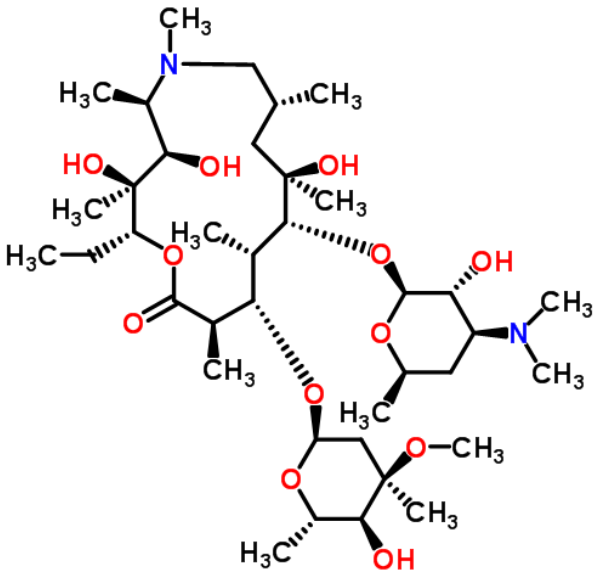
Miljøministeriet
Miljøstyrelsen

Fastsættelse af kvalitetskriterier for vandmiljøet

Azithromycin

CAS nr. 83905-01-5



Vandkvalitetskriterium	$VKK_{ferskvand}$	0,019 µg/l
Vandkvalitetskriterium	$VKK_{saltvand}$	0,0019 µg/l
Korttidsvandkvalitetskriterium	$KVKK_{ferskvand}$	0,18 µg/l
Korttidsvandkvalitetskriterium	$KVKK_{saltvand}$	0,018 µg/l
Sedimentkvalitetskriterium	$SKK_{ferskvand}$	16,92 µg/kg tørvægt (5% OC) 338,4 µg/kg tørvægt x f_{oc}
Sedimentkvalitetskriterium	$SKK_{saltvand}$	1,692 µg/kg tørvægt (5% OC) 33,84 µg/kg tørvægt x f_{oc}
Biota-kvalitetskriterium, sekundær forgiftning	$BKK_{sek.forgiftn.\ ferskvand\ og\ saltvand}$	1,8 mg/kg vådvægt (musling) 6,6 mg/kg vådvægt (fisk)
Biota-kvalitetskriterium, human konsum	HKK	Ikke muligt

December 2022

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

Dansk resumé og konklusioner

Azithromycin er et organisk stof, der tilhører gruppen af semisyntetiske macrolider og underklassen azalider. Stoffet anvendes som et antibiotikum overfor bakterielle infektioner i luftvejene, mave- og tarmsystemet, samt infektioner omkring kønsdele og i urinveje.

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

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

Indledningsvist indeholder rapporten en sammenfatning af grundlag og viden om forekomsten af stoffet Azithromycin i relevante eksterne miljøer. Baseret på indrapporterede koncentrationer af Azithromycin 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 at stoffet Azithromycin udgør en risiko for alle EU landes indlands overfladevande.

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.

Den udførte screening for stoffets tilstedeværelse og koncentration i det eksterne miljø danner baggrunden for, at stoffet er prioriteret til fastlæggelse af relevante kvalitetskriterier.

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

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

[2] Scientific committee on Health, Environmental and Emerging Risks (SCHEER) of the Commission of the European Union: final opinion on azithromycin (Publication date 6 May 2022), available on-line at: https://health.ec.europa.eu/publications/draft-environmental-quality-standards-priority-substances-under-water-framework-directive-3_en

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

bredde af de udførte undersøgelser i forhold til undersøgte akutte og kroniske effekter på specifikke organismer, trofiske niveauer og forskellige miljøer.

Azithromycin er undersøgt for toksikologiske og økotoksikologiske effekter i en 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. 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 dette reducerede datamateriale af studier med høj kvalitet og troværdighed (R1/R2) for stoffet Azithromycin, findes der fortsat relevante og solide studier af såvel akutte som kroniske effekter på minimum 3 taksonomiske grupper, men det samlede datagrundlag er dog stærkt begrænset.

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

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 reducerede datasæt omfatter for den anvendte deterministiske metode relevante studier af akutte effekter, der indeholder få studier fra det fastsatte minimum af 3 trofiske niveauer (alger, krebsdyr og fisk), hvor følgende tre ferskvandsarter og en saltvandsart er repræsenteret: (*Pseudokirchneriella subcapitata, Microcystis aeruginosa, Daphnia magna* og *Dicentrarchus labrax*). Det reducerede datasæt er relativt svagt, men omfatter dog taksonomiske grupper af potentielt sensitive arter såsom alger og cyanobakterier. Da der er få data for saltvandsarter, kombineres data for fersk- og saltvandsarter og den anvendte usikkerhedsfaktor er på baggrund heraf sat til 10 for ferskvand og 100 for saltvand jf. vejledningen (EC, 2018).

Med udgangspunkt i laveste EC_{50} værdi på 1,8 µg/l for studier af vækstrate i kulturer af cyanobakterien *Microsystis aeruginosa,* kan der med afsæt i den deterministiske tilgang fastlægges følgende KVKK-værdier:

$KVKK_{ferskvand}$ = 1,8 µg/l / 10 = 0,18 µg/l
$KVKK_{saltvand}$ = 1,8 µg/l / 100 = 0,018 µg/l

[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.

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 reducerede datasæt omfatter for den deterministiske metode relevante studier af kroniske effekter, der indeholder få studier fra det fastsatte minimum af 3 trofiske niveauer, hvor følgende fire ferskvandsarter er repræsenteret (*Pseudokirchneriella, Microcystis aeruginosa, Ceriodaphnia dubia og Pimephales promelas*). Der foreligger ikke studier af kroniske effekter for saltvandsarter. Det reducerede datasæt er relativt svagt, men omfatter dog taksonomiske grupper af potentielt sensitive arter (alger og cyanobakterier). Den anvendte usikkerhedsfaktor er på baggrund heraf sat til 10 for ferskvand og 100 for saltvand jf. vejledningen (EC, 2018).

Med udgangspunkt i laveste NOEC-værdi på 0,19 µg/l for studier af vækstrate i kulturer af cyanobakterien *Microsystis aeruginosa,* kan der med afsæt i den deterministiske tilgang fastlægges følgende VKK-værdier:

$VKK_{ferskvand}$ = 0,19 µg/l / 10 = 0,019 µg/l
$VKK_{saltvand}$ = 0,19 µg/l / 100 = 0,0019 µg/l

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

Azithromycin har estimerede og eksperimentelt bestemte værdier for log Koc omkring 3-4 l/kg og tilsvarende for log Kow omkring 3-4 l/kg, 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 Azithromycin 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 Koc på 17782,79 l/kg bestemmes fordelingskoefficienten mellem fast stof og vand i sediment, Kp_{sed} = Foc_{sed} x Koc = 0,05 x 17782,79 l/kg = 889,1395 l/kg og fordelingskoefficienten mellem sediment og vand, $K_{sed\text{-}water}$ kan beregnes som følgende:

$K_{sed\text{-}water} = Fair_{sed} \times K_{air\text{-}water} + F_{water\text{-}sed} + F_{solid\text{-}sed} \times (Kp_{sed} / 1000) \times RHO_{solid}$
$= 0 + 0{,}8 + 0{,}2 \times (889{,}1395 / 1000) \times 2500$
$= 445{,}36975\ m^3/m^{-3}$

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):

$SKK_{ferskvand}$ = (445,36975 / 1300) x 0,019 µg/l x 1000 x 2,6 = 16,92 µg/kg tørvægt (5% OC)
= 338,4 µg/kg tørvægt x f_{oc}

$SKK_{saltvand}$ = (445,3697 / 1300) x 0,0019 µg/l x 1000 x 2,6 = 1,692 µg/kg tørvægt (5% OC)
= 33,84 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.

Azithromycin har estimerede og eksperimentelt bestemte værdier for log Kow omkring 3-4 l/kg, og værdierne overskrider den udløsende værdi på 3. For stoffet Azithromycin er der tillige konstateret feltbaserede organ-specifikke Biokoncentrations Faktorer (BCF) på 24 – 254 l/kg for en søpølse *Apostichopus japonicus*. Tilsvarende er konstateret feltbaserede Bioakkumulerings Faktorer (BAF) på 204 – 575 l/kg for fisk og muslinger. Disse oplysninger udløser beregning af kvalitetskriterier for biota baseret på indtag, der kan føre til sekundær forgiftning for biota ($BKK_{sek.\ forgiftn.}$).

Baseret på det frembragte datagrundlag med bestemmelse af oral toksikologi i mus for stoffet Azithromycin ved indtag, er der bestemt en LD_{50} værdi på 3.000 mg/kg kropsvægt. Beregningsgrundlaget i Method A i Europa-Kommissionens tekniske vejledning (EC, 2018) er anvendt:

Det daglige energibehov (DEE) bestemmes ved anvendelse af en antaget kropsvægt på 30 g for mus

log DEE [kJ/d]= 0,8136 + 0,7149 x log (30) = 1,86959
DEE = 74,06

Den energinormaliseret føde-koncentration kan bestemmes på baggrund af LD_{50}, DEE og kropsvægten

$K_{energi\ normaliseret}$ [mg/kJ] = 3000 mg/kg x (0,030 kg / 74,06) = 1,215 mg/kJ

Den energinormaliseret værdi skal konverteres til en koncentration i det kritiske fødeemne. For Azithromycin 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}] = 1,215 mg/kJ x 19000 kJ/kg x (1-0,92) = 1847 mg/kg_{vv}
K_{fisk} [mg/kg_{vv}] = 1,215 mg/kJ x 21000 kJ/kg x (1-0,74) = 6635 mg/kg_{vv}

Der anvendes en usikkerhedsfaktor på 1.000 baseret dels på anvendelse af et akut-studie (faktor 100) og dels på ekstrapolation til det eksterne miljø fra toksikologiske studier i laboratorier (faktor 10), som leder frem til følgende kvalitetskriterier for biota:

$BKK_{sek.\ forgiftn.\ ferskvand}$ = 1847 mg/kg / 1000 = 1,8 mg/kg vådvægt (musling)
$BKK_{sek.\ forgiftn.\ ferskvand}$ = 6635 mg/kg / 1000 = 6,6 mg/kg vådvægt (fisk)

Det er for det marine miljø konstateret, at selvom den marine fødekæde indeholder et led mere ved tilstedeværelse af top-prædatorer, så forventes Azithromycin 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).

$BKK_{sek.\ forgiftn.\ saltvand}$ = 1,8 mg/kg vådvægt (musling)
$BKK_{sek.\ forgiftn.\ saltvand}$ = 6,6 mg/kg vådvægt (fisk)

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

For stoffet Azithromycin har det ikke været muligt at tilvejebringe data om NOAEL-værdier, kun et mikrobiologisk ADI (Acceptable Daily Intake) var til stede. Det følger af den konstaterede manglende viden om et acceptabelt eller tolerabelt dagligt humant indtag eller en referencedosis, at der ikke kan udledes et kvalitetskriterium for human konsum ved anvendelse af beregningsgrundlaget fastsat i Europa-Kommissionens tekniske vejledning (EC, 2018).

HKK = - µg/kg biota vådvægt

Supplerende kan det fastslås, at der på baggrund af langtidsstudier i forsøgsdyr for stoffet Azithromycin ikke er konstateret et potentiale for at stoffet er kræftfremkaldende, mutagent eller reproduktionsskadende.

Vandkvalitetskriterium baseret på $BKK_{sek.\ forgiftn.}$ og HKK
Der er beregnet et kvalitetskriterium for sekundær forgiftning af vandlevende organismer (biota) for beskyttelse af dyrelivet ($BKK_{sek.\ forgiftn.}$) i henholdsvis muslinger og fisk i både ferskvand og saltvand, mens det for samme vandlevende organismer ikke har været muligt at beregne et kvalitetskriterium for beskyttelse ved human konsum (HKK).

Bestemmelserne i Europa-Kommissionens tekniske vejledning (EC, 2018) indeholder som sidste led, at der om muligt skal foretages sammenligning og vurdering af frembragte kvalitetskriterier 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).

I ferskvand er det for de frembragte $BKK_{sek.\ forgiftn.}$-værdier fastslået, at der for fisk med en BAF-værdi på 12,5 l/kg er beregnet en koncentration af stoffet Azithromycin i vand på 0,53 mg/l, og for

muslinger med en BAF-værdi på 204,3 l/kg er beregnet en koncentration af stoffet Azithromycin i vand på 9,1 µg/l. Tilsvarende beregninger for det marine miljø har ikke været muligt.

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 Azithromycin 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 humant indtag eller referencedosis, og på grundlag af standard human konsum af drikkevand.

For stoffet Azithromycin har det ikke været muligt at tilvejebringe data om NOAEL-værdier, ligesom et mikrobiologisk ADI (Acceptable Daily Intake) ikke er til stede. Det følger af den konstaterede manglende viden om et acceptabelt eller tolerabelt dagligt humant indtag eller en referencedosis, at der ikke kan udledes et kvalitetskriterium for human konsum af drikkevand ved anvendelse af beregningsgrundlaget fastsat i Europa-Kommissionens tekniske vejledning (EC, 2018).

$HKK_{Drikkevand}$ = - µg/l

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)[5]. 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)[6] 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

[5] Forenede Nationer (UN, 2017): Deklaration vedtaget af FN's Generalforsamling den 22. september 2017. Tilgængelig online her: https://digitallibrary.un.org/record/842813
[6] 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).

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 Azithromycin er der tilvejebragt et datagrundlag for beregning af PNEC-MIC med en værdi på 0,25 µg/l.

Denne PNEC-MIC værdi for Antimikrobiel Resistens er højere end PNEC (0,019 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 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)[7].

Effekter af stoffets ionisering ved relevante pH værdier i det eksterne miljø
Stoffet Azithromycin er et ikke-ladet molekyle, der dog som en meget svag syre kan protolysere med en pKa værdi på 8,74. Stoffet forekommer derfor under miljørelevante forhold med pH værdier mellem 5 og 9, som et ikke-ladet stof.

[7] 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)

Konklusion
Følgende kvalitetskriterier for vandmiljøet er udregnet for Azithromycin:

Vandkvalitetskriterium

$VKK_{ferskvand}$	0,019 µg/l
$VKK_{saltvand}$	0,0019 µg/l

Korttidsvandkvalitetskriterium

$KVKK_{ferskvand}$	0,18 µg/l
$KVKK_{saltvand}$	0,018 µg/l

Sedimentkvalitetskriterium

$SKK_{ferskvand}$	16,92 µg/kg tørvægt (5% OC)
	338,4 µg/kg tørvægt x f_{oc}
$SKK_{saltvand}$	1,692 µg/kg tørvægt (5% OC)
	33,84 µg/kg tørvægt x f_{oc}

Biotakvalitetskriterium, sekundær forgiftning

$BKK_{sek.\ forgiftn.\ ferskvand}$	1,8 mg/kg vådvægt (musling)
$BKK_{sek.\ forgiftn.\ ferskvand}$	6,6 mg/kg vådvægt (fisk)
$BKK_{sek.\ forgiftn.\ saltvand}$	1,8 mg/kg vådvægt (musling)
$BKK_{sek.\ forgiftn.\ saltvand}$	6,6 mg/kg vådvægt (fisk)

Biotakvalitetskriterium, human konsum

HKK	Ikke muligt

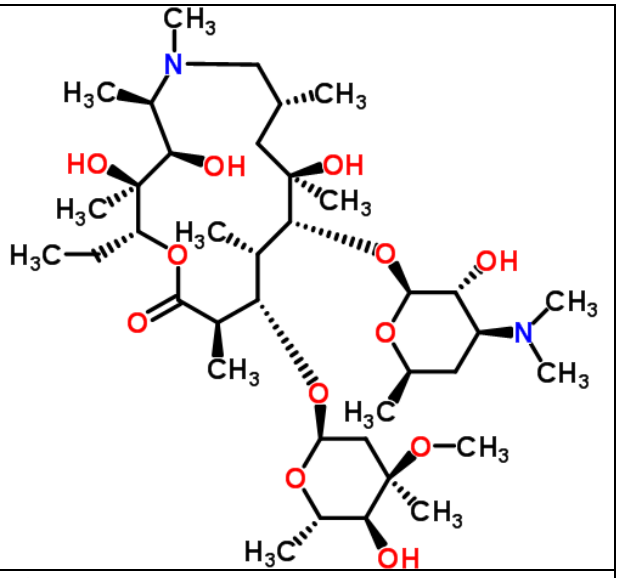
AZITHROMYCIN

Changes on the dossier after SCHEER final opinion:
Following the final SCHEER opinion published on 6th May 2022 (SCHEER, 2022)[8], the dossier has been updated by the JRC in the Section 7.5 QS for secondary poisoning.
The SCHEER agreed that there was not enough data to apply a probabilistic approach to derive acute and chronic toxicity related QS. The SCHEER supported the MAC-$EQS_{fw,eco}$ of 0.18 µg/L, MAC-$EQS_{sw,eco}$ of 0.018 µg/L, AA-$EQS_{fw,eco}$ of 0.019 µg/L and AA-$EQS_{sw,eco}$ of 0.0019 µg/L and benthic community QS of 17 and 1.7 µg/kg but with reservations. The SCHEER recommended that the data used in the MAC-QS and AA-QS derivation relied on a peer-reviewed document available for public consultation and not on personal communications. However, the data were within the range of other authors, so this reduced SCHEER's concern in both cases.
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 azithromycin is not expected to biomagnify in small birds or mammals within marine food chains, and no data are available to perform calculations or for the back calculation to water.

1 Chemical identity

Common name	Azithromycin
Chemical name (IUPAC)	(2R,3S,4R,5R,8R,10R,11R,13S,14R)-11-[(2S,3R,4S,6R)-4-dimethylamino-3-hydroxy-6-methyloxan-2-yl]oxy-2-ethyl-3,4,10-trihydroxy-13-[(2R,4R,5S,6S)-5-hydroxy-4-methoxy-4,6-dimethyloxan-2-yl]oxy-3,5,6,8,10,12,14-heptamethyl-1-oxa-6-azacyclopentadecan-15-one
Synonym(s)	--
Chemical class (when available/relevant)	Azalide, a subclass of macrolide antibiotics
CAS number	83905-01-5
EU number	617-500-5
Molecular formula	C38H72N2O12

[8] SCHEER final opinion on azithromycin (Publication date 6 May 2022), available on-line at: https://health.ec.europa.eu/publications/draft-environmental-quality-standards-priority-substances-under-water-framework-directive-3_en

Molecular structure	
Molecular weight (g.mol^{-1})	748.98

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 azithromycin is available.

3 Proposed Quality Standards (QS)

1.1 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}]**	**0.019** **0.0019**	**See Section 7.2 and 7.4.**
Proposed MAC-EQS for [freshwater] [µg·L^{-1}] **Proposed MAC-EQS for [marine waters] [µg·L^{-1}]**	**0.18** **0.018**	**See Section 7.1 and 7.4.**

1.2 3.2. Specific Quality Standard (QS)

Protection objective	Unit	Value	Comments
Predators (secondary poisoning)	[mg·kg^{-1}biota $_{ww}$]	**Freshwater:** 1.8 mg.kg^{-1}$_{biota\ ww}$ (bivalves) 6.6 mg.kg^{-1}$_{ww}$ (fish) **Marine water:** 1.8 mg.kg^{-1}$_{biota\ ww}$ (bivalves) 6.6 mg.kg^{-1}$_{ww}$ (fish)	**See section 7.5**
	[µg·L^{-1}]	**Freshwater:** 9.1 µg.L^{-1}(bivalves) -- (fish) **Marine water:** --	
Benthic community (freshwater)	[µg.kg^{-1} $_{dw}$]	16.92	**See section 7.3**
Benthic community (marine)	[µg.kg^{-1} $_{dw}$]	1.692	
Human health via consumption of fishery products	[µg·kg^{-1}biota $_{ww}$]	--	**See section 7.6**
	[µg·L^{-1}]	--	
Human health via consumption of water	[µg·L^{-1}]	--	

4 Major uses

Azithromycin is a semi-synthetic macrolide antibiotic of the azalide subclass that inhibits bacterial protein synthesis by binding to the 50S ribosomal subunit of the bacterial 70S ribosome. Azithromycin is widely used in clinical practice indicated for the treatment of respiratory tract, enteric and genitourinary infections[9]. It is currently authorised in the following European Member States (MS) and European Free Trade Association (EFTA) countries: Austria, Belgium, Bulgaria, Croatia, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Iceland, Ireland, Italy, Latvia, Liechtenstein, Lithuania, Luxembourg, Malta, Netherlands, Norway, Poland, Portugal, Romania, Slovakia, Slovenia, Spain, Sweden, and Switzerland.[10]

Azithromycin is also proposed as a potential therapy for SARS-CoV-2 due to the immunomodulatory action of azithromycin in a wide variety of respiratory viral infections that could be applicable to COVID-19 pandemic.[11]

5 Environmental Behaviour

1.3 5.1. Environmental distribution

		Master reference
Water solubility (mg.l^{-1})	2.37 (estimated)	PubChem[12]
	7.09 at 25 °C (estimated, ChemIDplus Lite	REACH dossier[13]; SRC PhysProp Database 2010 (In Oekotoxzentrum, 2015)
	0.062 (estimated, EPI-Suite 4.0)	Oekotoxzentrum (2015)
	514 (estimated)	Drugbank 2016 (In Oekotoxzentrum, 2015)
	Solubility at24 °C) : 10.7 mg/mL (pH 5.0) 5.4 mg/mL (pH 7.0) 1.9 mg/mL (pH 9.0)	Pfizer (2021)[14]
Volatilisation	Volatilisation from surface water is not expected to be an important fate process	

[9] Drugbank [accessed on March 2021]: https://go.drugbank.com/drugs/DB00207

[10] List of nationally authorised medicinal products available online at: https://www.ema.europa.eu/en/medicines/human/paediatric-investigation-plans/emea-001777-pip01-15 (Accessed on April 2021).

[11] Echeverría-Esnal, D., Martin-Ontiyuelo, C., Navarrete-Rouco, M. E., De-Antonio Cuscó, M., Ferrández, O., Horcajada, J. P., and Grau, S. (2021). Azithromycin in the treatment of COVID-19: a review. Expert review of anti-infective therapy, 19(2), 147-163. https://doi.org/10.1080/14787210.2020.1813024

[12] Available online at: https://pubchem.ncbi.nlm.nih.gov/compound/447043 t (Accessed on April 2021).

[13] Available online at: https://echa.europa.eu/registration-dossier/-/registered-dossier/6141/4/9 (Accessed on April 2021).

[14] Pfizer, personal communication (2021). Report No. 2438.6151, Azithromycin – Determination of the Water Solubility Following FDA Technical Assistance Document 3.01.

Vapour pressure (Pa)	$3.53x10^{-22}$ (estimated, EPI-Suite 4.0) ($2.65x10^{-24}$ mm Hg)	Oekotoxzentrum (2015); PubChem
	$5.21x10^{-25}$ ($3.91x10^{-27}$ mmHg) at 25°C (Estimated, ChemIDplus Lite)	REACH dossier[15]
Henry's Law constant ($Pa.m^3.mol^{-1}$)	$5.37x10^{-24}$ (estimated, EPI-Suite 4.0)	Oekotoxzentrum (2015)
Adsorption	Based on the experimental and estimated Koc values, azithromycin is expected to adsorb to suspended solids and sediment. Therefore, the sediment toxicity assessment should be performed.	
Organic carbon – water partition coefficient (K_{OC})	LogKoc: 4.25 (experimental)	Vermillion Maier and Tjeerdema (2018)
	3100 (estimated)	PubChem
	LogKoc: 3.50 (estimated, MCI method), LogKoc: 1.68 (estimated, KOW Method) EPI-Suite 4.0	Oekotoxzentrum (2015)
	Soil: California Clay Koc = 59,600 (log Koc = 4.78) Kansas Silt loam = 41,500 (log Koc = 4.62) Texas Silt loam Koc = 47,100 (log Koc = 4.67) Sludge Koc = 59.6 (log Koc = 1.78)	Pfizer (2021)[16]
sediment– water partition coefficient ($K_{sed\text{-}water}$)	No data	
Bioaccumulation	Based on the experimental and estimated LogKow and BCF values, the secondary poisoning assessment should be performed.	
Octanol-water partition coefficient (Log Kow)	3.24 (experimental, "Sirius PCA 101 Potentiometric System“)	McFarland et al. 1997 (In Oekotoxzentrum, 2015)
	4.02 (experimental, ChemIDplus Lite)	REACH dossier[17]
Bioconcentration Factor (BCF) [L/kg]*	200 (estimated, fish, US EPISuite, v4.1)	PubChem
	522 (estimated, fish)	Oekotoxzentrum, 2015

[15] Available online at: https://echa.europa.eu/registration-dossier/-/registered-dossier/6141/4/7 (Accessed on April 2021).
[16] Pfizer, personal communication (2021). Report No. 2438.6154, Azithromycin – Determination of the Sorption and Desorption Properties Following FDA Technical Assistance Document 3.08.
[17] Available online at: https://echa.europa.eu/registration-dossier/-/registered-dossier/6141/4/7 (Accessed on April 2021).

	At exposure of 1 µg/L: 619 in body wall; 170.4 in mouth; 23.9 in digestive tract; 151.4 in respiratory tract. At exposure of 10 µg/L: 58.4 in body wall; 112 in mouth; 24.4 in digestive tract; 254.6 in respiratory tract (experimental, *Apostichopus japonicus*)	Zhu M. et al. (2020)
Bioaccumulation Factor (BAF) [L/kg]*	575.44 L/kg for marine fish, field-derived	Zhang, R. et al., (2020)
	204.3 L/kg (97.8–346.4) for freshwater mussel, field-derived	de Solla et al. (2016)
	At exposure 8 ±7 ng /L,5000 L/Kg; at 6±5 ng /L, 14000; at 6±5 ng/L, 34000; for freshwater insect, field-derived	Grabicova et al., 2015

*Additional BCF and BAF values are reported in Tables 7.2 and 7.3 of section 7.5.

1.4 5.2. Abiotic and Biotic degradations

		Master reference
Hydrolysis	Abiotic degradation DT_{50}=38.2 months in aqueous solution (potassium phosphate buffered) at pH 6.3 and 25° C.	Zhang et al., 2009 (In Oekotoxzentrum, 2015)
Photolysis	Photolytic degradation under strong artificial irradiation in HPLC water or various artificial fresh water media, DT_{50}=1.1 - 20 hours. Photolytic degradation in natural river water and direct exposure to sunlight, DT_{50}=5 days	Tong et al., 2011 (In Oekotoxzentrum, 2015)
Biodegradation	Not readily biodegradable	NORMAN, 2014 (In Carvalho et al., 2015);
	Aerobic biodegradation in water under laboratory conditions. Inoculum: municipal secondary effluent, no acclimation. Mean DT50 of 9.2 days (using kinetic software CAKE)	Pfizer (2021)[18]
Distribution in water/sediment systems	Stability and retention under aerobic and anaerobic conditions in water or sediment-water systems (according to OECD 308): Water DT_{50}= 21.4 d aerobic (17.1 d anaerobic)	Oekotoxzentrum (2015)

[18] Pfizer, personal communication (2021). Report 2438.6160 Azithromycin – Aerobic Biodegradation in Water Following FDA Technical Assistance Document 3.11.

	Sediments DT_{50}=23.1 d aerobic	
	Aerobic and Anaerobic Transformation in Aquatic Sediment Systems: Water DT_{50}= 20.8 – 22.0 days aerobic (15 – 19.2 days anaerobic)	Pfizer (2021)[19]
Metabolites	*N*'-desmethyl azithromycin	Senta et al. (2017)

[19] Pfizer, personal communication (2021). Report 260E-134, Azithromycin: Aerobic and Anaerobic Transformation in Aquatic Sediment Systems, Following OECD 308.

6 Measured environmental concentrations

1.5 6.1. Freshwater

Note: This section has been revised and updated by the JRC after the final adoption of azithromycin QS values by the SCHEER committee in the plenary meeting on 6 May 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=0.019 µg/L..

6.1.1 Data availability and data scenarios

To update the information on exposure in the azithromycin'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). In addition, recent data for one MS have been retrieved from Naiades database via IPCheM portal (maintained by the JRC). 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)	561 samples (about 25.5% quantified) from 40 sites in 3 MS (2008 – 2014; not monitored in all years). Range of LOQs of non-quantified samples 0.01 – 0.05 µg/L.
EEA, Watch List (2019)	4588 samples (about 16% quantified) from 473 sites in 25 MS (2015 - 2019). Range of LOQs of non-quantified samples 0.00005 – 0.4 µg/L
EEA, WISE (2020)	3345 samples (about 13% quantified) from 375 sites in 25 MS (2008 – 2019; not monitored in each year). Range of LOQs of non-quantified samples 0.00005 – 0.4 µg/L.
Additional data received or retrieved after the 18th meeting of WFD CIS WG Chemicals (held in October 2020)	MS #12 (from Naiades database via IPCheM portal): 18046 samples (1.4% quantified) from 1444 sites (2016 - 2021). Range of LOQs of non-quantified samples 0.005 – 2.5 µg/L WISE (2022): 2921 samples (about 4.9% quantified) from 521 sites in 20 MS (2020 – 2021). Range of LOQs of non-quantified samples 0.00005 – 0.5 µg/

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 (2015-2019) and this dataset is in detail described in a dedicated report (Marinov and Lettieri, 2020; https://circabc.europa.eu/ui/group/9ab5926d-bed4-4322-9aa7-9964bbe8312d/library/deabbcb4-c001-4855-b503-04f27996ca7d/details).

The monitoring data from the WISE 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 2022-2021. The data from Naiades database are freely accessible on http://www.naiades.eaufrance.fr/acces-donnees#/physicochimie.

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[20] 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**

[20] 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″.

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 data from Naiades database were not included in the combined dataset because they would "flood" it and would make it not EU-representative. Instead, the Naiades data were considered separately (the same approach was applied to additional data from WISE 2022). 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_Azithromycin_dossier** (including the number of sites, number samples, fraction from all samples, number of quantified samples, info about LOQ values, statistics of MEC, etc.). It evidenced that one MS (#06) is overrepresented in the combined dataset (Sc2 scenario) holding about 58.8% of all samples.

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). The data from Naiades database are considered separately since if be included they would unbalance the combined dataset.

Scenario	Member States (MS)	Sites	Samples	Quantified samples (% from all for this scenario)
Sc1	20	220	886	100
Sc2	25	519	5207	16

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; 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) samples.

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 some MS have not monitored always with sufficiently sensitive analytical methods but majority of reported censored measurements have fulfilled the LOQ-PNEC criterion (excluding the censored samples

from MS #05 and #21). 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=0.019 µg/L. The Table 6.1.4 presents a summary information for Sc3 scenario while a more detailed statistics for Sc3 dataset is provided in the complementary Excel file. Seemingly, there is a sufficient amount of representative data with a good quality in Sc3 for making a union-wide risk assessment. The MS (#06) is overrepresented in the combined dataset (Sc3 scenario) holding about 53.5% of all samples.

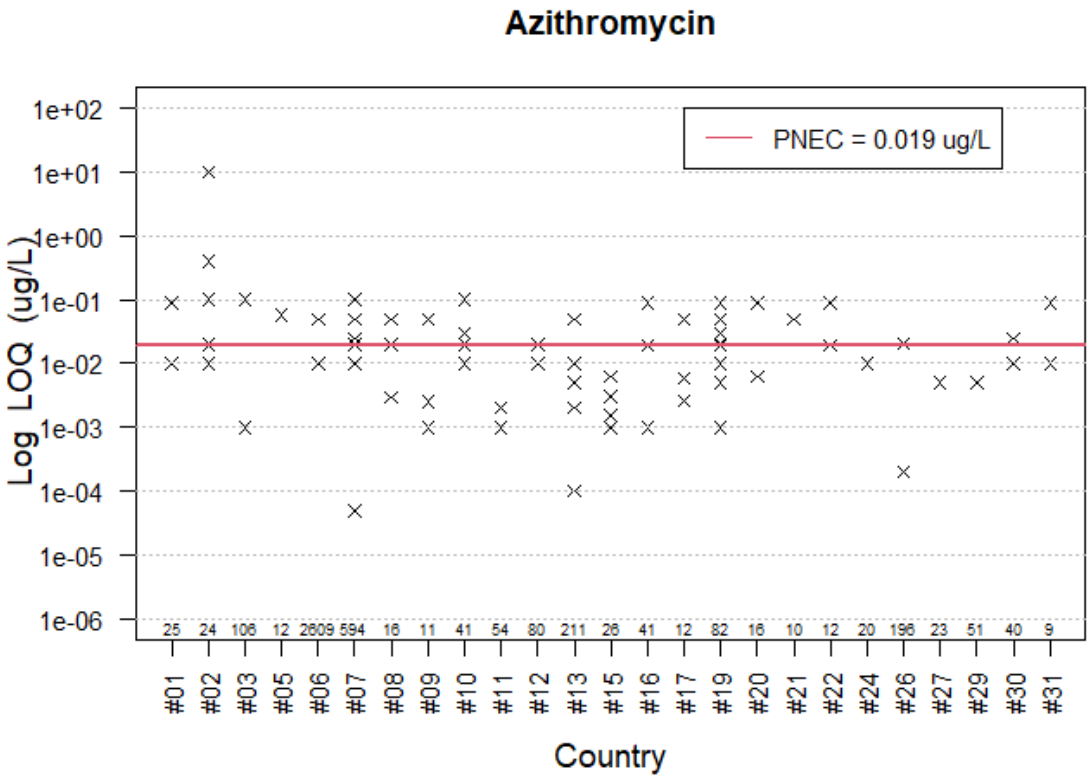


Figure 6.1.1: Range of LOQs for non-quantified samples in Sc2 of the combined dataset per country. The lowermost line of the figure shows the overall number of non-quantified samples in each reporting MS. For confidentiality the countries' names are coded. The red line indicates the PNEC value.

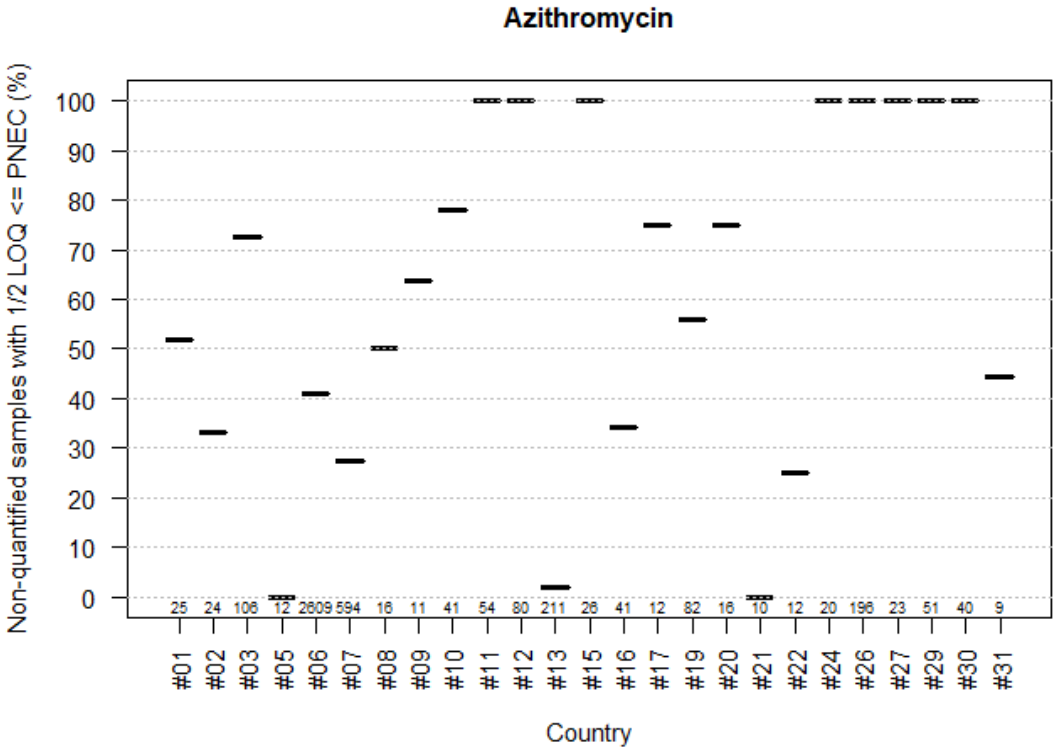


Figure 6.1.2: Number of non-quantified samples fulfilled LOQ-PNEC condition (½ LOQ≤PNEC) as percentage from all reported non-quantified samples per country in Sc2 of the combined dataset. The lowermost line of the figure shows the overall number of non-quantified samples in each reporting MS. For confidentiality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

Table 6.1.4: Available disaggregated data for the measured environmental concentrations across EU MS (jointly data from all countries) for the period 2008 – 2019 in Sc3 scenario of the combined dataset (PNEC=0.019 µg/L).

Scenario	Member States (MS)	Sites	Samples	Quantified samples (% of all samples for this scenario)
Sc3	24	399	2845	31.1

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 scenario of the combined dataset undertaking a substitution by half of LOQs for censored data. About 46.5% of all samples are non-quantified records having LOQ=0.01 µg/L which explains the high amount of 0.005 µg/L concentrations (Figure 6.1.3). In addition, the cumulative frequency (Figure 6.1.4) is compared to a log-normal distribution with the same mean and standard deviation as for monitoring data and it was found that the empirical distribution is not far away from the log-normal distribution.

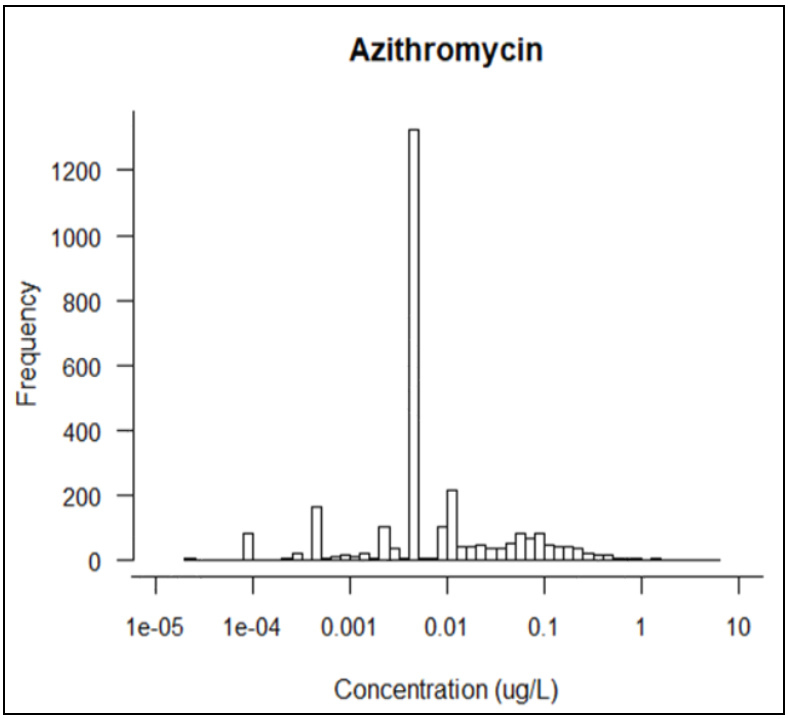


Figure 6.1.3: Histogram of concentrations (data from all MS together) for Sc3 of the combined dataset. About 46.5% of all samples are non-quantified records having LOQ=0.01 µg/L which explains the high amount of 0.005 µg/L concentrations in the combined dataset.

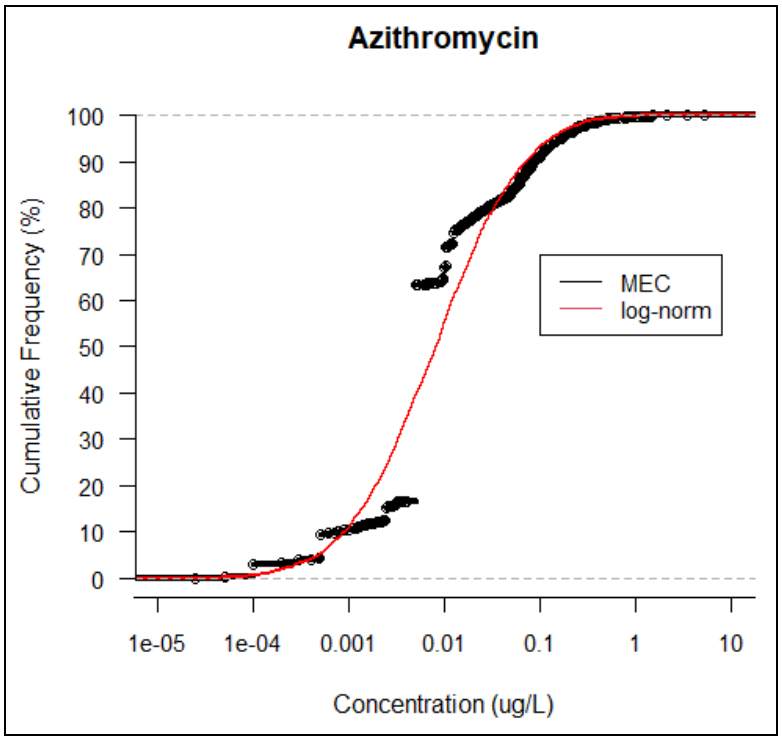


Figure 6.1.4: Cumulative frequency of concentrations (data from all MS together) for Sc3 of the combined dataset. The red line represents a cumulative frequency of log-normal distribution with the same mean and standard deviation as for monitoring data.

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 2008-2019). 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 closer to the lower bound of substitution (1% of LOQ) while the median and higher percentiles (for example P90 and P95) 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 $2.56*10^{-2}$ µg/L. The 95% upper confidence limit (95% UCL) of mean concentration, estimated by KM, is 0.0403 µg/L through bootstrapping and 0.0482 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 0.306 µg/L by KM approach assuming normal distribution and higher, 0.733 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, statistics for Sc3 derived by the substitution approach for censored data considering two extreme cases (lower bound 1% of LOQ and upper bound 99% of LOQ) alongside with the common "central" approach (50% of LOQ) is also presented.

Concentration (µg/L)	Kalpan-Meier method (ProUCL 5.1)	Scenario 1% LOQ	Scenario 50% LOQ	Scenario 99% LOQ
Min	5.00E-05	5E-07	2.5E-05	4.95E-05
Mean	3.51E-02	3.46E-02	3.81E-02	4.15E-02
StDev	1.60E-01	1.60E-01	1.60E-01	1.59E-01
Median	0.01	0.0001	0.005	0.0099
P90	0.0866	0.0866	0.0866	0.0866
P95	0.162	0.1616	0.1616	0.1616
P99	0.471	0.4708	0.4708	0.4708
Max	5.32	5.32	5.32	5.32

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 and also the hypothetical scenario of excluding the most data-rich country (MS#06). These statistics are estimated by Kaplan-Meier method for dataset containing censored data (ProUCL 5.1 tool). Similar statistical estimates were obtained for high percentiles of MECs (≥P90) when the overrepresented MS was excluded from the combined dataset. Furthermore, the table provides descriptive statistics of measured concentrations considering the additional monitoring data for MS#12 retrieved from Naiades database and for 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 high percentiles of MECs (for example percentiles ≥ P90).

Table 6.1.6. Summary statistics of measured environmental concentrations (µg/L) 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	2.00E-04	2.50E-05	5.00E-05
Mean	1.11E-01	3.47E-02	3.51E-02
StDev	2.72E-01	1.53E-01	1.60E-01
Median	0.056	0.025	0.01
P90	0.23	0.05	0.0866
P95	0.3775	0.0987	0.162
P99	1.0135	0.3494	0.471
Max	5.32	5.32	5.32

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 for MS#12 retrieved from Naiades database and for 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)	Only additional data for MS #12 retrieved from Naiades database (Sc3 scenario)	Only additional data from WISE for the period 2020-2021 (Sc3 scenario)
Min	5.00E-05	5.00E-05	0.005	5.00E-05
Mean	3.51E-02	3.91E-02	5.67E-03	9.00E-02
StDev	1.60E-01	2.12E-01	5.89E-03	3.98E-02
Median	0.01	0.01	0.005	0.01
P90	0.0866	0.09	0.005	0.025
P95	0.162	0.162	0.005	0.0548
P99	0.471	0.5	0.021	0.17
Max	5.32	5.32	0.165	0.55

6.1.4 Temporal trend

The temporal trend of azithromycin is verified in the period 2008-2019 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), it was found no trustworthy temporal trend in the period 2008-2013 because the missing information for 2009 and the limited amount of available data for the remaining years of the considered interval (for example the year 2008 is presented with only 2 samples). Onwards 2014, the 95th percentiles of MECs showed generally a gradual diminishing trend with some annual variability and oscillations. However, in recent years (after 2016) the P95 remain almost constant and always exceeded the PNEC value.
No substantial change of temporal pattern of P95 of MECs was observed if the most data-abundant MS (#06) was eliminated from the combined dataset Sc3 (see Figure 6.1.6).
The additional monitoring data from WISE dataset (see Table 6.1.1) showed a further decrease of annual P95 to 0.055 µg/L in 2020 and 0.04 µg/L in 2021, but the estimated P95-values are still higher than the PNEC=0.019 µg/L. Regarding data for MS#12 (Naiades dataset), annually invariable and almost constant P95 of MECs were observed in the period 2018-2021 but for this country the yearly P95<PNEC.

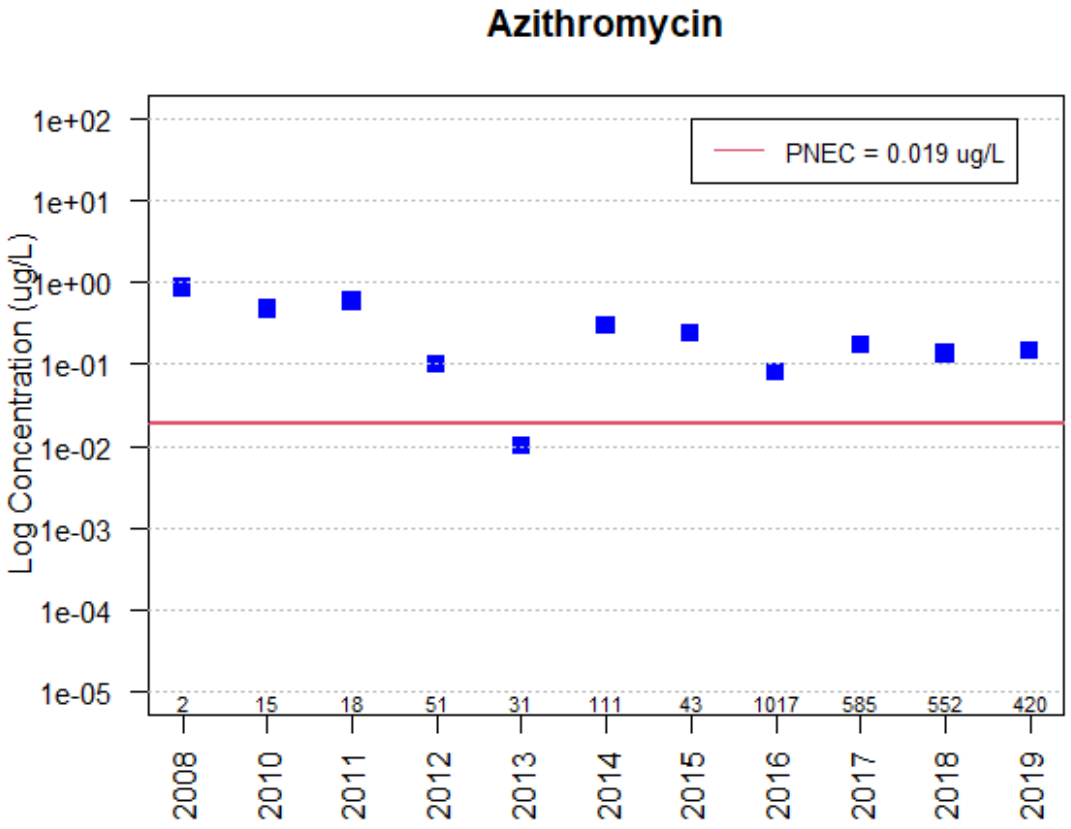


Figure 6.1.5: Plot for 95th percentiles of measured environmental concentrations per year for Sc3 scenario of the combined dataset considering **data from all MS**. No trustworthy temporal trend was observed in the period 2008-2013 because the missing information for 2009 and the limited amount of available data for the remaining years of this period. Onwards 2014, the 95th percentiles of MECs

showed generally a gradual diminishing trend with some annual variability and oscillations. In the recent years (after 2016) the P95 remain almost constant and always exceeded the PNEC value

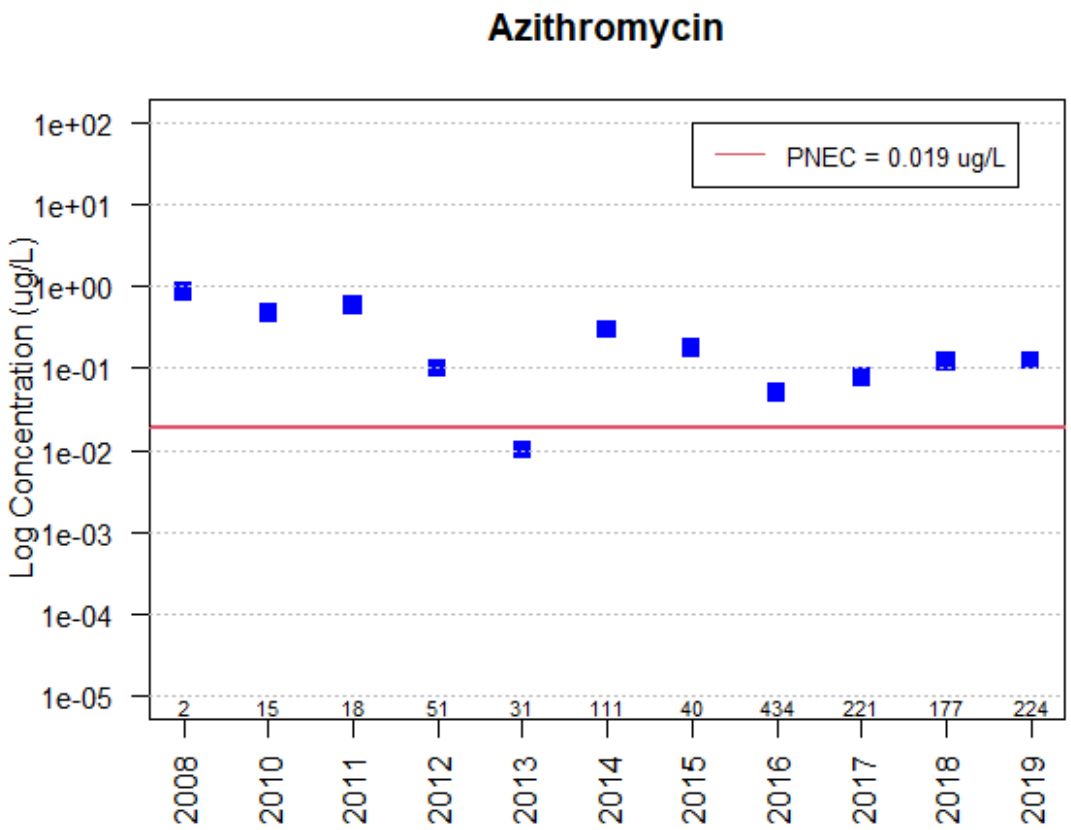


Figure 6.1.6: Plot for 95th percentiles of measured environmental concentrations per year for Sc3 scenario if the **most data-abundant MS (#06) is eliminated from the combined dataset**. No substantial change of temporal pattern of P95 of MECs is observed comparing to the complete dataset.

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=0.019 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=0.019 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	8.53	0.203	0.804	0.28	1.287	14 (58.3%)	24

The performed risk screening indicated a presence of risk for inland surface waters at EU level because the overall RQ(P95)=8.53, viz. it is considerably higher than one, the STE score is elevated (>1) and 14 MS out of the 24 reporting countries in Sc3 could be specified as exceeding MS (about 58.3% from all MS).
Notes:

1. The EU-wide concern for freshwaters is confirmed also if the most data-abundant MS (#06) is excluded from the combined dataset (Sc3 scenario) because the corresponding P95 of MECs exceeds the PNEC=0.019 µg/L (see Table 6.1.7). Respectively, RQ(P95)=8.53 and exceedances were observed in 13 reporting MS.
2. The available latest data for exposure from WISE 2022 (see Tables 6.1.1 and 6.1.7) likewise confirmed that azithromycin continues to pose an EU-wide risk in the recent years (2020-2021) since RQ(P95)=2.88 and 9 out of the 20 reporting MS showed exceedances.
3. According to additional data for exposure in MS #12 during the period 2018-2021 (retrieved from Naiades dataset; see Tables 6.1.1 and 6.1.7), the overall RQ(P95)=0.23, which indicated a low concern in this MS.

Compliance check

The compliance check, which is a core part of the developed risk assessment, was performed according to the EQS Directive[21] (amended by the Directive 2013/39/EU). The compliance is based on MECs in Sc3 data scenario of the combined dataset and is considered to be fulfilled (not failed) if the annual average measured concentrations at monitoring sites in the participating MS do not exceed the AA-EQS and when the maximum concentrations (or 99th percentile[22] 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[23] i.e. the substitution approach, adopted by the Directives 2009/90/EC and 2013/39/EU, was applied.
At first, a boxplot of annual average concentrations at monitoring sites (Sc3 data scenario) for the considered time period 2008-2019 is visualized on Figure 6.1.7 comparing to the freshwater AA-EQS=0.019 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. For instance, onwards 2015 (when exposure data from more reporting MS are available), yearly from 36 up to 199 monitoring sites, corresponding to 16.7-32.2% (on average 24.1%) of all sampling locations, showed annual mean concentrations higher

[21] 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''.
[22] 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".
[23] 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".

than the freshwater AA-EQS (in the period before 2015 the averaged percentage of exceeding annual mean concentrations at sites is about 70.6%).
Furthermore, according to the available latest data for exposure in 20 MS from WISE 2022 (see Table 6.1.1) the annual percentages of exceeding mean concentrations at sites in the period 2020-2021 vary from 20% to 23.6%.
Therefore, the above observations confirm distinctly the failure of compliance in regard to the freshwater AA-EQS.
Finally, regarding the compliance with the freshwater MAC-EQS=0.18 µg/L, the 99th percentiles of MECs from individual MS per year (Sc3 scenario of the combined dataset) were compared with the MAC-EQS threshold. The results are presented in Table 6.1.10. In the time-period 2015-2019, every year from 3 up to 5 MS showed P99 exceeding the freshwater MAC-EQS (corresponding to 16.7 - 50 % of the number of annually reporting MS). According to the additional recent data for exposure in 20 MS from WISE 2022, the MAC-EQS exceedances happened in 2 MS only in 2020. All these allow concluding a failure of compliance in regard to the freshwater MAC-EQS.

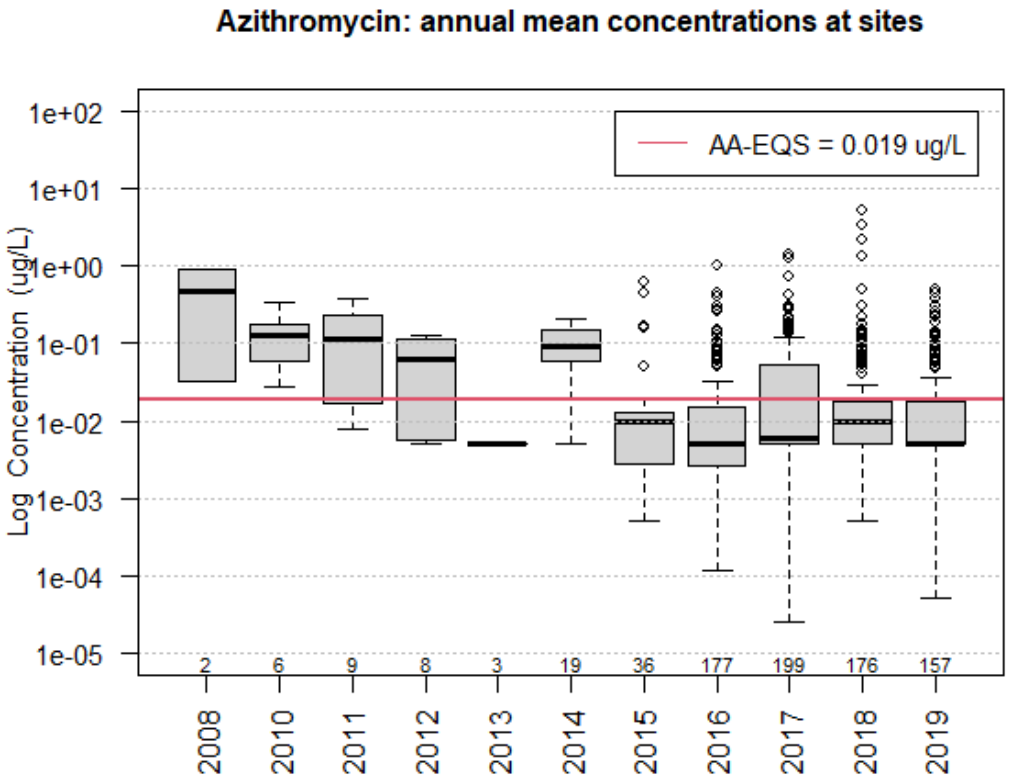


Figure 6.1.7: Boxplot of annual average values of measured concentrations at monitoring sites in Sc3 scenario for the considered time period. In this analysis the non-quantified concentrations are assumed to be equal to a half of LOQ (Directives 2009/90/EC and 2013/39/EU). The lowermost line of the figure gives the overall number of monitoring sites in each year. The red line indicates a limit equal to 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

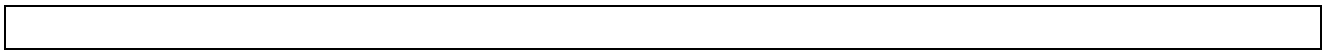
locations). In this analysis the non-quantified concentrations are assumed to be equal to a half of LOQ (Directives 2009/90/EC and 2013/39/EU).

Year	Number of reporting MS	Total number of sites	Number of exceeding sites	% of exceeding sites from all
2006	4	56	31	55.36
2008	2	2	2	100
2010	1	6	6	100
2011	1	9	6	66.7
2012	2	8	5	62.5
2013	1	3	0	0
2014	1	19	18	94.7
2015	6	36	6	16.7
2016	18	177	40	22.6
2017	20	199	64	32.2
2018	18	176	44	25
2019	20	157	38	24.2

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	1	1	100
2008	2	1	50
2010	1	1	100
2011	1	1	100
2012	2	0	0
2013	1	0	0
2014	1	1	100
2015	6	3	50
2016	18	3	16.7
2017	20	5	25
2018	18	4	22.2
2019	20	4	20

Conclusion:
The performed risk screening and the observed 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 Azithromycin poses an EU-wide risk in inland surface waters.



1.6 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 (31.6% quantified) from 14 sites in 6 MS for the period 2015-2019.
EEA, WISE (2020)	19 samples (all non-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	23	53	22.6

Regarding the quality of available monitoring data in Sc2 scenario, the range of LOQs of non-quantified samples is from 0.0025 µg/L to 0.05 µg/L. About 62.3% of non-quantified samples (33 out of 53 samples) are taken with LOQs≥0.005 µg/L which might indicate an insufficient sensitivity of applied analytical methods in regard to the marine water AA-EQS (0.0019 µg/L). Moreover, the total amount of data is scarce for making a reliable risk assessment. However for a sake of completeness, the descriptive statistic of measured concentrations was estimated and it is presented in Table 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.

	Min	Mean	StDev	Median	P90	P95	P99	Max
Concentration (µg/L)	$1.25*10^{-3}$	0.0193	0.0542	0.005	0.022	0.023	0.24	0.241

7 Effects and Quality Standards

Literature data were collected from the reports of Carvalho et al. (2015) and Oekotoxzentrum (2015), and the studies which were considered as reliable 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 azithromycin published among 2015-2021. Three 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 classified 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 azithromycin for freshwater and marine water organisms are reported in the tables below. Studies which are shown in grey colour 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 they are 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.

1.7 7.1. Acute aquatic ecotoxicity

ACUTE EFFECTS			**Master reference**
Algae & aquatic plants ($\mu g \cdot L^{-1}$)	**Freshwater**	Algae, *Pseudokirchneriella subcapitata* / 72h EC_{50}: 3.7 (biomass) Reliability evaluation:1	Mattson, 2016 (In Oekotoxzentrum 2015)
		Algae, *Pseudokirchneriella subcapitata* / 72h **EC_{50}: 8.4 (growth rate)[24]** **Reliability evaluation:1, key study**	**Mattson, 2016 (In Oekotoxzentrum 2015)**
		Algae, *Pseudokirchneriella subcapitata* / 96h EC_{50}: 19 (biomass; growth, area under curve) Reliability (R3) and relevance (C1), additional information (Oekotoxzentrum, 2015)	Harada et al. 2008 (In Carvalho et al., 2015; Oekotoxzentrum 2015)
		Algae, *Pseudokirchneriella subcapitata* / 96h EC_{50}: 26 (growth rate) Reliability evaluation: 2	Zhou, H. et al. (2016)
		Algae, *Pseudokirchneriella subcapitata* / 96h EC_{50}: 500 (growth rate, fluorescence) Reliability evaluation: reliability (R3) and relevance (C2), additional information	Minguez et al. 2014 (In Oekotoxzentrum 2015)
		Cyanobacteria, *Microcystis aeruginosa* / 72h EC_{50}: 0.94 (biomass) Reliability evaluation:1	Mattson, 2016 (In Oekotoxzentrum 2015)
		Cyanobacteria, *Microcystis aeruginosa* / 72h **EC_{50}: 1.8 (growth rate) (10)** **Reliability evaluation:1, key study**	**Mattson, 2016 (In Oekotoxzentrum 2015)**
	Marine	Algae, *Skeletonema marinoi* / 72h EC_{50}: 500 (growth) Reliability evaluation: reliability (R3) and relevance (C2), additional information	Minguez et al. 2014 (In Oekotoxzentrum, 2015)
Invertebrates ($\mu g \cdot L^{-1}$)		Crustaceans, *Daphnia magna* / 48h EC_{50}: >10000 (Immobilisation) Reliability evaluation: reliability (R3) and relevance (C1), additional information	Harada et al. 2008 (In Oekotoxzentrum, 2015)
		Crustaceans, *Daphnia magna* / 48h EC_{50}: >100000 (Immobilisation) Reliability evaluation: reliability (R3) and relevance (C2), additional information	Minguez et al. 2014 (In Oekotoxzentrum, 2015)
		Crustaceans, *Daphnia magna* / 48h **EC_{50}: 120000 (Immobilisation)** **Reliability evaluation: 1, key study**	**Mattson, 2010 (In Oekotoxzentrum, 2015; Carvalho et al., 2015)**

[24] According to the EQS Technical Guidance (EC, 2018), the growth rate endpoint is the more robust endpoint for algae tests, and thus preferred to biomass. Therefore, when data were available for both endpoints from the same study, growth rate was selected, such as in this case.

		Crustaceans, *Daphnia magna* / 24h EC_{50}: 148000 (Immobilisation) Reliability evaluation: 2	Li, Y. et al. (2020)
		Crustaceans, *Daphnia* sp. / (no information) EC_{50}: >120000 (no information) Reliability evaluation: 4 (not reliable), additional information	FDA-CDER 1996 (In Oekotoxzentrum, 2015)
		Crustaceans, *Amphipoda* / (no information) EC_{50}: >100000 (Immobilization) Reliability evaluation: 4 (not reliable), additional information	FDA-CDER 1996 (In Oekotoxzentrum, 2015)
	Marine	Crustaceans, *Artemia salina* / 48 h EC_{50}:> 100000 Reliability evaluation: reliability (R3) and relevance (C2), additional information	Minguez et al. 2014 (In Oekotoxzentrum 2015)
	Sediment	*No data*	
Fish ($\mu g \cdot L^{-1}$)	**Freshwater**	*Oncorhynchus mykiss* / 96h LC_{50}:> 84000 (mortality) Reliability evaluation: 1, key study	Mattson, 2016 (In Oekotoxzentrum 2015)
		Oncorhynchus mykiss / 96h NOEC: 84000 (mortality) Reliability evaluation: 1	Mattson, 2016 (In Oekotoxzentrum 2015)
	Marine	***Dicentrarchus labrax* / 96h** **LC_{50}: 30880 (mortality)** **Reliability evaluation: 2, key study**	**Mhadhbi et al. (2020)**
	Sediment	*No data*	
Other taxonomic groups ($\mu g \cdot L^{-1}$)		Amphibians, *Xenopus laevis* /freshwater/ 96h EC_{50}:> 10000 (Embryoteratogenicity) Reliability evaluation: reliability (R3) and relevance (C1), additional information	Harada et al. 2008 (In Oekotoxzentrum 2015)
		Nematode, *Caenorhabditis elegans* / 72h / freshwater LC_{50}: 411000 (Survival) Reliability evaluation: reliability (R4) and relevance (C4), additional information	Zhou, Y. et al. 2012 (In Oekotoxzentrum 2015)
		Bacteria, *Aliivibrio fischeri* (*Vibrio fischeri*) / 15 min. / marine water EC_{50}: >10000 (Luminescence) Reliability evaluation: reliability (R3) and relevance (C1), additional information	Harada et al. 2008 (In Oekotoxzentrum 2015)

Note: Studies reported in grey were not considered for the EQS derivation.

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

Due to the limited data available for marine water, freshwater and marine water data were combined for the maximum acceptable concentration quality standard (MAC-QS) derivation without statistical analysis, both for the acute and the chronic datasets, respectively (EC, 2018).

Deterministic approach

Acute ecotoxicity data are available for three freshwater species, representing the base set (algae, invertebrates and fish). Furthermore, it can be argued that potentially sensitive taxa are covered by algae and cyanobacteria. Therefore, an AF of 10 was applied to the lowest EC_{50} of 1.8 µg/L for the endpoint of growth rate in the cyanobacteria species *Microcystis aeruginosa* (Mattson, 2016), resulting in an **MAC-QS$_{fw,eco}$** of **0. 18 µg/L.**

Probabilistic approach

The dataset does not meet the criteria for construction of a Species Sensitivity Distribution (SSD) as listed in the EQS Technical Guidance (EC, 2018). According to the guidance, the output from an SSD-based quality standard is considered reliable if the database contains preferably more than 15, but at least 10 data points, from different species covering at least eight taxonomic groups. Below, the criteria are reported, together with the representative species from the present dataset, giving five taxonomic groups:

- Fish: *Oncorhynchus mykiss* (order *Salmoniformes*, family *Salmonidae*)
- A second family in the phylum Chordata: *Dicentrarchus labrax* (order *Perciformes*, Family *Moronidae*)
- A crustacean: *Daphnia magna* (order *Cladocera*, Family *Daphniidae*)
- An insect: no data
- A family in a phylum other than Arthropoda or Chordata: no data
- A family in any order of insect or any phylum not already represented: *Microcystis aeruginosa* (phylum cyanobacteria, order *Chroococcales*, family *Microcystaceae*)
- Algae: Pseudokirchneriella subcapitata (phylum Chlorophyta, order *Sphaeropleales*, family *Selenastraceae*)
- Higher plants: no data

The probabilistic assessment was therefore not carried out in the present evaluation due to the acute toxicity dataset for azithromycin was not sufficient for performing an SSD.

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-EQS derivation, there was at least one short-term $L(E)C_{50}$ from each of the three trophic levels representing the base set (algae, invertebrates and fish),. Potentially sensitive taxa are represented in the dataset, but no specifically marine species are present (i.e. marine test organisms other than algae, crustaceans and fish, and/or having a life form or feeding strategy differing from that of algae, crustaceans or fish). Therefore, an AF of 100 was applied to the lowest

EC_{50} of 1.8 µg/L for the endpoint of growth rate measured for the cyanobacteria species *Microcystis aeruginosa* (Mattson, 2016), thus resulting in a **MAC-QS$_{sw,eco}$** of **0.018 µg/L.**

Probabilistic approach

No species sensitivity distribution could be derived for the acute ecotoxicity dataset based on azithromycin. Details are reported above in the freshwater section.

1.8 7.2. Chronic aquatic ecotoxicity

CHRONIC EFFECTS			Master reference
Algae & aquatic plants ($\mu g \cdot L^{-1}$)	**Freshwater**	**Algae, *Pseudokirchneriella subcapitata* / 72h NOEC: 1.8 (Growth rate) Reliability evaluation:1, key study**	**Mattson, 2016 (In Oekotoxzentrum, 2015)**
		Algae, *Pseudokirchneriella subcapitata* / 96h NOEC: 5.2 (Growth, area under curve) Reliability (R3) and relevance (C1), additional information (Oekotoxzentrum,2015)	Harada et al. 2008 (In Carvalho et al., 2015; Oekotoxzentrum,2015)
		Cyanobacteria, *Microcystis aeruginosa* / 72h EC_{10}: 0.33 (growth rate) Reliability evaluation:1-2	Pfizer (2021)[25]
		Cyanobacteria, *Microcystis aeruginosa* / 72h NOEC: 0.19 (biomass)[26] Reliability evaluation:1	Mattson, 2016 (In Oekotoxzentrum 2015)
		Cyanobacteria, *Microcystis aeruginosa* / 72h NOEC: 0.19 (growth rate) Reliability evaluation:1, key study	**Mattson, 2016 (In Oekotoxzentrum 2015)**
	Marine	No data	
Invertebrates ($\mu g \cdot L^{-1}$)	**Freshwater**	**Crustaceans, *Ceriodaphnia dubia* / 7 days NOEC: 4.4 (reproduction) Reliability evaluation:1, key study**	**Mattson, 2010 (In Oekotoxzentrum 2015; Carvalho et al., 2015)**
	Marine	No data	
Fish ($\mu g \cdot L^{-1}$)	**Freshwater**	***Pimephales promelas* / 32 days NOEC: 4600 (early life stage toxicity test, OECD 210) Reliability evaluation:1, key study**	**Mattson, 2016 (In Oekotoxzentrum 2015)**
	Marine	No data	

Note: Studies reported in grey were not considered for the EQS derivation.

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

As mention in the section above, freshwater and marine water data were combined for quality standard (QS) derivation without statistical analysis (EC, 2018).

[25] Pfizer, personal communication (2021).

[26] According to the EQS Technical Guidance (EC, 2018), the growth rate endpoint is the more robust endpoint for algae tests, and thus preferred to biomass. Therefore, when data were available for both endpoints from the same study, growth rate was selected, such as in this case.

Deterministic approach
The chronic ecotoxicity data are available for four species, representing three trophic levels: algae, invertebrates and fish. Furthermore, representative species for the most sensitive taxonomic group are included in the dataset. The application of an assessment factor (AF) of 10 (Table 3 in EC, 2018) to the lowest NOEC of 0.19 µg/L for the endpoint of growth rate for the cyanobacteria species *Microcystis aeruginosa* (Mattson, 2016) resulted in an **AA-QS$_{fw,eco}$ of 0.019 µg/L.**

Probabilistic approach
Since only five data points are available for four taxonomic groups, the probabilistic assessment could not be carried out.

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

Deterministic approach

For the marine water QS derivation based on the deterministic approach, there are long-term results from four freshwater species representing three trophic levels (Table 4 in EC, 2018). However, no specifically marine species are present (i.e. marine test organisms other than algae, crustaceans and fish, and/or having a life form or feeding strategy differing from that of algae, crustaceans or fish). Therefore, an AF of 100 was chosen. The application of an AF of 100 to the lowest NOEC of 0.19 µg/L for the endpoint of growth rate in the cyanobacteria species *Microcystis aeruginosa* (Mattson, 2016) resulted in an **AA-QS$_{sw,eco}$ of 0.0019 µg/L** for marine water.

Probabilistic approach

No species sensitivity distribution could be derived for the chronic ecotoxicity dataset based on azithromycin. Details are reported above in the freshwater section.

1.9 7.3. Sediment ecotoxicity

Based on the experimental and estimated Koc values (section 5.1.), azithromycin is expected to adsorb to suspended solids and sediment. Hence, the sediment toxicity assessment should be performed. No sediment toxicity data are available for azithromycin. 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 7.1).

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

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

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

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

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

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

Table 7.1. 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	17782.79 $L \cdot kg^{-1}$ (experimental)	Vermillion Maier and Tjeerdema (2018) (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	889.1395 $L \cdot kg^{-1}$	Equation 1
H	Henry's law constant	5.37E-24 $Pa \cdot m^3 \cdot mol^{-1}$	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)
$K_{air-water}$	air-water partition coefficient	2.26631E-27 $m^3 \cdot m^{-3}$	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	445.36975 $m^3 \cdot m^{-3}$	Equation 3
FRESHWATER			
$QS_{fw,eco}$	quality standard for direct ecotoxicity on freshwater aquatic organisms	1.9E-05 $mg \cdot L^{-1}$	In this dossier (see section 7.3)
$QS_{sed,EqPww}$	wet weight quality standard for sediment based on equilibrium partitioning	0.00650925 $mg \cdot kg_{ww}^{-1}$	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	0.01692 $mg \cdot kg_{dw}^{-1}$	Equation 6
MARINE WATER			
$QS_{mw,eco}$	quality standard for direct ecotoxicity on marine aquatic organisms	1.9E-06$mg \cdot L^{-1}$	In this dossier (see section 7.3)
$QS_{sed,EqPww}$	wet weight quality standard for sediment based on equilibrium partitioning	0.000650925 $mg \cdot kg_{ww}^{-1}$	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	0.001692 $mg \cdot kg_{dw}^{-1}$	Equation 6

The derived $QS_{sediment}$ for azithromycin resulted in a **$QS_{sedEqPdw}$ for freshwater of 16.92 µg/kg_{dw}** and **$QS_{sedEqPdw}$ for saltwater of 1.692 µg/kg_{dw}**.
Based on the Log Kow values, azithromycin was not considered as a highly lipophilic substance, and therefore the additional AF of 10 was not applied to the $QS_{sediment}$ (EC, 2018).

1.10 7.4. Tentative QS_{water}

The following table shows the tentative QS_{water} calculated for azithromycin in the present dossier.

Tentative QS_{water}	**Relevant study for derivation of QS**	**Assessment factor**	**Tentative QS**
$MAC_{freshwater, eco}$	*Microcystis aeruginosa* / 72 h EC_{50}: 1.8 µg·l^{-1} (growth rate)	10	0.18 µg·L $^{-1}$
$MAC_{marine\ water, eco}$		100	0.018 µg·L^{-1}
$AA\text{-}QS_{freshwater, eco}$	*Microcystis aeruginosa* / 72 h NOEC: 0.19 µg·l^{-1} (growth rate)	10	0.019 µg·L^{-1}
$AA\text{-}QS_{marine\ water, eco}$		100	0.0019 µg·L^{-1}
$AA\text{-}QS_{freshwater, sed}$	EqP	-	16.92 µg·kg_{dw}^{-1}
$AA\text{-}QS_{marine\ water, sed}$	EqP	-	1.692 µg·kg_{dw}^{-1}

1.11 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 azithromycin is indicated by the experimental LogKow values of 3.24 (McFarland et al. 1997) and 4.02 (REACH dossier), which both exceed the trigger value of 3 (EC, 2018), and by the several BCF and BAF values listed in Tables 7.2 and 7.3 below, which are above the trigger value of 100 (EC, 2018). 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	Rat / Oral / acute LD_{50} >2000 mg/kg bw (mortality) Reliability : 2 Gastrointestinal: nausea or vomiting. Lungs, thorax or respiration: respiratory depression. Behavioural: somnolence (general depressed activity).	Yakuri, 1996 (In REACH[27])
	Mouse/ Oral / acute **LD_{50} : 3000 mg/kg bw (mortality)** Reliability : 2, key study Gastrointestinal: nausea or vomiting. Lungs, thorax or respiration: respiratory depression. Behavioural: convulsions or effect on seizure threshold.	Yakuri, 1996 (In REACH[28])
	Reproduction studies in rats and mice / oral / the highest dose of 200 mg/kg/day was associated with moderate maternal toxicity / no effects on foetus and no evidence of impaired fertility	REACH[29]; RxList[30]; U.S. FDA Zithromax label[31]
	In three fertility and general reproduction studies in rats, there was decreased fertility at doses of 20 and 30 mg/kg/day. Azithromycin was not fetotoxic or teratogenic in mice and rats at doses that were moderately maternotoxic (up to 200 mg/kg/day).	New Zealand's MedSafe Authority[32]

[27] Available online at : https://echa.europa.eu/registration-dossier/-/registered-dossier/6141/7/3/2/?documentUUID=860059f5-ce42-4466-afd4-32751773b442 (Accessed on April 2021)
[28] Available online at : https://echa.europa.eu/registration-dossier/-/registered-dossier/6141/7/3/2/?documentUUID=6bc2dc1f-40c5-406c-ac0c-99ec2bc0042a (Accessed on April 2021)
[29] Available online at : https://echa.europa.eu/registration-dossier/-/registered-dossier/6141/7/9/1 (Accessed on April 2021)
[30] Zithromax medical leaflet, available online at: https://www.rxlist.com/zithromax-drug.htm#description (Accessed on April 2021)
[31] Available online at : https://www.accessdata.fda.gov/drugsatfda_docs/label/2013/050710s039,050711s036,050784s023lbl.pdf (Accessed on April 2021)
[32] Available online at: https://www.medsafe.govt.nz/profs/Datasheet/z/zithromaxiv.pdf (Accessed on April 2021)

	No teratogenic effects were observed in animal studies of embryotoxicity in mice and rats. In rats, azithromycin doses of 100 and 200 mg/kg bw/day led to mild retardations in foetal ossification and in maternal weight gain. In peri-/postnatal studies in rats, mild retardations following treatment with 50 mg/kg/day azithromycin and above were observed.	Medical leaflet (Sandoz)[33]
Avian oral toxicity	No data	

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

For the derivation of the $QS_{biota,\ sec\ pois,\ fw}$, the **$LD_{50}$ of 3000 mg/kg bw in mice** (Yakuri, 1996) was selected, since the rat LD_{50} was an unbound value, and possible LOAEL values were mostly identified from reproduction toxicity studies. No access to the full study reports and/or publications was possible, due to lack of the original references. Most of the sources identified a maternal toxicity effect at 200 mg/kg bw/day in rats and/or mice. In a few cases, a decreased fertility at 20 mg/kg/day in rats, and mild retardations in foetal ossification at 100 mg/kg bw/day were mentioned. However, it is unknown if these effects were statistically relevant and/or treatment related, and no NOAEL values were provided. Therefore, the LD_{50} of 3000 mg/kg_{bw} observed in mice was chosen, despite the use of acute toxicity studies is not encouraged for deriving a QS_{biota} (EC, 2018).

For the derivation of a biota standard for secondary poisoning, firstly the critical food item should be selected according to the energy contents of the food items, and the bioaccumulation characteristics of the substance through the food chain (EC, 2018). Experimental BMF were not identified in the literature. Based on whole fish concentrations of azithromycin in the marine food web, a trophic magnification factor (TMF) (wet weight) of 0.7 with a 95 % CI (0.5−0.9) was derived (Liu S. et al., 2017).

Available BCF and BAF are listed in Tables 7.2 and 7.3. BCFs derived for the different organs of a sea cucumber (*Apostichopus japonicus*) ranged from 23.9-254.6 L/kg, with BCFs being lower at the higher exposure concentration of 10 µg/L (Zhu M. et al., 2020). A geometric mean cannot be derived, as the weight fraction of the individual organs is unknown, but it can be expected that the mean would be >100 L/kg. Similar BCF values for fish were estimated by the U.S. EPISuite software (v. 4.1, as reported in PubChem), along with a predicted BAF of 12.50 L/kg_{ww} for fish (U.S. EPISuite Software in Ortiz de García et al. 2017). Zhang, R. et al. (2020) derived a mean field BAF of 575.44 L/kg for offshore fish based on average azithromycin concentrations 0.12 ± 0.22 ng/L (Mean ± SD). Whereas, a mean BAF of 204.3 L/kg was measured for the freshwater mussel *Lasmigona costata* in a river receiving wastewater effluent (de Solla et al., 2016).

A higher BAF value is noted for marine fish species, rather than freshwater mussels. However, according to the EQS Technical Guidance (EC, 2018), if either the freshwater or marine water TMF (lipid) is below 0.8, the risk limit should be calculated for bivalves. However, the reported TMF is not lipid normalised and, as experts of the subgroup noted, the biota used for its derivation was not sampled at the same time. Therefore, the trophic magnification study by Liu S. et al. (2017) was not considered reliable, and the TMF value was not used as a guiding value to determine which food item is the most critical one in the $QS_{secpois}$ derivation.

[33] Available online at : https://www.medicines.org.uk/emc/product/6541/smpc#gref (Accessed on April 2021)

Table 7.2.: BCF values reported for azithromycin.

Species	BCF [L/kg]	Tissue	Exposure	Further information	Reference
Sea Cucumber (*Apostichopus japonicus*), Echinoderm	619.0 170.4 23.9 151.4	Body wall Mouth Digestive tract Respiratory tract	1 µg/L	Juveniles, 4 months old (body length: 2−3 cm, wet weight: ~1.0 g) Composite-sand filtered natural sea water 14.3 ± 1.2 °C photoperiod of 12:12 (light/dark) commercial Laboratory, semistatic, daily replacement of 1/3 volume	Zhu M. et al. (2020)[34]
	58.4 112.0 24.4 254.6	Body wall Mouth Digestive tract Respiratory tract	10 µg/L		
Fish	200	Whole body	--	Estimated value	PubChem, 2014 (In Carvalho et al., 2015);
Fish	522	Whole body	--	Estimated value	Oekotoxzentrum (2015)

Table 7.3.: BAF values reported for azithromycin.

Species	BAF [L/kg]	Tissue	Exposure	Further information	Reference
Hydropsyche sp., freshwater insect	site E 5000; site B 14,000; site R 34,000	Whole body	site E 8 ±7 ng /L site B 6±5 ng /L site R 2±1 ng /L LOQ range 1-2ng /L	Czech Republic, water samples collected from the WWTP stream from 24th April to 10th May	Grabicova et al., 2015
Fluted shell (*Lasmigona costata),* freshwater mussel	204.3 (97.8–346.4) wet weight	Whole body	Caged, field, 4 weeks, Mean (SD): 16.73 ng/L (19.78), n=5	Grand River, Ontario, Canada, downstream of a WWTP, from year 2009 to 2011	de Solla et al. (2016)

[34] Azithromycin was taken up slowly and concentrations increased gradually within the 28 day exposure in most of the tissues under study. In the depuration period, the target antibiotics seem to follow biphasic elimination patterns with an initial rapid decrease of the internal concentrations, followed by a slower decrease (Zhu M. et al., 2020).

Species	BAF [L/kg]	Tissue	Exposure	Further information	Reference
Marine fish, a total of 18 species of coral reef fishes	575.44 (logBAF 2.76) wet weight	Whole body	0.12 ± 0.22 ng/L (Mean ± SD)	seawater and coral reef fish samples from the South China Sea[35]	Zhang, R. et al., 2020
Freshwater cyanobacteria and zooplankton	3030–130,000	Whole body	Concentration of azithromycin in surface water <7 ng/L.	Freshwater food web of Lake Taihu, China. September 2015	Zhou, L. J. et al., 2020
Freshwater zooplankton	4800	Whole body	Concentration in water 7.5 ng/L	Czech Republic, wastewater stabilization ponds (WSP)	Grabicova et al., 2020
Freshwater fish, common carp (*Cyprinus carpio*),	1200 1500	Liver Kidney	Concentration in water 7.5 ng/L	n=12, (mean total length 430 ± 20 mm, weight 1760 ± 250 g)	Grabicova et al., 2020
Freshwater fish, Pikeperch (*Sander lucioperca*)	770 690 160	Liver Kidney Brain	Concentration in water 7.5 ng/L	n=12, (mean total length 370 ± 10 mm, weight 480 ± 20 g)	Grabicova et al., 2020
Freshwater fish	12.50 wet weight		--	estimated by EPI Suite™ (US EPA, 2012)	Ortiz de García et al., 2017

For the derivation of the $QS_{biota,\ sec\ pois,\ fw}$, the method A of the EQS Technical Guidance was followed (EC, 2018), due to the selected endpoint of the toxicity test the **LD_{50} of 3000 mg/kg bw in mice** is expressed as a daily dose and no information on food consumption is available. For normalisation of the azithromycin 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 30 g for mice.

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

Equation 7

The diet concentration on an energy basis (mg/kJ) for azithromycin can now be calculated with toxicological endpoint expressed as daily dose (3000 mg/kg bw/d) and the body weight (bw; 0.030 kg), using equation 8.

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

Equation 8

This results in an energy content normalised concentration of azithromycin of **1.215 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

[35] The concentrations of azithromycin and other antibiotics in the coastal fishes were generally lower than those in the offshore fishes, although in the coastal seawater were higher than in the offshore seawater while the lipid contents were higher in the coastal fish than in the offshore fish (Zhang R. et al., 2020).

azithromycin's dossier 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:

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

The standard moisture content and energy content of invertebrates (bivalves) are 92% and 19kJ/g_{dw}, respectively (see Table 7 in EC, 2018). The concentration in the critical food item for bivalves is **1847 mg/kg_{ww}** (bivalves). For fish the standard moisture is 74% and energy content 21 kJ/g_{dw} (Table 7 in EC, 2018), therefore the $C_{food\ item}$ for fish resulted in **6635 mg/kg_{ww}** (fish)**.**
To extrapolate to the required protection level of the ecosystem, the $QS_{biota,\ secpois}$ will be derived by applying an assessment factor of 1000 to the lowest value selected (AF 100 from Table 9, being an acute study, and AF 10 from Table 10 in EC, 2018).

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

The application of the AF of 1000 to the lowest credible chronic datum resulted in a **$QS_{Biota,\ sec\ pois,\ fw}$** of **1.8 mg/$kg_{ww}$ for bivalves** and **6.6 mg/kg_{ww} for fish**.
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:

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

The water standard (**$QS_{water,\ biota}$**) for bivalves using the BAF value of 204.3 L/kg_{ww} for freshwater mussels (de Solla et al., 2016), was calculated to be **9.1 µg/L for bivalves**.
The water standard (**$QS_{water,\ biota}$**) for fish, using the estimated BAF value of 12.5 L/kg_{ww} (Ortiz de García et al., 2017), was calculated to be 0.53 mg/L. However, a higher uncertainty is associated with this value, since it is based on a predicted BAF value.

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. However, as mentioned above the reported TMF by Liu S. et al. (2017) was not considered reliable by the expert's subgroup, and it was not used as a guiding value to determine which food item is the most critical one in the $QS_{secpois}$ derivation. Nevertheless, azithromycin is not expected to biomagnify in small birds or mammals within marine food chains, 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 **1.8 mg/kg_{ww} for bivalves** and **6.6 mg/kg_{ww} for fish**.
For the back calculation to water, using the BAF value of 575.44 L/Kg for marine fish (Zhang, R. et al., 2020) the $QS_{water,\ biota}$ for fish resulted to be 0.0115 mg/L (11.5 µg/L). However, it was agreed among the experts

subgroup on azithromycin to consider the BAF of Zhang, R. et al. (2020) as not reliable, due to uncertainties in the calculation of this BAF value[36].

Tentative QS_{biota}

Tentative QS_{biota}	Relevant study for derivation of QS	Assessment Factor	Tentative QS
Biota	Mouse/ Oral / acute **LD_{50} : 3000 mg/kg $_{bw}$ (mortality)**	1000	**Freshwater:** 1.8 $mg.kg^{-1}_{biota\ ww}$ (for bivalves) corresponding to 9.1 $\mu g.L^{-1}$ 6.6 $mg.kg^{-1}_{ww}$ (for fish) **Marine water:** 1.8 $mg.kg^{-1}_{biota\ ww}$ (for bivalves) 6.6 $mg.kg^{-1}_{ww}$ (for fish)

[36] As experts pointed out, azithromycin was measured in only 2% of the samples, while at all of these sites azithromycin was not detected in water. Furthermore, the reported maximum concentration in one fish is below the reported LOQ in fish.

1.12 7.6. Human health

Human health via consumption of fishery products

Human health via consumption of fishery products		**Master reference**
Mammalian oral toxicity	Rat / Oral / acute / Endpoint not specified LD_{50} >2000 mg/kg bw Reliability : 2 Gastrointestinal: nausea or vomiting. Lungs, thorax or respiration: respiratory depression. Behavioural: somnolence (general depressed activity). Remarks: Migrated information Criteria used for interpretation of results: EU.	Yakuri, 1996 (In REACH[37])
	Mouse/ Oral / acute / Endpoint not specified LD_{50} : 3000 mg/kg bw Reliability : 2 Gastrointestinal: nausea or vomiting. Lungs, thorax or respiration: respiratory depression. Behavioural: convulsions or effect on seizure threshold. Remarks: Migrated information Criteria used for interpretation of results: EU.	Yakuri, 1996 (In REACH[38])
	Reproduction studies in rats and mice / oral / the highest dose of 200 mg/kg/day was associated with moderate maternal toxicity / no effects on foetus and no evidence of impaired fertility	REACH[39]; RxList[40]; U.S. FDA Zithromax label[41]
	In three fertility and general reproduction studies in rats, there was decreased fertility at doses of 20 and 30 mg/kg/day. Azithromycin was not fetotoxic or teratogenic in mice and rats at doses that were moderately maternotoxic (up to 200 mg/kg/day).	New Zealand's MedSafe Authority[42]

[37] Available online at : https://echa.europa.eu/registration-dossier/-/registered-dossier/6141/7/3/2/?documentUUID=860059f5-ce42-4466-afd4-32751773b442 (Accessed on April 2021)
[38] Available online at : https://echa.europa.eu/registration-dossier/-/registered-dossier/6141/7/3/2/?documentUUID=6bc2dc1f-40c5-406c-ac0c-99ec2bc0042a (Accessed on April 2021)
[39] Available online at : https://echa.europa.eu/registration-dossier/-/registered-dossier/6141/7/9/1 (Accessed on April 2021)
[40] Zithromax medical leaflet, available online at: https://www.rxlist.com/zithromax-drug.htm#description (Accessed on April 2021)
[41] Available online at : https://www.accessdata.fda.gov/drugsatfda_docs/label/2013/050710s039,050711s036,050784s023lbl.pdf (Accessed on April 2021)
[42] Available online at: https://www.medsafe.govt.nz/profs/Datasheet/z/zithromaxiv.pdf (Accessed on April 2021)

	No teratogenic effects were observed in animal studies of embryotoxicity in mice and rats. In rats, azithromycin doses of 100 and 200 mg/kg bw/day led to mild retardations in foetal ossification and in maternal weight gain. In peri-/postnatal studies in rats, mild retardations following treatment with 50 mg/kg/day azithromycin and above were observed.	Medical leaflet (Sandoz)[43]
CMR	Long-term studies in animals have not been performed to evaluate carcinogenic potential. Azithromycin has shown no mutagenic potential in standard laboratory tests: mouse lymphoma assay, human lymphocyte clastogenic assay, and mouse bone marrow clastogenic assay. No evidence of impaired fertility due to azithromycin was found. Reproduction studies have been performed in rats and mice at doses up to moderately maternally toxic dose concentrations (i.e., 200 mg/kg/day). In the animal studies, no evidence of harm to the foetus due to azithromycin was found. There are, however, no adequate and well-controlled studies in pregnant women	US FDA, Zithromax label[44]

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, azithromycin is not mutagenic, and its carcinogenic potential was not investigated. Most of the sources identified a maternal toxicity effect at 200 mg/kg bw/day in rats and/or mice. In a few cases, a decreased fertility at 20 mg/kg/day in rats, and mild retardations in foetal ossification at 100 mg/kg bw/day were mentioned. However, it is unknown if these effects were statistically relevant and/or treatment related, and no NOAEL values were provided. No access to the full study reports and/or publications was possible, due to lack of the original references.
A microbiological ADI value of 0.0017 mg/kg day was calculated for azithromycin based on the concentration inhibiting 50% of *Costridium* spp. in the human intestinal flora for a person of about 60 kg, and following a daily water ingestion of 126 mL/kg per day (Leung et al., 2013).
According to the EQS Technical Guidance (EC, 2018), the $QS_{biota,\ hh,\ food}$ should be derived based on the following equation (EC, 2018):

$$QS_{biota,hh\ food}\ [\mu g/\ kg_{biota}] = \frac{0.2 \cdot TL_{hh}}{0.00163}$$ **Equation 13**

[43] Available online at : https://www.medicines.org.uk/emc/product/6541/smpc#gref (Accessed on April 2021)

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, no NOAEL values were identified, and a microbiological ADI was only available. Therefore, based on this data gap, the $QS_{biota,hh}$ could not be derived.

Tentative $QS_{biota, hh}$	**Relevant study for derivation of $QS_{biota, hh}$**	**Assessment Factor**	**Tentative $QS_{biota, hh}$**
Human health	---	---	-- µg.kg^{-1}$_{biota\ ww}$ (-- µg.L^{-1})

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 the following equation:

$$QS_{dw,hh}\ [\mu g/\ L] = \frac{0.2 \cdot TL_{hh} \cdot bw}{Uptake_{dw}} \qquad \textbf{Equation 14}$$

A human body weight (bw) of 70 kg and a daily uptake of drinking water ($uptake_{dw}$) of 2 litres are recommended in the EQS Technical Guidance (EC, 2018). The value for the TL_{hh} should be the acceptable daily intake (ADI) or tolerable daily intake (TDI), if these are available, a reference dose (RfD), or a benchmark dose. If no ADI or TDI is available, the TL_{hh} could be calculated from the $NOAEL_{min}$ (the lowest no observed adverse effect level value from a review of mammalian toxicology data) using the following equation:

$$TL_{hh} = \frac{NOAEL_{min}}{100} \qquad \textbf{Equation 15}$$

Due to the lack of toxicological human health based guidance values and NOAEL values, the TL_{hh} could not be estimated, and therefore the $QS_{dw, hh}$ could not be derived.

Human health via consumption of drinking water		**Master reference**
Existing drinking water standard(s)	-- µg.L^{-1}	
Any guideline		

[44] Available online at: https://www.accessdata.fda.gov/drugsatfda_docs/label/2013/050710s039,050711s036,050784s023lbl.pdf (Accessed on April 2021)

8 Additional Considerations

1.13 8.1. pH-effects

Azithromycin is an ionisable organic chemical, with a dissociation constant pKa value of 8.74, indicating that this compound will exist almost entirely in the cation form in the environment at pH values of 5 to 9 (PubChem[45]).
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 might affect the partition coefficient of azithromycin (i.e., the pH dependent log D_{ow}), 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).

1.14 8.2. Contribution of Azithromycin to antimicrobial resistance

Azithromycin is a broad-spectrum antibiotic, largely used to treat Gram-positive and Gram-negative infections in human medicine (Section 4). Azithromycin was included in the first surface water Watch List (WL) of the European Water Framework Directive (WFD) in 2015 (EU, 2015/495), together with erythromycin and clarithromycin as they belong to the same class (macrolide antibiotics), sharing the same mode of action and analytical method (Carvalho et al., 2015; Loos et al., 2015).
The PNEC value obtained for azithromycin (0.019 µg/L, see Section 7.3) is based on an NOEC of 0.19 $\mu g \cdot L^{-1}$ (*Microcystis aeruginosa*/ 72 h/ growth rate) and using an assessment factor (AF) of 10. It has been observed a rise of azithromycin resistance in *Salmonella* in clinical samples from South Asia (Sajib et al., 2021), and this could pose a serious threat to the health system. Microorganisms exposed to sub-inhibitory concentrations of antibiotics can develop, or acquire, antimicrobial resistance (AMR), which has been identified as a global threat to public health (WHO 2014). Currently, the PNEC derivation for azithromycin is based on ecotoxicology data and it does not take into account the contribution of this substance to the spread of resistance in freshwater environments.
Recently, Bengtsson and Larsson derived a PNEC value for antibiotics using the Minimum Inhibitory Concentration (MIC) data collected from the European Committee on Antimicrobial Susceptibility Testing database (EUCAST) (Bengtsson-Palme & Larsson 2016). This PNEC derivation (PNEC-MIC) is based on the assumption that selective concentrations of antibiotics need to be lower than those that inhibit the bacterial growth. For the calculation, the lowest MIC value was identified and an assessment factor (AF) of 10 was applied considering that the selective concentration must be lower than the inhibitory concentration (Bengtsson-Palme and Larsson 2016). To date, this is the first approach for PNEC derivation taking into consideration the

[45] Available online at: https://pubchem.ncbi.nlm.nih.gov/compound/Azithromycin

contribution to antimicrobial resistance. In the case of azithromycin, the PNEC-MIC (0.25 µg/L) is above the available PNEC value for ecotoxicological effects (see Table 8.1.). In order to be protective with the environment and to lower the pressure on the maintenance of AMR, it has been recommended to use the lower of the two PNEC values (AMR Alliance, 2018; Tell et al., 2019).

Table 8.1. Comparison of Predicted no-effect concentration (PNEC) derived from ecotoxicology data and the new approach using the Minimum Inhibitory Concentrations (PNEC-MIC) for azithromycin.

Azithromycin	
PNEC (µg/L)	**PNEC-MIC (µg/L)**
0.019	0.25*

*(Bengtsson-Palme and Larsson, 2016)

Data from the literature indicates that azithromycin is present in influent and effluent of wastewater treatment plants (WWTP) in concentrations ranging from 45.2 to 597.5 ng/L (Rodriguez-Mozaz et al., 2020; Verlicchi et al., 2014). In some studies, azithromycin was detected at the highest concentrations among the investigated antibiotics analysed in WWTP (Aydin et al., 2019; Kulkarni et al., 2017). A study in Italy showed that azithromycin was the only pharmaceutical measured that was detected slightly higher in the effluent than in the influent of the WWTP, at concentrations 120 ng/L and 130 ng/L respectively, and it was also detected in the receiving waters in a concentration of 7 ng/L (Verlicchi et al., 2014).

The study of Aydin et al. (2019) pointed out an inadequate removal efficiency at the WWTP for antibiotics and the potential risk of azithromycin may have in the receiving waters, especially to fish and algae. The presence of macrolides-resistant has been also detected in aquatic environments (Szczepanowski et al., 2009; Valáriková et al., 2020). A study using the analysis of antibiotic resistance genes (ARG) as an endpoint to evaluate the risk assessment generates a PNEC value based on the detection of different resistance genes (Stanton et al., 2020). For macrolides, the PNEC generated was significantly higher (50 µg/L) than the one derived by MIC data and ecotoxicology data (Table 8.1). This could suggest that the current ecological PNEC may be protective of resistance selection for macrolides, however, this may not be the case for other antibiotics and further investigation may be required.

These data highlight the importance of studies to monitor the impact of anthropogenic sources of antibiotics and its contribution to 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 et al., 2020).

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hexopyranosyl)oxy]-2-ethyl-3,4,10-trihydroxy-3,5,6,8,10,12,14-heptamethyl-11-[[3,4,6-trideoxy-3(dimethylamino)-.beta.-D-xylo-hexopyranosyl]oxy],(2R,3S,4R,5R,8R,10R,11R,12S,13S,14R)- EC number: 617-500-5 | CAS number: 83905-01-5. Available online at: https://echa.europa.eu/registration-dossier/-/registered-dossier/6141 [Consulted 25 February 2021].

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