Master reference**
Existing drinking water standard(s)	not available	--
Any guideline		

8 Additional considerations

8.1 Ph-effects

The pKa of erythromycin is 8.9, indicating that this compound will exist almost entirely in the cation form in the environment at pH values of 5 to 9 (PubChem)[25].
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). 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 erythromycin (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_{ow} values are considered in the environmentally relevant pH-range of approximately 5 to 9 prior to the commencement of testing (Chapter 6.1).

8.2 Contribution of Erythromycin to antimicrobial resistance

Erythromycin is a natural antibiotic which belongs to the macrolide class. It is a wide-spectrum antibiotic acting against gram-positive and gram-negative bacteria and it is widely used both in human and veterinary medicine for treating a variety of infections, including respiratory tract diseases (Section 4). Erythromycin was included in the first surface water Watch List (WL) of the European Water Framework Directive (WFD) in 2015 (EC, 2015) as a high-risk substance for prioritisation, together with clarithromycin and azithromycin as they belong to the same class, sharing the same mode of action and analytical method (Carvalho et al., 2015; Loos, 2015).
Erythromycin enters in the environment mainly via excretion in feces and urine of humans/animals. Most of the administrated erythromycin is metabolised by the liver (Farzam et al., 2021); and only about 2.5% of an oral dose is recovered unchanged in the urine (Bryskier, 2010). Erythromycin reaches the wastewater treatment plants (WWTP) through the sewage system and since plants are not designed to eliminate antibiotics, they may be only partly removed and can therefore reach and contaminate the aquatic environment.
The main concern associated to the ever increasing use of antibiotics is the spread of antibiotic resistant bacteria (ARB) and antibiotic resistant genes (ARG) in humans, animals and the environment.
The derivation of the Predicted No Effect Concentration (PNEC) for antibiotics is currently based on ecotoxicology data and does not consider their contribution to the dissemination of ARB and ARG in water environments; indeed, no guidelines are available for deriving the minimum levels of antibiotics that may result in the development of antibiotic resistance.
In this dossier, the PNEC value obtained for erythromycin (0.5 µg/L, see section 7.2.) is based on an EC_{10} of 5 µg/L (*Anabaena* sp., 72 h, growth) with an assessment factor (AF) of 10.
In a recent publication, Bengtsson and Larsson derived PNEC values for antibiotics by collecting Minimum Inhibitory Concentrations (MIC) from the public European Committee on Antimicrobial Susceptibility Testing database (EUCAST) (Bengtsson-Palme and Larsson, 2016). The lowest MIC

[25] Available online at: https://pubchem.ncbi.nlm.nih.gov/compound/Erythromycin (Accessed on April 2021)

value was identified and an assessment factor of 10 was then applied considering that the selective concentration must be lower than the inhibitory concentration (Bengtsson-Palme and Larsson, 2016) . To date, this is the only derivation of PNEC for antibiotics that addresses the resistance. The PNEC-MIC values are intended to be protective for both humans and the environment and have also been adopted by the AMR Industry Alliance with the recommendation to use the lower of the two values: PNEC and PNEC-MIC (Tell et al., 2019).
In the case of erythromycin, the PNEC-MIC (1 µg/L) is above the available PNEC value for ecotoxicological effects (see Table 8.1.). It should be pointed out that the PNEC-MIC proposed by Bengtsson and Larsson does not account for multidrug resistant bacteria and does not consider the exposure of bacteria to mixtures of antibiotics. In addition, other pollutants such as biocides and metals may also contribute to the selection of ARG.

Table 8.1: PNEC (Predicted No Effect Concentration) and PNEC-MIC (Minimum Inhibitory Concentrations) values for erythromycin. PNEC and PNEC-MIC were derived for erythromycin and the lower value was selected for the risk assessment.

Erythromycin	
PNEC (µg/L)	**PNEC-MIC (µg/L)**
0.5	1*

*(Bengtsson-Palme and Larsson, 2016)

Data from the literature underline the need to carry out surveillance of environmental sources of antibiotics. A research performed in a Portuguese WWTP identified different antibiotics, including erythromycin, in the influent and effluent of a WWTP receiving urban and industrial wastewater (de Jesus Gaffney et al., 2017). Treatment processes of the plant included a pre-treatment step followed by a primary (removal of suspended solids) and secondary treatment (activated sludge). During the eleven campaigns performed in three seasons (autumn, winter and spring), the erythromycin concentration in WWTP influent samples was found to show a peak in winter (2.3 µg/L) (de Jesus Gaffney et al., 2017). The efficiency of the plant in removing pharmaceuticals was also monitored and a negative removal efficiency (-50%) was observed for erythromycin (de Jesus Gaffney et al., 2017). Lower concentrations of erythromycin were detected in influents of two WWTP in Tehran (from 0.02 to 0.159 µg/L) during the summer season (Mirzaei et al., 2018). The two plants were assessed for their efficiency in removing antibiotics and one of them, including the activated sludge process, was found to be not efficient in removing erythromycin. The authors found a possible explanation in the different rates of biodegradation processes which erythromycin could undergo in the two WWTP, one including the anoxic, anaerobic, and aerobic tanks followed by a secondary clarifier, and the other based on a combined activated sludge and filtration processes (Mirzaei et al., 2018). Indeed, removal of antibiotics from WWTP could be influenced by different factors such as adsorption onto sludge, the antibiotic consumption, treatment process, variation in influent composition, hydraulic retention time, pH and temperature. Therefore, different removal efficiencies can be observed for the same antibiotic even in plants containing the same processes (Aydin et al., 2019). However, chlorination, a widespread tertiary treatment is generally effective in removing erythromycin (Burch et al., 2019). Since the Logarithm of the solid liquid coefficient (Log Kd) for macrolides is < 2.7, sorption on the sludge only accounts for a minor part in their removal in the WWTP (Aydin et al., 2019).
Data from the literature indicate that erythromycin can be present in hospital effluents, influents and effluents of WWTP in the range of below detection limit (BDL) to 7.54 µg/L, 0.003 to 2.3 µg/L and BDL to 2.7 µg/L, respectively (de Jesus Gaffney et al., 2017; Aydin et al., 2019; Burch et al., 2019).

Aydin et al. also pointed out the potential risk erythromycin may pose to the receiving environment, in particular to organisms like *Daphnia* and fish (Aydin et al., 2019). Presence of macrolide-resistant genes has been also detected in surface water in different studies (Zhang et al., 2009; Stoll et al., 2012; Su et al., 2020).
These data underline the importance to continue monitoring how anthropogenic sources can impact the dissemination of antibiotic resistance in the environment.
Further research is however required to better understand how information on resistance can be used in the process of the environmental risk assessment for antibiotics. Finally, it should be noted that this evaluation should also consider the contribution of ARG and mobile genetic elements (MGE) to the spread of resistance, considering that gene transfer is the way by which the microbial community become resistant. In this context, measurements of ARG by quantitative polymerase chain reaction (qPCR) and sequencing methods were proposed in the 3rd WL Report by the JRC as an endpoint for the evaluation of risk assessment (Gómez Cortés et al., 2020).

9 Bibliography, Sources and supportive information

Alexy, R. (2003). Antibiotika in der aquatischen Umwelt: Eintrag, Elimination und Wirkung auf Bakterien. Fakultät für Chemie, Pharmazie und Geowissenschaften der Albert-Ludwigs-Universität Freiburg.

Ando, T., H. Nagase, K. Eguchi, T. Hirooka, T. Nakamura, K. Miyamoto, and K. Hirata (2007). A novel method using cyanobacteria for ecotoxicity test of veterinary antimicrobial agents. Environ.Toxicol.Chem. 26:601-606. https://doi.org/10.1897/06-195R.1

Aydin, S., Aydin, M. E., Ulvi, A., and Kilic, H. (2019). Antibiotics in hospital effluents: occurrence, contribution to urban wastewater, removal in a wastewater treatment plant, and environmental risk assessment. Environmental Science and Pollution Research, 26(1), 544-558. https://doi.org/10.1007/s11356-018-3563-0

Barron, L., J. Havel, M. Szpak, M. Purcell, and B. Paull (2008). Using artificial neural networks to predict the fate of pharmaceuticals and personal care products in the soil environment.

Barron, L., J. Havel, M. Purcell, M. Szpak, B. Kelleher, and B. Paull (2009). Predicting sorption of pharmaceuticals and personal care products onto soil and digested sludge using artificial neural networks. Analyst 134:663-670. https://doi.org/10.1039/B817822D

Bengtsson-Palme, J., and Larsson, D. J. (2016). Concentrations of antibiotics predicted to select for resistant bacteria: proposed limits for environmental regulation. Environment International, 86, 140-149. https://doi.org/10.1016/j.envint.2015.10.015

Bills, T. D., L. L. Marking, and G. E. Howe (1993). Sensitivity of Juvenile Striped Bass to Chemicals Used in Aquaculture. Fish and Wildlife Service, US Department of the Interior, Washington, DC, USA.

Boström, M. L. and O. Berglund (2015). Influence of pH-dependent aquatic toxicity of ionizable pharmaceuticals on risk assessments over environmental pH ranges. Water Res 72: 154-161.

Bryskier, A. (ed) (2010) Antibiotic and Chemotherapy. Chapter 22. Macrolides.

Burch, K. D., Han, B., Pichtel, J., and Zubkov, T. (2019). Removal efficiency of commonly prescribed antibiotics via tertiary wastewater treatment. Environmental Science and Pollution Research, 26(7), 6301-6310. https://doi.org/10.1007/s11356-019-04170-w

Calma, M.L., Asis, J.L.B., Medina, P.M.B. (2018). Erythromycin Exposure Disrupts the Life Cycle Stages of Aedes aegypti L. (Diptera: Culicidae). Water, Air, & Soil Pollution volume 229, Article number: 159 (2018)

Carman, R. J., Simon, M. A., Petzold III, H. E., Wimmer, R. F., Batra, M. R., Fernández, A. H., ... and Bartholomew, M. (2005). Antibiotics in the human food chain: establishing no effect levels of tetracycline, neomycin, and erythromycin using a chemostat model of the human colonic microflora. Regulatory Toxicology and Pharmacology, 43(2), 168-180.

Carvalho, R. N., Ceriani, L., Ippolito, A., and Lettieri, T. (2015). Development of the 1st Watch List under the Environmental Quality Standards Directive. Report EUR 27142 EN. Publications Office of the European Union. EUR 27142 EN. JRC 95018. JRC Science Hub. https://doi.org/10.2788/101376

Carvalho, R.N., Marinov, D., Loos, R., Napierska, D., Chirico, N., and Lettieri, T. (2016). Monitoring-based exercise: second review of the priority substances list under the Water Framework Directive, Available at https://circabc.europa.eu/w/browse/52c8d8d3-906c-48b5-a75e-53013702b20a.

ChemIDPlus website at http://chem.sis.nlm.nih.gov/chemidplus/rn/114-07-8

De Jesus Gaffney, V., Cardoso, V. V., Cardoso, E., Teixeira, A. P., Martins, J., Benoliel, M. J., and Almeida, C. M. M. (2017). Occurrence and behaviour of pharmaceutical compounds in a

Portuguese wastewater treatment plant: Removal efficiency through conventional treatment processes. Environmental Science and Pollution Research, 24(17), 14717-14734. https://doi.org/10.1007/s11356-017-9012-7

Drugbank website at http://www.drugbank.ca/drugs/DB00199

EC (2015), European Commission. Commission Implementing Decision (EU) 2015/495 of 20 March 2015 establishing a watch list of substances for Union-wide monitoring in the field of water policy pursuant to Directive 2008/105/EC of the European Parliament and of the Council. Official Journal of the European Union, L 78/40-42, 24.3.2015. (2015).

EFSA (2010), European Food Safety Authority. Management of left-censored data in dietary exposure assessment of chemical substances. EFSA J 8:1–96. https://doi.org/10.2903/j.efsa.2010.1557

Eguchi, K., H. Nagase, M. Ozawa, Y. S. Endoh, K. Goto, K. Hirata, K. Miyamoto, and H. Yoshimura (2004). Evaluation of antimicrobial agents for veterinary use in the ecotoxicity test using microalgae. Chemosphere 57:1733-1738. https://doi.org/10.1016/j.chemosphere.2004.07.017

El-Nahhal, Y., and El-Dahdouh, N. (2015). Toxicity of amoxicillin and erythromycin to fish and mosquito. Ecotoxicology and Environmental Contamination, 10(1), 13-21.

EMA (2000a), European Agency for the Evaluation of Medicinal Products, Veterinary Medicines and Information Technology Unit. Erythromycin - erythromycin thiocyante - erythromycin stereate: Summary report. EMEA/MRL/720/99-FINAL, January 2000. Available from: https://www.ema.europa.eu/en/documents/mrl-report/erythromycin-summary-report-2-committee-veterinary-medicinal-products_en.pdf

EMA (2000b), European Agency for the Evaluation of Medicinal Products, Veterinary Medicines and Information Technology Unit. Erythromycin: Summary report 2. EMEA/MRL/720/99-FINAL, January 2000. Available from: https://www.ema.europa.eu/en/documents/mrl-report/erythromycin-summary-report-2-committee-veterinary-medicinal-products_en.pdf

ETX 2.3 software by RIVM. A program to calculate hazardous concentrations and fraction affected, based on normally distributed toxicity data.

Farzam K, Nessel TA, and Quick J. Erythromycin. [Updated 2020 Nov 27]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2021 January. Available from: https://www.ncbi.nlm.nih.gov/books/NBK532249/

Förster, B., I. Löffler, and A. Witte (2013). Ökotoxikologische Effektstudien mit dem Antibiotikum Erythromycin zur Einschätzung der Umweltwirkung. Umweltbundesamt, Berlin.

Gao, L., Y. Shi, W. Li, J. Liub, and Y. Cai (2012). Occurrence, distribution and bioaccumulation of antibiotics in the Haihe River in China. J.Environ.Monit. 14:1248-1255. https://doi.org/10.1039/C2EM10916F

Gardner, M. 2011. Improving the interpretation of ‘less than’ values in environmental monitoring. Water and Environment Journal, CIWEM, pp.1-6.

Gómez Cortés, L., Marinov, D., Sanseverino, I., Navarro Cuenca, A., Niegowska, M., Porcel Rodríguez, E., and Lettieri, T. (2020) Selection of substances for the 3rd Watch List under the Water Framework Directive.

González-Pleiter, M., S. Gonzalo, I. Rodea-Palomares, F. Leganés, R. Rosal, K. Boltes, E. Marco, and F. Fernández- Pinas (2013). Toxicity of five antibiotics and their mixtures towards photosynthetic aquatic organisms: Implications for environmental risk assessment. Water Research 47:2050-2064. https://doi.org/10.1016/j.watres.2013.01.020

Helsel D. 2012. Statistics for Censored Environmental Data Using Minitab and R. John Wiley & Sons, Hoboken, NJ, USA

Isidori, M., M. Lavorgna, A. Nardelli, L. Pascarella, and A. Parrella (2005). Toxic and genotoxic evaluation of six antibiotics on non-target organisms. Sci.Total Environ. 346:87-98. https://doi.org/10.1016/j.scitotenv.2004.11.017

Ji, K., S. Kim, S. Han, J. Seo, S. Lee, Y. Park, K. Choi, Y. L. Kho, P. G. Kim, J. Park, and K. Choi (2012). Risk assessment of chlortetracycline, oxytetracycline, sulfamethazine, sulfathiazole, and erythromycin in aquatic environment: are the current environmental concentrations safe? Ecotoxicology 21:2031-2050. https://doi.org/10.1007/s10646-012-0956-6

KIWA. 2000. Inventory on the presence of pharmaceuticals in dutch water.

Klasmeier, J., N. Kehrein, J. Berlekamp, and M. Matthies (2011). Mikroverunreinigungen in oberirdischen Gewässern: Ermittlung des Handlungsbedarfs bei kommunalen Kläranlagen. Institut für Umweltsystemforschung, Universität Osnabrück, Osnabrück.

Klimisch, H. J., Andreae, M., and Tillmann, U. (1997). A systematic approach for evaluating the quality of experimental toxicological and ecotoxicological data. Regulatory toxicology and pharmacology, 25(1), 1-5.

Klosterhaus, S. L., R. Grace, C. Hamilton, and D. Yee (2013). Method validation and reconnaissance of pharmaceuticals, personal care products, and alkylphenols in surface waters, sediments, and mussels in an urban estuary. Environment International 54:92-99.

Kociolek, J.P., Spaulding, S.A., Lowe, R.L. (2015). Chapter 16 - Bacillariophyceae: The Raphid Diatoms. Freshwater Algae of North America (Second Edition) Ecology and Classification Aquatic Ecology 2015, Pages 709-772. https://doi.org/10.1016/B978-0-12-385876-4.00016-5

Kümmerer, K. (2003). Eintrag von Antibiotika in die aquatische Umwelt: Prüfung der biologischen Abbaubarkeit ausgewählter Antibiotika, ihr Vorkommen im Abwasser und ihr möglicher Einfluss auf die Reinigungsleistung kommunaler Kläranlagen - Identifizierung von Risikofeldern. Freiburg.

Kwon, J. W. (2016). Environmental impact assessment of veterinary drug on fish aquaculture for food safety. Drug testing and analysis, 8(5-6), 556-564. https://doi.org/10.1002/dta.2007

LANUV NRW (2007). Eintrag von Arzneimitteln und deren Verhalten und Verbleib in der Umwelt Literaturstudie. Ministerium für Umwelt und Naturschutz, Landwirtschaft und Verbraucherschutz des Landes NordrheinWestfalen, Düsseldorf.

Le Page, G., Gunnarsson, L., Snape, J., Tyler, C.R. (2017). Integrating human and environmental health in antibiotic risk assessment: A critical analysis of protection goals, species sensitivity and antimicrobial resistance. Environment International, 109:155-169. https://doi.org/10.1016/j.envint.2017.09.013

Li, W., Shi, Y., Gao, L., Liu, J., and Cai, Y. (2012). Occurrence of antibiotics in water, sediments, aquatic plants, and animals from Baiyangdian Lake in North China. Chemosphere, 89(11), 1307-1315. http://dx.doi.org/10.1016/j.chemosphere.2012.05.079

Loos, R. (2015) Analytical methods for possible WFD first Watch List substances. EUR 27046EN. JRC 94012.

Loos R., D. Marinov, I. Sanseverino, D. Napierska and T. Lettieri (2018). Review of the 1st Watch List under the Water Framework Directive and recommendations for the 2nd Watch List, EUR 29173 EN, Publications Office of the European Union, Luxembourg, ISBN 978-92-79-81839-4, doi: 10.2760/614367, JRC111198

Machado, M. D., and Soares, E. V. (2019). Sensitivity of freshwater and marine green algae to three compounds of emerging concern. Journal of Applied Phycology, 31(1), 399-408. https://doi.org/10.1007/s10811-018-1511-5

Manallack, D.T., (2008). The pK(a) distribution of drugs: application to drug discovery. Perspect. Med. Chem. 1, 25 -38.

Marinov D., and T. Lettieri (2020). Results of the Watch List under the Water Framework Directive from the 4th reporting year and the combined dataset. Part A: Data quality, EUR xxxxxx EN, Publications Office of the European Union, Luxembourg (under printing). Available at https://circabc.europa.eu/ui/group/9ab5926d-bed4-4322-9aa7-9964bbe8312d/library/deabbcb4-c001-4855-b503-04f27996ca7d/details

McFarland, J.W. et al. 1997. Quantitative structure-activity relationships among macrolide antibacterial agents: in vitro and in vivo potency against *Pasteurella multicida*. J. Med. Chem. 40: 1340-6.

Merrington Graham, Adam Peters, Iain Wilson, Mike Gardner, Stuart Rutherford, Stijn Baken, Christian Schlekat, Chris Cooper, Jelle Mertens, William Adams, Lara Van de Merckt, Jaap van Nes, Leondina Della Pietra, Jim Ryan, 2021. Using Exposure Data to Identify Priority Substances Under the European Water Framework Directive: The Quest to Reflect Uncertainties, ET&C, Wiley, https://doi.org/10.1002/etc.4987

Meinertz, J. R., T. M. Schreier, J. A. Bernardy, and J. L. Franz (2010). Chronic Toxicity of Diphenhydramine Hydrochloride and Erythromycin Thiocyanate to *Daphnia*, *Daphnia magna*, in a Continuous Exposure Test System. Bull.Environ.Contam.Toxicol. 85:447-451. https://doi.org/10.1007/s00128-010-0117-7

Mirzaei, R., Yunesian, M., Nasseri, S., Gholami, M., Jalilzadeh, E., Shoeibi, S., and Mesdaghinia, A. (2018). Occurrence and fate of most prescribed antibiotics in different water environments of Tehran, Iran. Science of the Total Environment, 619, 446-459. https://doi.org/10.1016/j.scitotenv.2017.07.272

Moermond CTA, Kase R, Korkaric M, and Ågerstand M. (2016). CRED: Criteria for Reporting and Evaluationg Ecotoxicity Data. Environ. Toxicol. and Chem. 25 (5): 1297-1309

Muñoz, I., Bueno, M. J. M., Agüera, A., and Fernández-Alba, A. R. (2010). Environmental and human health risk assessment of organic micro-pollutants occurring in a Spanish marine fish farm. *Environmental pollution*, *158*(5), 1809-1816. https://doi.org/10.1016/j.envpol.2009.11.006

NORMAN (2014). Factsheet on erythromycin, version of 31.08.2014. Available on CIRCA BC.

OECD (2019), the Organisation for Economic Co-operation and Development. Environmental Health and Safety Publications, Series on Testing and Assessment No. 23: Guidance Document on Aquatic Toxicity Testing of Difficult Substances, Test Chemicals and Mixtures, Organisation for Economic Co-Operation and Development, Paris, pp. 81.

Ortiz de Garcia, S., G. P. Pinto, P. A. Garcia-Encina, and R. I. Mata (2013). Ranking of concern, based on environmental indexes, for pharmaceutical and personal care products: An application to the Spanish case. Journal of Environmental Management 129:384-397. https://doi.org/10.1016/j.jenvman.2013.06.035

Pfizer (2007). Material Safety Data Sheet: Erythromycin Capsules, revision date 28-sep-2007.

Pomati, F., A. G. Netting, D. Calamari, and B. A. Neilan (2004). Effects of erythromycin, tetracycline and ibuprofen on the growth of *Synechocystis* sp. and *Lemna minor*. Aquat.Toxicol. 67:387-396. https://doi.org/10.1016/j.aquatox.2004.02.001

PubChem, National Center for Biotechnology Information (2021). PubChem Compound Summary for CID 12560, Erythromycin. Available online at: https://pubchem.ncbi.nlm.nih.gov/compound/Erythromycin. [Consulted 6 March 2021].

REACH. Registered substance factsheets: Erythromycin EC number: 204-040-1 | CAS number: 114-07-8. Available online at: https://echa.europa.eu/registration-dossier/-/registered-dossier/19599 [Consulted 26 February 2021].

Regulation 1272/2008/EC. Regulation (EC) No 1272/2008 of the European Parliament and of the Council of 16 December 2008 on classification, labelling and packaging of substances and

mixtures, amending and repealing Directives 67/548/EEC and 1999/45/EC, and amending Regulation (EC) No 1907/2006.
Sanderson, H., D. J. Johnson, C. J. Wilson, R. A. Brain, and K. R. Solomon (2003). Probabilistic hazard assessment of environmentally occurring pharmaceuticals toxicity to fish, daphnids and algae by ECOSAR screening. Toxicology Letters 144:383-395. https://doi.org/10.1016/S0378-4274(03)00257-1
SCHEER (Scientific Committee on Health, Environmental and Emerging Risks) (2022). Preliminary Opinion on Draft Environmental Quality Standards for Priority Substances under the Water Framework Directive - Erythromycin, 28 February 2022.
Schlüsener, M. (2005). Fate, pathways and methods for the determination of selected antibiotics and steroid hormones in the environment. Universität Duisburg-Essen, Essen, Germany.
Schwab, B. W., Hayes, E. P., Fiori, J. M., Mastrocco, F. J., Roden, N. M., Cragin, D., ... and Anderson, P. D. (2005). Human pharmaceuticals in US surface waters: a human health risk assessment. Regulatory Toxicology and Pharmacology, 42(3), 296-312.
Sendra, M., Damián-Serrano, A., Araújo, C. V., Moreno-Garrido, I., and Blasco, J. (2018). Erythromycin sensitivity across different taxa of marine phytoplankton. A novel approach to sensitivity of microalgae and the evolutionary history of the 23S gene. Aquatic Toxicology, 204, 190-196. https://doi.org/10.1016/j.aquatox.2018.09.008
Sendra, M., Moreno-Garrido, I., Blasco, J., and Araújo, C. V. (2018b). Effect of erythromycin and modulating effect of CeO2 NPs on the toxicity exerted by the antibiotic on the microalgae *Chlamydomonas reinhardtii* and *Phaeodactylum tricornutum*. Environmental pollution, 242, 357-366. https://doi.org/10.1016/j.envpol.2018.07.009
Senta, I., Krizman-Matasic, I., Terzic, S., and Ahel, M. (2017). Comprehensive determination of macrolide antibiotics, their synthesis intermediates and transformation products in wastewater effluents and ambient waters by liquid chromatography–tandem mass spectrometry. *Journal of Chromatography A*, *1509*, 60-68. https://doi.org/10.1016/j.chroma.2017.06.005
Shoari N, and Dubé J.S. 2018. Toward Improved Analysis of Concentration Data: Embracing Nondetects. Environmental Toxicology and Chemistry, Volume 37, Number 3, pp. 643–656
Stoll, C., Sidhu, J. P., Tiehm, A., and Toze, S. (2012). Prevalence of clinically relevant antibiotic resistance genes in surface water samples collected from Germany and Australia. Environmental science & technology, 46(17), 9716-9726. https://doi.org/10.1021/es302020s
Su, S., Li, C., Yang, J., Xu, Q., Qiu, Z., Xue, B., ... and Shen, Z. (2020). Distribution of antibiotic resistance genes in three different natural water bodies-a lake, river and sea. International journal of environmental research and public health, 17(2), 552. https://doi.org/10.3390/ijerph17020552
Tell, J., Caldwell, D. J., Häner, A., Hellstern, J., Hoeger, B., Journel, R., ... and Vestel, J. (2019). Science-based targets for antibiotics in receiving waters from pharmaceutical manufacturing operations. Integrated environmental assessment and management, 15(3), 312-319. https://doi.org/10.1002/ieam.4141
Tesson, B., Gaillard, C., and Martin-Jezequel, V. (2009). Insights into the polymorphism of the diatom *Phaeodactylum tricornutum* Bohlin. https://doi.org/10.1515/BOT.2009.012
UBA (2014) EQS Datasheet: Environmental Quality Standard, Erythromycin EQS proposal. May 2014. FKZ 3712 28 23. Available on-line at: https://webetox.uba.de/webETOX/public/basics/literatur/download.do;jsessionid=5ABC79729770788A08390BD09CBB997C?id=33
US EPA (2012a), United States Environmental Protection Agency. EPI Suite v4.11. U.S. Environmental Protection Agency, Washington, DC.
US EPA (2012b). Toxicity Estimation Software Tool (T.E.S.T.), version 4.1.

Wang, G., Zhang, Q., Li, J., Chen, X., Lang, Q., and Kuang, S. (2019). Combined effects of erythromycin and enrofloxacin on antioxidant enzymes and photosynthesis-related gene transcription in *Chlorella vulgaris*. Aquatic Toxicology, 212, 138-145. https://doi.org/10.1016/j.aquatox.2019.05.004

Webb, S., T. Ternes, M. Gibert, and K. Olejniczak. 2003. Indirect human exposure to pharmaceuticals via drinking water. Toxicology Letters 142:157-167.

Williams, R. R., T. A. Bell, and D. V. Lightner (1992). Shrimp Antimicrobial Testing. II. Toxicity Testing and Safety Determination for Twelve Antimicrobials with *Penaeid* Shrimp Larvae. J. Aquat. Animal Health 40 262-270.

Wu, Y., Wan, L., Zhang, W., Ding, H., and Yang, W. (2020). Resistance of cyanobacteria *Microcystis aeruginosa* to erythromycin with multiple exposure. Chemosphere, 249, 126147. https://doi.org/10.1016/j.chemosphere.2020.126147

Xie, Z., Lu, G., Liu, J., Yan, Z., Ma, B., Zhang, Z., and Chen, W. (2015). Occurrence, bioaccumulation, and trophic magnification of pharmaceutically active compounds in Taihu Lake, China. Chemosphere, 138, 140-147. https://doi.org/10.1016/j.chemosphere.2015.05.086

Xie, Z., Lu, G., Yan, Z., Liu, J., Wang, P., and Wang, Y. (2017). Bioaccumulation and trophic transfer of pharmaceuticals in food webs from a large freshwater lake. Environmental Pollution, 222, 356-366. http://dx.doi.org/10.1016/j.envpol.2016.12.026

Zhang, X. X., Zhang, T., and Fang, H. H. (2009). Antibiotic resistance genes in water environment. Applied microbiology and biotechnology, 82(3), 397-414. https://doi.org/10.1007/s00253-008-1829-z

Zhang, R., Yu, K., Li, A., Wang, Y., Pan, C., and Huang, X. (2020). Antibiotics in coral reef fishes from the South China Sea: Occurrence, distribution, bioaccumulation, and dietary exposure risk to human. Science of the Total Environment, 704, 135288. https://doi.org/10.1016/j.scitotenv.2019.135288