2021

oekotoxzentrum
centre ecotox
Schweizerisches Zentrum für angewandte Ökotoxikologie
Centre Suisse d'écotoxicologie appliquée

CQC (AA-EQS) and AQC (MAC-EQS) – Proposal by the Ecotox Centre for: Fipronil

First proposal: October 2020 (last bibliographic research)
September 2021 (implementation of the expertise)

Imprint

Publisher

Swiss Centre for Applied Ecotoxicology, 8600 Duebendorf/1015 Lausanne

Commissioned by

FOEN, Federal Office of the Environment, Water Quality Section, 3003 Bern

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Please note that the suggested EQS and contents of this dossier do not necessarily reflect the opinion of the external reviewer.

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Citation Proposal

Alena Tierbach, Alexandra Kroll, Marion Junghans. 2021. CQC (AA-EQS) and AQC (MAC-EQS) – Proposal by the Ecotox Centre for: Fipronil. Dübendorf (CH): Swiss Centre for Applied Ecotoxicology; 112 pp.

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Executive summary

CQC (AA-EQS): 0.00077 µg/L

AQC (MAC-EQS): 0.0032 µg/L

The chronic quality criterion (CQC) and the acute quality criterion (AQC) were derived according to the TGD for EQS of the European Commission (EC 2018a). In order to ensure that the dossiers are internationally comparable, the English terminology of the TGD will be used in the remainder of the dossier. The AQC corresponds to the MAC-EQS ("maximum allowable concentration environmental quality standard") and the CQC corresponds to the AA-EQS ("annual average environmental quality standard"). According to the Swiss Water Protection Ordinance (The Swiss Federal Council, 2020), the CQC should not be compared with an annual average value but with the averaged concentration over two weeks.

Zusammenfassung

CQC (AA-EQS): 0.00077 µg/L

AQC (MAC-EQS): 0.0032 µg/L

Das chronische Qualitätskriterium (CQK) und das akute Qualitätskriterium (AQK) wurden nach dem *TGD for EQS* der Europäischen Kommission (EC 2018a) hergeleitet. Damit die Dossiers international vergleichbar sind, wird im Weiteren die englische Terminologie des TGD verwendet. Der AQK entspricht dabei dem MAC-EQS (“maximum allowable concentration environmental quality standard”) und der CQK entspricht in der Herleitung dem AA-EQS (“annual average environmental quality standard”) soll aber gemäss Schweizer Gewässerschutzverordnung (Der Schweizerische Bundesrat, 2020) nicht mit einem Jahresmittelwert sondern mit der gemittelten Konzentration über 2 Wochen verglichen werden.

Résumé

CQC (AA-EQS): 0.00077 µg/L

AQC (MAC-EQS): 0.0032 µg/L

Le critère de qualité chronique (CQC) et le critère de qualité aiguë (AQC) ont été dérivés selon le TGD for EQS de la Commission européenne (EC 2018a). Afin que les dossiers soient comparables au niveau international, la terminologie anglaise du TGD est utilisée ci-dessous. La CQA correspond à la MAC-EQS (“maximum allowable concentration environmental quality standard”) ou NQE-CMA ("norme de qualité environnementale de la concentration maximale admissible") et la CQC correspond à la AA-EQS (“annual average environmental quality standard”) ou NQE-MA ("norme de qualité environnementale de la moyenne annuelle"). Selon l'ordonnance suisse sur la protection des eaux (Le Conseil fédéral suisse, 2020), la CQC ne doit cependant pas être comparée à une valeur moyenne annuelle, mais à la concentration moyenne sur deux semaines.

Sommario

CQC (AA-EQS): 0.00077 µg/L

AQC (MAC-EQS): 0.0032 µg/L

Il criterio di qualità cronica (CQC) e il criterio di qualità acuta (CQA) sono stati derivati secondo il TGD for TGD della Commissione Europea (EC 2018a). Per garantire che i dossier siano comparabili a livello internazionale, viene utilizzata la terminologia inglese del TGD. Il CQA corrisponde al MAC-EQS (“maximum allowable concentration environmental quality standard”) oppure SQA-CMA ("standard di qualità ambientale a concentrazione massima ammissibile") e il CQC corrisponde al AA-EQS (“annual average environmental quality standard”) oppure SQA-MA ("standard di qualità ambientale medio annuo"). Secondo l'ordinanza svizzera sulla protezione delle acque (Il Consiglio federale svizzero, 2020), tuttavia, il CQC non deve essere confrontato con un valore medio annuo, ma con la concentrazione media su due settimane.

1General Information

Selected information on the substance fipronil relevant for the aquatic environment is presented in this chapter. Registration information and risk assessments referred to are:

- Draft Assessment Report (DAR), Initial risk assessment provided by the rapporteur Member State France for the existing active substance fipronil of the second stage of the review programme referred to in Article 8(2) of Council Directive 91/414/EEC, Rapporteur: France, 2004 (EC, 2004).
- Assessment Report, Directive 98/8/EC concerning the placing biocidal products on the market, Inclusion of active substances in Annex I or IA to Directive 98/8/EC, fipronil, Product-Type 18 (insecticides, acaricides and products to control other arthropods), 2011 (EC, 2011).
- EFSA - Conclusion on the peer review of fipronil, EFSA Scientific Report (2006) 65, 1-110 (EFSA, 2006)
- CLH report for fipronil - Proposal for Harmonised Classification and Labelling, Based on Regulation (EC) No 1272/2008 (CLP Regulation), Annex VI, Part 2, Substance fipronil, 2014 (CLH, 2014).
- United State Environmental Protection Agency (EPA), Registration Review – Preliminary Problem Formulation for Ecological Risk and Environmental Fate, Endangered Species, and Drinking Water Assessments for fipronil (PC Code 129121; DP 387319) 2011 (EPA, 2011).
- Food and Agriculture Organization of the United Nations (FAO), Specifications and Evaluations for Agricultural Pesticides, fipronil, 2009 (FAO, 2009).
- Fipronil –review scope document, Part 2: environmental considerations, Australian Pesticides and Veterinary Medicines Authority (APVMA) 2012 (APVMA, 2012).
- BASF study summary, active substance: fipronil (BAS 350 I), Document III Section A1 to A7, 2011 (BASF, 2011).
- Review of Aquatic Invertebrate Toxicity Studies for fipronil, United States Environmental Protection Agency Washington, D.C. 20460, PC Code: 129121, DP Barcode: D06156 March 22, 2006 (U. EPA, 2006)
- Commission Implementing Regulation (EU) No 781/2013 of 14 August 2013, amending Implementing Regulation (EU) No 540/2011, as regards the conditions of approval of the active substance fipronil, and prohibiting the use and sale of seeds treated with plant protection products containing this active substance, Official Journal of the European Union, 2013 (EC, 2013).
- Commission Delegated Regulation (EU) 2019/330 of 11 December 2018 amending Annexes I and V to Regulation (EU) No 649/2012 of the European Parliament and of the Council concerning the export and import of hazardous chemicals, 2018 (EC, 2018)

- Bower JC, Tjeerdema RS. 2017. Water and Sediment Quality Criteria Report for fipronil. Final Report. Report prepared by the University of California Davis for the Central Valley Regional Water Quality Control Board. March (Bower JC, 2017).

1.1 Identity and physico-chemical properties

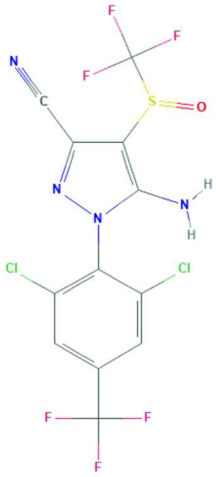
The compound fipronil (ISO) is also referred to by its IPAC name (±)-5-amino-1-(2,6-dichloro-α,α,α-trifluoro-para-tolyl)-4-trifluoromethylsulfinyl-pyrazole-3-carbonitrile, CAS name 5-amino-[2,6-dichloro-4-(trifluoromethyl)phenyl]-4-[(1R,S)-(trifluoromethyl)sulfinyl]-1H-pyrazole-3-carbonitrile, RM1601, AEF124964, M&B46030 or MB46030, (EC, 2004). The active substance is a white powder at room temperature (Chabassol, Hunt,1991a cited in EC (2004) with a relative density of 1.477-1.705 at 20 °C (PubChem, 2021a) (Chabassol and Hunt, 1991a cited in FAO (2009), Nobuhiro, (2001c) cited in EC (2004) Volume 3 –Annex B2, p12) and does not contain inactive isomers (EC, 2004).

The water solubility of fipronil is 3.3 mg/l (geometric mean of valid data provided in **Table 1**). In comparison to water, fipronil has a higher solubility in organic solvents such as acetone ($5.46*10^5$ mg/L at 20 °C), dichloromethane ($22.3*10^3$ mg/L at 20 °C), methanol ($1.38*10^5$ mg/L at 20 °C) and toluene ($3.0*10^3$ mg/L at 20 °C) (PubChem, 2021a) (Chabassol and Reynaud, 1991d cited in EC (2004) Volume 3 –Annex B2, p14).

Fipronil is a chiral molecule and is released into the environment as a 1:1 racemic mixture (equal amounts of two enantiomers). The molecular structure of fipronil contains one chiral center, which is located at the sulfoxide functional group. Chiral molecules can be designated as R or S, based on their configuration (Cahn-Ingold system) and (+) or (-), based on their optically activity. In case of fipronil, the corresponding enantiomers are (+/S) and (-/R) (Teicher, Kofoed-Hansen, & Jacobsen, 2003).

Table 1 summarizes identity and physico-chemical parameters for fipronil required for EQS derivation according to the EU TGD for EQS (EC, 2018). Where available, experimentally collected data is identified as (exp.) and estimated data as (est.). When not identified, no indication is available in the cited literature.

Table 1 Information required for EQS derivation according to the EU TGD for EQS (EC, 2018).

Characteristics	Values	References
Common name	fipronil	EC (2004)
IUPAC name	5-amino-1-[2,6-dichloro-4-(trifluoromethyl)phenyl]-4-(trifluoromethylsulfinyl)pyrazole-3-carbonitrile	PubChem (2021a)
Chemical group	Pyrazole (Phenylpyrazole)	PubChem (2021a)
Structural formula		PubChem (2021a)
Molecular formula	$C_{12}H_4Cl_2F_6N_4OS$	EC (2004)
CAS	120068-37-3	EC (2004)
CIPAC number	581	EC (2004)
EC Number	424-610-5 and 601-663-4	PubChem (2021a)
SMILES code	C1=C(C=C(C(=C1Cl)N2C(=C(C(=N2)C#N)S(=O)C(F)(F)F)N)Cl)C(F)(F)F	PubChem (2021a)
Molecular weight [g/mol]	437.15 g/mol	EC (2004)
Melting point [°C]	203 (99.3 %)[a] (mean melting point of technical fipronil) 195.5–203[b] 204.1 – 204.5 (exp.), capillary method in metal block and DSC[c] 200.5[d] 203.92[d]	a) Chabert, Lecourt, 1996a cited in EC (2004), Volume 3 – Annex B2, p12. b) APVMA (2012) c) Daum, A. 2004, cited in CLH (2014) d) USEPA 2011, cited in UCDAVIS
Boiling point [°C]	Not required (solid)[a] At ca. 220°C decomposition started indicated by an exothermic effect and gas evolution at 238°C. No boiling point was observed, (exp.), measured, capillary method in metal block and DSC[b]	a) EC (2004), Volume 3 –Annex B2, p12. b) Daum, A. 2004, cited in CLH (2014)

Characteristics	Values	References
Vapour pressure [Pa]	$2*10^{-6}$ at 25°C (exp.), gas saturation method [1a] 3.5*10-5 Pa at 50°C (exp.) gas saturation method[a] $3.7*10^{-7}$ at 25°Cgas saturation method, not accepted, purity <98 [b,] < 2.0×10^{-6} at 25°C (exp), gas flow method[c] 1.51×10^{-7} at 25°C[d] 3.71×10^{-7} at 25°C[d] 2.0×10^{-5} at 25°C[e] 1.9×10^{-6} at 25°C[f]	a) Nobuhiro, 2001a cited in EC (2004), Volume 3 –Annex B2, p12 b) Chabassol and Reynaud, 1991d cited inEC (2004), Volume 3 –Annex B2, p12, FAO (2009) c) Nobuhiro, K. 2001x, cited in CLH (2014) d) USEPA 2011, cited in Bower JC (2017) e) PPDB 2015, cited in Bower JC (2017) f) Goel 2007, cited in Bower JC (2017)
Henry's law constant [$Pa \cdot m^3 \cdot mol^{-1}$]	$2.31*10^{-4}$ at 25°C (est.)	Bascou, 2002f cited in EC (2004), Volume 3 –Annex B2, p12
Water solubility [$mg \cdot l^{-1}$]	pH 5: 2.4 at 20°C, column elution, (exp.)[a] pH 7: 1.9 at 20°C, column elution, (exp.)[a,] pH 9: 2.2 at 20°C, column elution, (exp.)[a] pH 4: 5.29 at 20°C, column elution, (exp.)[b] pH 7: 3.35 at 20°C, column elution, (exp.)[b] pH 9: 3.97 at 20°C, column elution, (exp.)[b] pH 5.7: 5.84 at 20°C, in deionised water, column elution, (exp.), [b] pH 6.6: 3.78 at 20°C, column elution, (exp.) in deionised water[c] pH 7: 3 at 25°C, buffered[d] **geometric mean: 3.3**	a) Chabassol, Reynaud, 1991c cited in EC (2004) Volume 3 –Annex B2, p14, CLH (2014) and FAO (2009) b) Daum, A. 2005, cited in CLH (2014) c) Nobuhiro, 2001b cited in EC (2004) Volume 3 –Annex B2, p14 and CLH (2014) d) Buddle, 1991 cited in FAO (2009)
Dissociation constant (pK_a)	cannot be determined[a] Not provided. As an amine, fipronil would be expected to be basic, but the lack of variation of solubility with pH suggests the degree of dissociation in aqueous media is not significant [b]	a) Cichy, 2001c cited in EC (2004), Volume 3 – Annex B2, p12 b) APVMA (2012)
Octanol-water partition coefficient (log K_{ow})	**4.0 at 20°C[a] (Flask method) (exp.)** 3.5 at 20°C[b] (HPLC method) (est.) 3.68-6.64 (geomean:4.45)[c]	a) Chabassol Reynaud, 1991b cited in EC (2004) Volume 3 –Annex B2, p12, CLH (2014) and FAO (2009) b) Cousin, 1997b EC (2004), Volume 3 – Annex B2, p12, CLH (2014) and FAO (2009) c) Bower JC (2017)

[1] Outside of the recommended range of 10^{-5}-10^{3} Pa (EC, 2018)

Characteristics	Values	References
Sediment/soil-water partition coefficient (log K_{oc} or K_p)	logK_{oc} 2.63 (Loamy sand) (est.)[a] logK_{oc} 3.10 (Sandy loam) (est.)[a] logK_{oc} 2.69 (Loam) (est.)[a] logK_{oc} 2.90 (Sandy clay loam 1) (est.)[a] logK_{oc} 2.83 (Sandy clay loam 2) (est.)[a] (5 soils, pH 5.6-8.2, OC 0.5-4.9%, batch sorption data. Freundlich isotherms calculated → Freundlich organic carbon normalized partitioning coefficient (Kfoc) values (l/kg))[a] logK_{oc} = 3.1 (est.) (logK_{OC}=0.52*logK_{OW}+1.02)[b] **Log K_{oc} (geometric mean) =2.87**	a) Godward P.J., Austin D.J., Quarmby D.L. 1992b, plus amendment April 1996, cited in EC (2004), Volume 3 - Annex B8, p466 b) Sabljic et al, 1995 cited in (EC 2018b)
Aqueous hydrolysis DT_{50}	pH 5: stable pH 7: stable pH 9: 28 days (turns to the corresponding amide RPA200766)	Corgier, Plewa, 1992a cited in EC (2004), Volume 3 – Annex B2, p15
Aqueous photolysis DT_{50}	pH 5, 25°C: 0.33 day (major transformation product is MB46513)	Corgier, Plewa 1992b cited in EC (2004), Volume 3 –Annex B2, p12
Biodegradation in aqueous environment DT_{50} [d]	DT_{50} water :14.2-93.6 DT_{50} whole system :16.4-119.6 (dissipation study in 5 water-sediment systems, pH range : 5.8-8.2 and OC range 0.4-3.2%)	Lowden P. and Mahay N.; 2000,cited in EC (2004), Volume 3 –Annex B8, p485

1.2 Regulatory context and environmental limits

The active substance fipronil has been approved under Regulation (EC) No 1107/2009. New insights on the active substance, however, indicated considerable risks to honeybees and resulted in the review of fipronil in 2013. Data collected during the review identified high acute risk for bees resulting from dust. Based on the data, unacceptable effects on colony survival and development could not be excluded. To provide risk mitigation measures for the protection of bees, authorities imposed further restrictions on the use of fipronil in plant protection products. Fipronil treated seeds of crops are not approved for the market. The only exceptions to this are seeds intended to be sown in greenhouses, and seeds of vegetables intended to be sown in fields and harvested before flowering (leek, onions, shallots, and brassica vegetables (EC, 2013).

During the process of approval renewal, a supplementary dossier was not submitted by the applicants resulting in expiration of fipronil approval. Consequently, the use of the active substance fipronil in plant protection products is prohibited in the EU and Switzerland (EC, 2018). In Switzerland, fipronil containing pesticide products were banned from the market already in 2014.

Nonetheless, fipronil is authorized and regulated by the Swiss Veterinary Medicinal Products Ordinance (TAMV) and Biocidal Products Regulation (VBP) as a treatment against fleas and ticks in domestic pets that are not food-producing animals (e.g. dogs and cats). Most fipronil products are

applied either as a spray or by drops administered on the skin of the animal (CliniPharm, 2020, Oct 17). In the Netherlands, veterinarians sell ~3000 kg of fipronil per year as an active ingredient in anti-parasitic products (Moermond C.T.A, 2020).

For the protection of aquatic freshwater communities and sustainable use of pesticides, the German Environment Agency (Umweltbundesamt – UBA) defined a regulatory acceptable concentration (RAK) for fipronil of 0.00077 µg/l. This value was derived based on the NOEC (0.0077 µg/L) in combination with an assessment factor of 10. The NOEC was derived in a toxicity assay by monitoring changes in the body length of the crustacean *Americamysis bahia* (synonym to *Mysidopsis bahia*) during a 28 day exposure period (Machado, 1995).

In the Netherlands, an *ad hoc* maximum permissible risk level (MTR) of 0.00007 µg/l was derived for surface freshwater based on a toxicity assay with the most sensitive species, the crustacean *Americamysis bahia* (synonym to *Mysidopsis bahia*). In this case casethe 96-h LC_{50} value of 0.00014 mg/l for *Americamysis bahia* was used in combination with the assessment factors AF_{water} = 1000 and Af_{dv}[2] =2 (Margriet Beek, 2008).

In view of the Directive 98/8/EC concerning the placing biocidal products on the market, the inclusion of the active substance fipronil in Annex I or IA to Directive 98/8/EC was assessed. In the assessment report, an $PNEC_{surface\ water}$ of 0.012 µg/L was derived based on the lowest reported NOEC of 0.121 µg/L (28 d) for the midge, *Chironomus riparius* in combination with an assessment factor of 10 (EC, 2011).

Table 2 summarizes existing regulation and environmental limits in Switzerland, Europe and elsewhere for fipronil. Existing PNEC/Environmental quality standards are listed in **Table 3**. Please note that the information provided in **Table 2** and **Table 3** may have changed since finalization of this dossier.

[2] Assessment factor for secondary poisoning: $Af_{doorvergiftiging}$

Table 2 Existing regulation and environmental limits for fipronil in Switzerland and Europe

Europe	
ECHA Classification and Labelling (EC, 2011)	Indication of danger N - dangerous for the environment T – toxic Risk phrases R23/24/25 - Toxic by inhalation, in contact with skin and if swallowed R48/25 - Toxic: danger of serious damage to health by prolonged exposure if swallowed R50/53 - Very toxic to aquatic organisms, may cause long-term adverse effects in the aquatic environment Safety phrases S1/2 - keep locked up and out of reach of children S36/37 - wear suitable protective clothing and gloves S45 - in case of accident or if you feel unwell, seek medical advice immediately (show the label where possible) S60 - this material and its container must be disposed of as hazardous waste S61 - avoid release to the environment. refer to special instructions/safety data sheets
International	
UN risk class (ref, UN2015)	6.1 – POISON Acute Tox. 3, H311 Acute Tox. 3, H331 Acute Tox. 3, H301 Aquatic Acute 1, H400 Aquatic Chronic 1, H410 STOT RE 1, H372

Table 3 PNEC and Environmental Quality Standards available from authorities and reported in the literature

Description	Value [µg/L]	Development method	References
Germany, RAC	0.00077	28 d, NOEC (0.0077 µg/L), body length, *A. bahia,* AF=10	(UBA, 2020)
Netherlands: ad hoc. MTR	0.00007	96-h, LC_{50}, (0.00014 mg/l), lethality, *A. bahia,* AF_{water} = 1000 and Af_{dv} = 2.	(Margriet Beek, 2008)
Inclusion of active substances in Annex I or IA to Directive 98/8/EC – Assessment Report: PNEC	0.012	28 d, NOEC (0.121 µg/L), *C. riparius,* AF=10	(EC, 2011)

1.3 Use and emissions

Fipronil is a broad-spectrum insecticide, which is effective against ants, beetles, roaches, fleas, ticks, termites, and other insects. The active substance is incorporated into various pesticide products, such as gel baits against roaches and ants (e.g. Goliath®, Nexa®), termite control products (e.g. Termidor®), as seed dressing and for soil treatment (e.g Mundial®, Regent 500FS®). Additionally, it is used as veterinary medicine, for the control of parasites on pets, such as dogs and cats (Frontline®, Effipro®, Fiproclear®). In Switzerland, fipronil is authorized exclusively as a treatment against fleas and ticks in domestic pets that are not food-producing animals (e.g. dogs and cats) (see 1.2).

Research has indicated that the application of fipronil containing veterinary products can result in fipronil wash off into surface waters at locations where treated pets swim. In addition, down-the-drain

transport is likely to occur during routine bathing of pets and through the washing of textiles in contact with the pets (clothes, interior surfaces or animal bedding). A study in California showed that, depending on the time passed since the initial application, the rinsate from the pets could contain 20% (2 d) to 4% (28 d) of the initially applied mass. This way, fipronil can enter a sewer system and can pose a risk to the aquatic organisms downstream of wastewater discharge (Teerlink, Hernandez, & Budd, 2017). Additionally, a study from the Netherlands (Guldemond et al. 2019 cited by Swissmedic (2020)) reports that fipronil has been detected in samples taken from dead great tit (*Parus major*) nestlings and was found on dog and cat hairs used by tits to build their nests. According to this study, these residues may be toxic to young great tits.

1.4 Mode of action

The active substance fipronil belongs to the phenyl-pyrazole family and, although phenyl-pyrazoles principally act as herbicides, fipronil has proven to be a highly potent systemic insecticide. Fipronil is taken up *via* contact or ingestion, and acts by binding to glutamate- and γ-aminobutiric acid (GABA) gated chloride channels. When functioning properly, these receptors have an inhibitory effect on neurotransmission by timely opening of chloride channels, which supports the nerves to return from excitation to their resting state. Binding of fipronil to the receptors however, disrupts the pre- and postsynaptic passage of chloride ions and leads to hyperexcitation of the nervous system. Depending on the dose, the continuously stimulation of neurons can induce adverse effects, including paralysis and death, of target and non-target organisms (R. C. Gupta & Milatovic, 2014) (FAO, 2009).

Glutamate-gated chloride channels can be found exclusively in protostome invertebrate phyla (such as in mollusks, flatworms, roundworms, ticks, or mites). Hovever, they are closely related to mammalian glycine receptors (Wolstenholme, 2012). Additionally, fipronil shows a higher binding affinity to invertebrate GABA receptors as compared with mammalian receptors (Ratra & Casida, 2001). The selectivity of fipronil for invertebrate receptors adds a level of safety for vertebrate animals and humans, which might come into contact with the active ingredient (R. C. Gupta & Milatovic, 2014) (FAO, 2009).

Within the environment, fipronil is readily metabolized to fipronil-sulfone (Tang, Usmani, Hodgson, & Rose, 2004) (Hodgson, 2012). Like the parent compound, fipronil sulfone is a potent inhibitor of glutamate-gated chloride channels in protostome organisms and also targets invertebrate GABA receptors as effective as fipronil (Zhao, Yeh, Salgado, & Narahashi, 2005). The affinity of fipronil sulfone towards mammalian GABA receptors is however, much higher than the parent compound (R. C. Gupta & Milatovic, 2014) (Hainzl, Cole, & Casida, 1998; Zhao et al., 2005). In the environment,

photodecomposition of fipronil leads to the formation of desulfinyl derivative. The fipronil desulfinyl derivative also shows a higher affinity to vertebrate GABA receptors when compared to the parent compound (Hainzl & Casida, 1996; Ying & Kookana, 2002). Despite the potency of fipronil transfromtion products to adverselyeffect mammalian GABA receptors, the intrinsic selectivity between insects and mammal still remains as the binding affinities of the transformation products towards mammalian receptors are less compared to the invertebrates[3] (Hainzl et al., 1998)

Fipronil is released into the environment as a mixture of two enantiomers (+/S and -/R). Toxicity data indicate that some organisms are affected by fipronil in an enantioselective manner. The results also show that the most toxic isomer is organism dependent (Baird et al., 2013; Konwick, Fisk, Garrison, Avants, & Black, 2005; Overmyer et al., 2007; Qu, Ma, Liu, Gao, et al., 2016; Qu et al., 2014; Wilson, Konwick, Garrison, Avants, & Black, 2008). Further information on enantioselective toxicity is provided in Annex II (13.1).

The estrogen antagonism effect of fipronil and its enatiomeres was tested using *in vitro* reporter gene assays. Data showed, that fipronil exhibited enantioselective behaviour with the (-/R) enantiomer showing the strongest effect followed by the racemate and the (+/S) enantiomer[4] (Song et al., 2017). However, in short-term and long-term toxicity studies in rodents and dogs, fipronil did not indicate endocrine disruption potential, based on histopathological endpoints, effects on fertility, reproductive performance and survival of the offsprings (Evans, 2005). To investigate the genotoxic potential of fipronil, *in vitro* and *in vivo* studies were performed. Data collected within these studies do not demonstrate genotoxic or carcinogenic potential (EC, 2004; EFSA, 2006). In a two-year rat study, the formation of thyroid tumors was observed (EC, 2004). However, in view of the sensitivity of the rat to substances causing thyroid hormone imbalance, it was concluded that the induction of thyroid tumors is an indirect rat-specific effect (EC, 2004; EFSA, 2006).

[3] The selectivity ratio of the GABA receptor (IC_{50} human/IC_{50} insect) is fipronil: 135, sulfone: 17 and desulfinyl derivative: 16 (Hainzl et al., 1998)

[4] Reative luciferase activity (%) decreased significantly at concentrtaions > 5*10-7 M in case of the (-/R) enantiomer and >5*10-6 in case of the racemate. The (+/S) enantiomer had significant effect on the Reative luciferase activity as compared to the control (Song et al., 2017).

2 Environmental fate

2.1 Stability and transformation products

***Air**:*

Fipronil has a low potential for volatilization based on its vapor pressure and Henry law constant (***Table 1***) is therefore expected to be present in the air only during application (e.g. if used as a spray) (EFSA, 2006). For instance, the emission of fipronil in fine particles/dust may originate from seed coatings, if pneumatic sowing equipment is used (EFSA 2006). However, fipronil can be enriched in fine particles (especially in dust with high organic carbon content) (Richards, Reif, Luo, & Gan, 2016). The parent compound and its transformation products desulfinyl fipronil (photolysis), fipronil sulfone (oxidation), and fipronil sulfide (reduction) were reported in dust samples and the data suggest a rapid transformation of fipronil to its biologically active intermediates. Occurrence of fipronil and its derivates on fine particles can facilitate dust-borne wind transport (Richards et al., 2016).

Soil:

The adsorption constant is strongly dependent on the soil type and ranges from K_a = 4.19 (sandy loam) to 20.69 (loam) (CLH, 2014). Fipronil can also bind to organic functional groups, leading to the observation that soil with high organic carbon content can retain fipronil more effectively than soils with low organic content (Log K_{oc} (geometric mean) =2.87, ***Table 1***). The sorption of fipronil is considered to be reversible with similar mechanisms being involved in adsorption and desorption (CLH, 2014). The soil binding properties of fipronil suggest medium to low soil mobility and provide some level of protection against runoff and ground/surface water contamination (CLH, 2014; EC, 2011).

Laboratory and field studies indicate that fipronil is persistent in soil, when the compound is shielded from light by soil particles. In the soil compartment, fipronil is subject to transformation reactions. The transformation rate is temperature dependent with higher transformation rates observed at 20°C than at 10°C (EC, 2011) (CLH, 2014). Degradation can also be related to the soil microbial biomass activity (CLH, 2014). For instance, enantioselective microbial transformation of fipronil is indicated by biodegradation experiments in anoxic sediments (Jones, Mazur, Kenneke, & Garrison, 2007). The half-life (DT_{50}) of fipronil under aerobic conditions was assessed in several studies, with a geometric mean value of 334 days (converted to the average EU outdoor temperature of 12°C) (CLH, 2014; EC, 2011).

Under dark, aerobic conditions, fipronil is transformed *via* hydrolysis to fipronil amide (RPA 200766) and *via* oxidation to fipronil sulfone (MB 46136); fipronil amide and fipronil sulfone are considered as major soil transformation products. Additionally, the transformation product produced *via* reduction (fipronil sulfide, MB 45950) can be found in low quantities (CLH, 2014; EFSA, 2006). Under laboratory

dark aerobic conditions fipronil amide (RPA 200766) accounted for max. 38.4% after 219 days, fipronil sulfone (MB 46136) accounted for max. 34.3% after 162 days and fipronil sulfide, MB 45950) accounted for max. 17% of AR after 91 days (EFSA 2006).

Exposure to sunlight at the surface of particles leads to the production of fipronil desulfinyl (MB 46513) and RPA 104615. The contribution of photolysis to the environmental dissipation of fipronil is strongly depended on the light intensity at the certain location (EFSA, 2006). Under laboratory aerobic conditions with UV irradiation fipronil desulfinyl (MB 46513) accounted for max. 6.9 % after 30 days and RPA 104615 accounted for max. 7.2 % AR after 21 days (EFSA 2006).

In soil, fipronil sulfone (MB 46136) is considered to be highly persistent (DT_{50} = 185 – 280.5 days) and immobile to slightly mobile (K_{oc}=1448–6745 L/kg). Similarly, fipronil sulfide (MB 45950) is medium to highly persistent (DT_{50} = 89 – 224 days) and immobile to slightly mobile (K_{oc}=1695–5621 L/kg). Fipronil desulfinyl (MB 46513) is moderately to medium persistent (DT_{50} = 46.5 – 98 days) and has a low mobility (K_{oc}= 1150–1498 L/kg) and fipronil amide (RPA 200766) is highly persistent (DT_{50} = 107 – 149 days), and medium to highly mobile (K_{oc} = 96–203 L/kg) (EFSA, 2006). In general, fipronil and its transformation products have been suggested to be moderately to highly persistent in soil (APVMA, 2012; EFSA, 2006).

Screening tests for biodegradation:

Fipronil has been tested for ready biodegradability in an aerobic aqueous medium OECD 301B (CO_2 evolution test). Fipronil attained 47% degradation after 28 days and thus, according to the conditions of the OECD guideline, cannot be considered readily biodegradable (CLH, 2014).

Water:

Fipronil has a relatively low water solubility (**Table 1**). The stability of fipronil in water is mainly influenced by the pH and light intensity. Fipronil has proven to be stable to hydrolysis under acidic (pH 5) and neutral (pH 7) conditions. At alkaline pH (pH 9) however, fipronil is hydrolysed to fipronil amide (RPA 200766) following the pseudo-first order kinetics with a half-life of 28 days (at 25°C, rate constant k = 0.0243 day^{-1}) and 75.2 days (at 12°C, rate constant k = 0.009 day^{-1}) (EC, 2011; EFSA, 2006).

In absence of light, fipronil has been reported to be stable. However, under environmental light conditions, photolysis leads to the formation of aniline derivates, with fipronil-desulfinyl (MB 46513, 43.4%) being the main photoproduct. Photolysis under natural light is the main transformation pathway of fipronil in natural waters. The photolysis rate depends on water depth and turbidity. Additionally dissolved organic matter can inhibit fipronil photolysis *via* light-shielding (S. S. Walse,

Morgan, Kong, & Ferry, 2004). Generally, photolysis follows the first order kinetics with a half-life in the range of hours (EC, 2011), depending on depth, OC content and light intensity.

The presence of organisms in water, can lead to an enantioselective degradation of fipronil. In algae suspensions for instance, fipronil was degraded in an enantioselective manner, with (-/R) enantiomer showing a shorter half-live as compared with the (+/S) enantiomer (Qu et al., 2014).

Water/Sediment System: In water sediment systems, fipronil steadily partitions into the sediment. In the water phase, the major metabolite is fipronil amide (RPA 200766, 20% at 244 days) followed by fipronil sulfide (MB 45950, 8.9 % at 93 days) and fipronil sulfone (MB 46136, 2.3 % at 244 days). Within the sediment, fipronil is partially degraded *via* reduction to fipronil sulfide (MB 45950, 80% at 120 days)[5] and *via* hydrolysis to fipronil amide (RPA 200766, 11% at 60 days). A small proportion of the parent compound is also oxidized to fipronil sulfone (MB 46136, 4.9 % at 244 days)[6] (CLH, 2014; EFSA, 2006). Due to sediment sorption and transformation, long-range transport and deposition of fipronil may be considered negligible (EFSA, 2006).

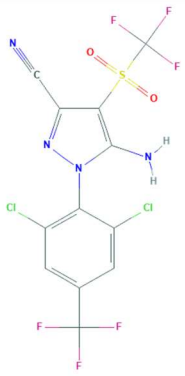
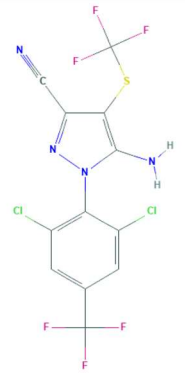
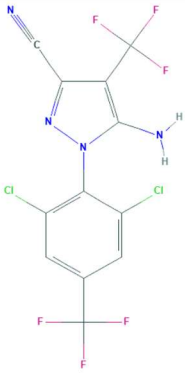
The transformation of fipronil in the water-sediment system follows linear first order kinetics with DT_{50} in water of 14.2 to 93.6 days and DT_{50} in the whole system of 16.4 to 119.6 days (EFSA, 2006). The concentration of fipronil sulfone (MB 46136) decreases steadily in the water phase with DT_{50} of 4.2 to 9.9 days. The compound is adsorbed to the sediment and slowly degraded (EFSA, 2006). The soil metabolite fipronil sulfide (MB 45950) however, is more persistent as compared to the parent compound. Due to a very slow decrease in concentration, a DT_{50} could not be measured and therefore was estimated (DT_{50} est. of 50.2 to 78.8 days, whole system (EFSA, 2006). In addition, the photolysis product, fipronil desulfinyl (MB 46513) adsorbs quickly to the sediment and is rather slowly degraded. The transformation products fipronil sulfide (MB 45950) and fipronil desulfinyl (MB 46513) tend to be persistent in the sediment.

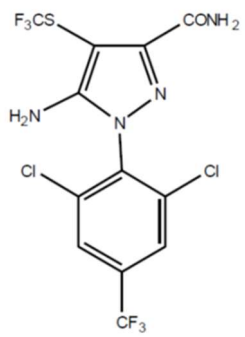
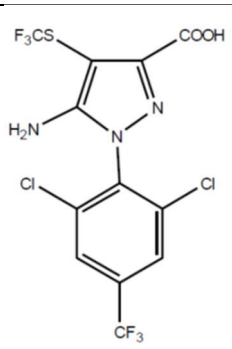
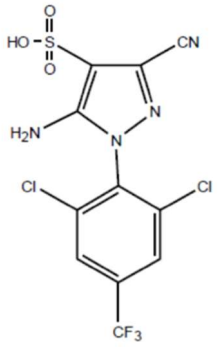
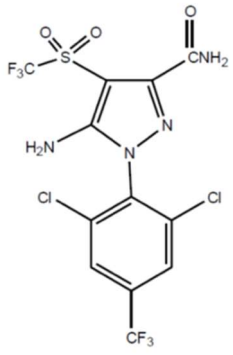
Table 4 summarizes the transformation products of fipronil and provides their CAS- number and chemical formulae, if available. A short summary of the transformation processes (photolysis, hydrolysis and redox reactions) with the resulting products is presented in Annex II (Section 13.2).

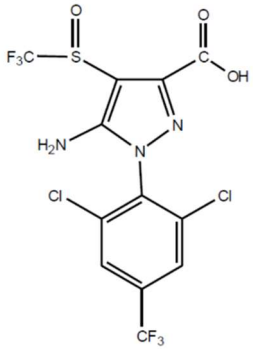
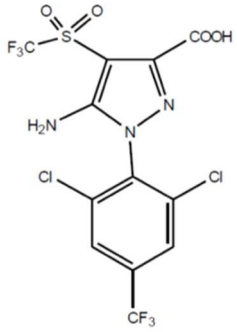
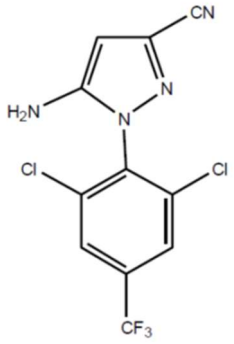
[5] Fipronil sulfide (MB 45950) can be further degraded *via* hydrolysis to MB 46126

[6] Fipronil amide (RPA 200766) and Fipronil sulfone (MB 46136) can be further transformed to RPA 105320

Table 4 Degradation products of fipronil with the underlying transformation reaction, CAS number and chemical formulae.

Name		Transformation reaction	CAS	Chemical formulae	References
Fipronil sulfone	MB 46136 5-amino-1-(2,6-dichloro-α,α,α-trifluoro-p-tolyl)-4-trifluoro-methylsulfonylpyrazole-3-carbonitrile	oxidation	120068-36-2		PubChem (2021b) EFSA (2006)
Fipronil sulfide	MB 45950 5-amino-1-(2,6-dichloro-4-(trifluoromethyl)phenyl)-4-trifluoromethylthio-1-pyrazole-3-carbonitrile	reduction	120067-83-6		PubChem (2021d) EFSA (2006)
Fipronil desulfinyl	MB 46513 5-amino-1-(2,6-dichloro-α,α,α-trifluoro-p-tolyl)-4-trifluoro-methylpyrazole-3-carbonitrile	photolysis	205650-65-3		PubChem (2021e) EFSA (2006)
Fipronil amide (carboxamide)	RPA 200766 5-amino-1-(2,6-dichloro-4-(trifluoromethyl)phenyl)-4-trifluoromethylsulfonyl-1H-pyrazole-3-carboxamide	hydrolysis	205650-69-7		PubChem (2021c) EFSA (2006)

Name		Transformation reaction	CAS	Chemical formulae	References
N.A.	MB 46126	Hydrolysis of MB 45950	N.A.		APVMA (2012)
N.A:	MB 46233	Hydrolysis of MB 46126	N.A.		APVMA (2012)
Fipronil sulfonate	RPA 104615 5-amino-3-cyano-1-(2,6-dichloro-4-trifluoromethylphenyl) pyrazole-4-sulfonic acid, potassium salt	photolysis of fipronil sulfone (MB 46136)	N.A.		APVMA (2012) EFSA (2006)
Fipronil sulfone-amide	RPA 105320 5-amino-3-carbamyl-1-(2,6-dichloro-4-trifluoromethylphenyl)-4-trifluoromethylsulfonylpyrazole	Hydrolysis of MB 46136	N.A.		APVMA (2012)

Name		Transformation reaction	CAS	Chemical formulae	References
N.A.	RPA 200761 5-amino-1-(2,6-dichloro-4-trifluoromethylphenyl)-4-trifluoromethylsulfonylpyrazole-3-carboxylic acid	hydrolysis of fipronil amide (RPA200766)	N.A.		APVMA (2012) EFSA (2006)
N.A.	RPA 106681	Hydrolysis of RPA 105320	N.A.		APVMA (2012)
N.A.	MB 45897 5-amino-1-(2,6-dichloro-α,α,α-trifluoro-p-tolyl)-1H-pyrazole-3-carbonitrile	Displacement product, possibly from RPA 104615	N.A.		APVMA (2012) EFSA (2006)

2.2 Bioavailability

Bioavailability is a complex process which depends on many factors including the sorption capacity of the dissolved organic carbon (DOC) in water and sediment (e.g. OC content), the hydrophobicity of the compound, and the physiology, feeding behaviour and activity of the organism considered (Warren, Allan, Carter, House, & Parker, 2003). As stated in the EU TGD for EQS, total and dissolved concentrations of very hydrophic substances with K_p values above 10000 L/kg or K_{oc} values for linear partitioning into amorphous organic matter above 100000 L/kg, may differ. Thus, for compounds with log K_p < 4 (or, if this value is not available, log K_{ow} < 6) the $EQS_{water,\ total}$ is equivalent to the $EQS_{water,\ dissolved}$ (EC, 2018).

Based on the available literature, no statement can be made regarding the bioavailabilty of fipronil and its transformation products. Information on how the sorption of these compounds on solids, dissobled solids or the freely dissolved state influences their bioavaiability, is not available. However, the log K_{ow} values for fipronil and some transformation productsare available (***Table 5***). In addition, summary of organic carbon sorption partition coefficients is provided in Bower JC (2017) and (EPA, 2011).

Table 5 summarizes the log K_{ow} and K_{oc} values for fipronil and its transformation products.

Table 5: Log K_{ow} and K_{oc} for fipronil and its transformation products.

Name	logK_{ow}		K_{oc}, L/kg	
Fipronil	3.5 (est.)[a] 4.0 (exp)[b] 3.68-6.64 (geomean:4.45)[c]	a) Chabassol Reynaud, 199 cited in EC (2004) Volume 3 –Annex B2, p12 b) Cousin, 1997, cited in EC (2004) Volume 3 – Annex B2, p12 c) Bower JC (2017)	K_{oc} (geometric mean) =741.31	Table 1
Fipronil sulfone (MB 46136)	3.8[a] 3.4[b] 4.82/4.92 (geomean:4.56)[c]	a) Cousin, 1997i cited in EC (2004) Volume 3 – Annex B2, p12b) Cousin, 1997g cited in EC (2004) Volume 3 –Annex B2, p12 c) Bower JC (2017)	153623[a] 1448–6745* (geomean: 3638.9) (exp)[b]	a) Bower JC (2017) b) (EPA, 2011)
Fipronil sulfide (MB 45950)	3.7[a] 4.82/4.92 (geomean:4.87)[b]	a) Cousin, 1998a cited in DAR Vol3, B1-B5, p14 b) Bower JC (2017)	40904[a] 1695-5621* (geomean: 4155.5) (exp)[b]	a) Bower JC (2017) b) (EPA, 2011)
Fipronil amide (RPA 200766)	5.43	U. EPA (2020)	96-203*	(EFSA, 2006)
Fipronil desulfinyl (MB 46513)	3.4[a] 4.22/4.16 (geomean:4.56)[b]	a) Cousin, 1997d cited in FAO b) Bower JC (2017)	1310a 11550-1498* (geomean: 1282.9) (exp)[b]	a) Bower JC (2017) b) (EPA, 2011)

* *Batch adsorption / desorption studies (exp.)*

The possibility that fipronil and its transformation products might compete for adsorption sites of particles in the water column (Masutti & Mermut, 2007) cannot be excluded. However, to fully assess to what extend the compounds are freely dissolved or sorbed to particles such as dissolved or suspended carbon, further data are required. Based on the logK_{ow} /K_{oc} values, it is recommended to assume the total concentration of fipronil and its transformation products as dissolved ($EQS_{water,\ total}$ equivalent to the $EQS_{water,dissolved}$), until more information is available to assess the bioavailability.

2.3 Bioaccumulation and biomagnification

Since fipronil and its transformation products have a log K_{ow} greater than three (***Table 1***, ***Table 5***), the potential for bioaccumulation needs to be considered. Additionally, as fipronil is a chiral insecticide,

organism dependent, enantioselective bioaccumulation and biomagnification is a possibility. In the mussel (*Anodonta woodiana*) and zebrafish (*Danio rerio*) an enantioselective enrichment of the (+/S) enantiomer was reported, which could result from enantioselective uptake and biotransformation (Qu, Ma, Liu, Gao, et al., 2016; C. Xu et al., 2019). In contrast, in the blackworm (*Lumbriculus variegatus*) the concentration of the (-/R) enantiomer was shown to be higher (Wang, Li, & You, 2019).

In a bioconcentration study with bluegill sunfish (*Lepomis macrochirus*), [14C]-labelled fipronil was used to estimate the whole body steady-state bioconcentration factor (BCF) (Chapleo S. & Hall B.E. 1992 cited in (EC, 2004) Volume 3, B9, p566). Based on the radioactivity from [14C]-fipronil an steady-state BCF of 321[7] was estimated in whole fish during a 35-day uptake study. Bioconcentration in relation to the fish lipid content is not provided by the respective study. Within a 14-day depuration phase, residues were rapidly and nearly completely (> 99%) eliminated from the tissue (Chapleo S. & Hall B.E. 1992 cited in (EC, 2004) Volume 3, B9, p566). Additionally, the bioconcentration potential of fipronil was analyzed in tilapia (*Oreochromis niloticus*) by using *in vivo* mass-spectrometry measurements (HPLC/MSMS) and multi-compartmental toxicokinetic model. The study consisted of a 96-h uptake phase, during which the steady state could be reached, and a subsequent 96-h elimination phase. The steady-state and kinetic based BCF were at 1016 L kg^{-1} lipid[8] and 1047 L kg^{-1} lipid[9], respectively (Li, You, & Wang, 2018a). Similarly, to bluegill sunfish, the study performed in tilapia reported a rapid depuration of fipronil (Li et al., 2018a). However, due to the short duration of the study performed by (Li et al., 2018a), the bluegill sunfish (*Lepomis macrochirus*) BCF of 321 (Chapleo S. & Hall B.E. 1992 cited in (EC, 2004) Volume 3, B9, p566) is selected for further use in EQS derivation.

In bluegill sunfish (*Lepomis macrochirus*), the absorbed fipronil was shown to be biotransformed to fipronil sulfone (MB 46136), fipronil sulfide (MB 45950) and fipronil amide (RPA 200766), with fipronil sulfone (MB 46136) being the major biotransformation product (EC, 2004). Also in rainbow trout, zebrafish, tilapia and silver eel, fipronil sulfone was identified as the major biotransformation product (Konwick, Garrison, Black, Avants, & Fisk, 2006; Li et al., 2018a; Michel et al., 2016; C. Xu et al., 2019). Data suggest that fipronil sulfone is eliminated form fish in a slower rate than the parent compound (Konwick et al., 2006; Li et al., 2018a). Nonetheless, all biotransformation products can be eliminated

[7] BCF was determined by measuring total radioactivity via HPLC, thus including potential transformation products.

[8] The BCF was calculated using the equation: BCF = steady state lipid normalized concentration of fipronil in the whole fish / water concentration of fipronil during exposure (lipid content whole fish:1.3 ± 0.09 %)

[9] The BCF is calculated using the equation: BCF = uptake rate of fipronil by the fish / (elimination rate + metabolism rate) / lipid content in the whole fish (1.3 ± 0.09 %)

from fish tissue following simple equilibria and kinetics (Roohi S., Coote A., Savage E.A. 1993 cited in (EC, 2004) Volume 3, B9, p567).

No literature is available on the bioaccumulation of fipronil or its transformation products. However, by using the structure of a chemical compound, a bioaccumulation factor (BAF) can be estimated (BCFBAF calculator (U. EPA, 2020)). Estimated BAF for fipronil (based on $logK_{ow}$ of four, lipid normalized, assuming lipid content of 5 % for fish) is on average 113 (102.8, 116.3 and 120 for the upper, mid and lower trophic level, respectively). Despite the chemical properties of fipronil ($LogK_{ow}$ > 3, estimated BAF ~100), the potential for bioaccumulation of fipronil is assumed to be low due to biotransformation and rapid depuration (EC, 2004; EFSA, 2006).

A study focusing on the food web in Vaccare` s Lagoon (Biosphere Reserve in Rhone Delta, France) reports fipronil biomagnification factors (BMF) for different trophic compartments. At the base of the food web, producer vs. consumer I, BMF of 5.31 was identified. However, at middle and upper trophic levels (TL) the BMF was lower. A BMF of 0.68, 1.14 and 0.48 were identified for consumer I vs. consumer II-1, consumer II-1 vs consumer II-2, and consumer II-2 vs. consumer II-3, respectively (Roche et al., 2009). One additional study performed with rainbow trout fed with contaminated food, reports a BMF of 0.04 indicating, that fipronil is unlikely to biomagnify in aquatic food webs (Konwick et al., 2006).

3 Analytics

For the analysis of fipronil and impurities within technical material, High-Performance Liquid Chromatography-Ultraviolet (HPLC-UV) methods have been developed and validated with respect to selectivity, linearity, accuracy and repeatability (BASF, 2011). Fipronil can be detected with UV-detector at a wavelength of 220 nm (BASF, 2011).

For the analysis of fipronil and its transformation products in drinking water, two gas chromatography (GC) methods were established for different analytical ranges (one for concentrations between 0.01 and 0.2 µg/l, and one for concentrations between 0.1 µg/L and 1.0 µg/L) (Diot and Kieken (2002) and Bourgade et al., (1998) cited in BASF (2011)). Both methods contain an enrichment and purification step by using a fipronil immunoaffinity cartridge. Gas chromatography – electron capture detector GC-ECD methods have also been established and validated for surface waters, such as river and pond water (Fuchsbichler (1999) and Lopes (1997) cited in BASF (2011)). Additionally, liquid chromatography in combination with tandem mass spectrometry (LC-MS/MS) methods are available for the analysis of fipronil and its transformation products in water samples. As the interface, electrospray ionization operating in the negative ion mode (ESI-) is sucesfully applied. Water samples that contain a high

amount of sediment should be filtered on a filter paper with an additional washing step of the sediment on filter paper with acetonitrile (Ibrahim (1999) cited in BASF (2011)). However, for turbid waters, a cleanup step based on liquid-liquid extraction is necessary, such as reported in Grote (2005b) and Grote (2006) cited in BASF (2011). An analysis of fipronil in parallel with other organic pollutants *via* LC-MS/MS is also possible and was demonstrated in previous studies (Huntscha, Singer, McArdell, Frank, & Hollender, 2012; Spycher et al., 2018). For sample clean-up and enrichment, solid phase extraction (SPE) is used. Analytes are detected with high resolution MS (HRMS) by using selected reaction monitoring or data dependent acquisition and as the interface, Electrospray Ionization operating in the negative ion mode (ESI-) is used. The calibration is reported from 0.5 to 1000 ng/L. For fipronil, an LOQ is provided in the range of 0.5 – 6 ng/L (Huntscha et al., 2012; Spycher et al., 2018).

As multiple methods are available for the analysis of fipronil in water samplesthe appropriate method should be selected based on the sample characteristics (matrix) and the expected concentration range of fipronil. For residues analysis in surface waters, however, lowest detection and quantification limits can be obtained with ESI (-) and LC-MS/MS.

The different analytical methods for fipronil detection and quantification are summarized in ***Table 6***.

Table 6 Methods for Fipronil analysis in water and corresponding limits of detection (LOD) and limits of quantification (LOQ). N.A. means not reported.

LOD	LOQ	Analytical method	Reference
N.A.	0.0005 – 0.006 µg/L	ESI(-)-LC-MS/MS	Spycher et al. (2018)
N.A.	0.004 µg/L	ESI(-)-LC-MS/MS	Grote (2005b) cited in BASF (2011)
N.A.	0.01 µg/kg	ESI(-)-LC-MS/MS	Grote (2006) cited in BASF (2011)
0.004 µg/L	0.01 µg/L	ESI(-)-LC-MS/MS	Ibrahim (1999) cited in BASF (2011)
N.A.	0.2 µg/L	GC-ECD	Fuchsbichler (1999) cited in BASF (2011)
N.A.	1 µg/L	GC-ECD	Lopes (1997) cited in BASF (2011)
0.01 µg/L	0.05 µg/L	GC-MS	Diot and Kieken (2002) cited in BASF (2011)
N.A.	0.1 µg/L	GC-ECD	Bourgade et al., (1998) cited in BASF (2011)
N.A.	not applicable	HPLC-UV	Robles and Cousin (1996) cited in BASF (2011)

4 Effect data

Only reliable and relevant data should be used for EQS derivation (EC, 2018). These data are often referred to as “valid”. Different approaches to assessment and classification of (eco)toxicological data have been published. An established method introduced by Klimisch, Andreae, and Tillmann (1997) uses four levels of validity: (1) reliable, (2) reliable with restrictions, (3) not reliable, (4) not assessable. The CRED approach published by (Moermond, Kase, Korkaric, & Ågerstrand, 2016) is based on a similar classification scheme but additionally takes into account the relevance of test results for the derivation of quality standards. Both methods are recommended in the EU TGD for EQS (EC, 2018). The relevant and reliable data that was considered for EQS derivation is presented in **Table 7**, whereas the complete

data-set is provided in **Table 13 (Annex I)**, i.e. also non-relevant/non-reliable effect data is provided in Annex I. Here, validity in terms of relevance ("C" in **Table 7**, **Table 13**) and reliability ("R" in **Table 7**, **Table 13**) of studies were evaluated according to the CRED-criteria.

A comprehensive review of literature up to the year 2014 is presented in Bower JC (2017). Therefore, a literature search (Scopus) was performed in October 2020 for the years 2014-2020 using the search terms fipronil, 120068-37-3 only and in combination with NOEC, EC10, EC50, LC50, ecotoxicity, ecotoxicology, aquatic toxicity, or toxicity. Studies fulfilling the reliability and relevance criteria were included in the assessment (**Table 7**).

Previous assessments published in EC (2004), or U. EPA (2006) were adopted as accepted without additional assessment (face value). The US EPA Office of Pesticide Programs (OPP) Pesticide Ecotoxicity Database contains effect data that have been rated as "C" (core") or "S" (supportive) with "C"-rated studies usually being used for risk assessments by the US EPA. "S"-rated studies may be used following careful assessment in case of lack of a "C" rated study (US EPA 2004)[10]. This classification has been adopted with "C"-rated studies being used in the same manner as Klimisch 1-rated studies and "S"-rated studies as supportive data. Studies cited in the "Water and Sediment Quality Criteria Report for Fipronil" by Bower JC (2017) were re-evaluated. However, the studies rated as "reliability R" were generally accepted and marked with reliability 2.

When selecting effect concentrations from algae growth inhibition tests, growth rate is preferred over growth, biomass, and cell density according to (EC, 2018). For *Lemna* sp., biomass and growth rate are preferentially used (EC, 2018), with growth rate being the preferred endpoint according to OECD test guideline (TG) 221 (OECD, 2006) and REACH guidance R. 7b (ECHA 2017, S. 28-29). The endpoint population abundance as used in the OPP database can be based on various readouts (e.g. biomass, growth rate, yield) and was thus not preferred.

Fipronil is a chiral molecule and is usually applied as a 1:1 racemic mixture of the (+/S) and (-/R) enantiomer. Studies investigating the enantioselective toxicity of fipronil showed that the most toxic isomer is organism dependent (Section 1.4, Annex II (13.1)). For EQS derivation, the mean value of the effect concentration for the racemate and the most toxic enantiomer was selected.

In aqueous systems, fipronil has shown to be stabe. Therefor, verification of the chemical concentration at the beginning of the test is considered to be sufficient for a study to be reliable.

[10] [Page 33]: […] In some instances, a core study may not be available for a particular data requirement listed in 40 CFR 158. In this case, the risk assessment team may consider other sources of information to address the data gap (e.g., submitted studies considered to be supplemental and data from other sources not submitted as part of fulfillment of 40 CFR 158). If supplemental or non-guideline study data are available to address the type of information described by the associated guideline, then it may be used in the risk assessment after its use is carefully considered. Professional judgment is used by the risk assessment team to determine the utility of the available supplemental data for the proposed risk assessment […].

Studies without any reporting of chemical analysis are considered “not assignable” and are not used for EQS derivation. Furthermore, only data above the water solubility threshold of fipronil (3.3 mg/l, **Table 1**) were considered as reliable. Studies conducted with formulations are not considered relevant because of the unknown formulation adjuvants and are therefore not considered for the risk assessment but are listed in section 13.3.

Table 7 Effect data collection for fipronil in µg/L.Data were evaluated for relevance and reliability according to the CRED criteria (Moermond et al., 2016).

Acute freshwater effect data											
Group	Species	Endpoint	Duration	Parameter	value (µg/L)	Analytics	Exposure	Purity (%)	Validity	Reference	Notes
cyanobacterium	Anabaena flos-aquae	growth rate	120 h	EC50	> 170	m-am	S	96.1	2/C2	Hoberg J.R. 1993 cited in EC (2004) Vol.3 B9 p.585	supportive
algae	Navicula pelliculosa	growth rate	120 h	EC50	> 120	m-am	S	96.1	2/C2	Hoberg J.R. 1993 cited in EC (2004) Vol.3 B9 p.586	
algae	**Scenedesmus subspicatus**	**biomass**	**96 h**	**EbC50**	**68**	**nom-m**	**S**	**> 95**	**2/C1**	Handley J.W., Mead C., Bartlett A.J. 1991 cited in EC (2004) Vol.3 B9 p.584	the 96 h EbC50 is selected since an ErC50 is only available for the endpoint growth rate for the time interval 24 - 48 h.
algae	Selenastrum capricornutum	growth rate	120 h	EC50	> 140	mm	S	96.1	2/C2	Hoberg J.R. 1993 cited in EC (2004) Vol.3 B9 p.585	
higher plant	Lemna gibba	growth rate	14 d	EC50	> 160	m-i	S	96.1	2/C2	Hoberg, J.R. 1993 cited in EC (2004) Vol.3 B9 p.596	
higher plant	**Lemna gibba**	**biomass**	**14 d**	**EC50**	**> 81**	**m-gm**	**S**	**96.1**	**2/C2**	Han Hoberg, J. R. (A.7.4.3.5.2/01) cited in (BASF, 2011) Document IIIA 7.4 page 240 Section 7.4.3.5, Annex Point IIIA, XIII.3.4, Aquatic plant toxicity	
insect	Baetis tricaudatus	mortality	48 h	LC50	0.105	m	S	99.5	2/C1	Weston and Lydy (2014) cited in Bower JC (2017)	
insect	**Baetis tricaudatus**	**immobilisation**	**48 h**	**EC50**	**0.0519**	**m**	**S**	**99.5**	**2/C1**	Weston and Lydy (2014) cited in Bower JC (2017)	endpoint immobilization selected as the most sensitive
insect	Chironomus dilutus	mortality	96 h	LC50	> 0.0815	m	S	99.5	2/C2	Weston and Lydy (2014) cited in Bower JC (2017)	
insect	Chironomus dilutus	immobilisation	96 h	EC50	0.035	m	S	99.5	2/C1	Weston and Lydy (2014) cited in Bower JC (2017)	endpoint immobilization selected as the most sensitive
insect	Chironomus dilutus	immobilisation	96 h	EC50	0.03	m	S	99.5	2/C1	Weston and Lydy (2014) cited in Bower JC (2017)	endpoint immobilization selected as the most sensitive
mean (immobilization)					**0.032**	**µg/L**					
insect	Chironomus riparius	mortality (larvae)	48 h	LC50	1.74	n-m	S	>= 97	R2/C1	Monteiro et al. (2019).	OECD guideline 235

Group	Species	Endpoint	Duration	Parameter	value (µg/L)	Analytics	Exposure	Purity (%)	Validity	Reference	Notes
insect	Chironomus tentans	mortality	10 d	LC50	0.43	mm	R	98.3	2/C2	Putt A.E (A.7.4.3.5.1/01) 2003 cited in (BASF, 2011) Document IIIA 7.4 page 220 Section 7.4.3.5, Annex Point IIIA, XIII.3.4, Effects on sediment dwelling organisms	Supportive short-term data. Sediment/water system. Sediment from: Glen Charlie Pond, Massachusetts, 2.8% organic carbon 94% sand 6% silt % clay, pH 5.7, exposure stage is L3 larvae.
insect	Chironomus tentans	growth	10 d	LC50	0.73	mm	R	98.3	2/C2	Putt A.E (A.7.4.3.5.1/01) 2003 cited in (BASF, 2011) Document IIIA 7.4 page 220 Section 7.4.3.5, Annex Point IIIA, XIII.3.4, Effects on sediment dwelling organisms	Supportive short-term data. Sediment/water system. Sediment from: Glen Charlie Pond, Massachusetts, 2.8% organic carbon 94% sand 6% silt % clay, pH 5.7, exposure stage is L3 larvae.
insect	Diphetor hageni	mortality	48 h	LC50	0.347	m	S	99.5	2/C1	Weston and Lydy (2014) cited in Bower JC (2017)	
insect	**Diphetor hageni**	**immobilisation**	**48 h**	**EC50**	**0.163**	**m**	**S**	**99.5**	**2/C1**	Weston and Lydy (2014) cited in Bower JC (2017)	endpoint immobilization selected as the most sensitive
insect	Ephemeralla excrucians	mortality	48 h	LC50	> 0.436	m	S	99.5	2/C2	Weston and Lydy (2014) cited in Bower JC (2017)	supportive
insect	Ephemeralla excrucians	immobilisation	48 h	EC50	> 0.436	m	S	99.5	2/C2	Weston and Lydy (2014) cited in Bower JC (2017)	supportive
insect	Fallceon quilleri	mortality	48 h	LC50	> 0.187	m	S	99.5	2/C2	Weston and Lydy (2014) cited in Bower JC (2017)	supportive
insect	**Fallceon quilleri**	**immobilisation**	**48 h**	**EC50**	**0.0707**	**m**	**S**	**99.5**	**2/C1**	Weston and Lydy (2014) cited in Bower JC (2017)	endpoint immobilization selected as the most sensitive
insect	Helicopsyche sp.	mortality	96 h	LC50	> 0.842	m	S	99.5	2/C2	Weston and Lydy (2014) cited in Bower JC (2017)	supportive

Group	Species	Endpoint	Duration	Parameter	value (µg/L)	Analytics	Exposure	Purity (%)	Validity	Reference	Notes
insect	**Helicopsyche sp.**	**immobilisation**	**96 h**	**EC50**	**0.267**	**m**	**S**	**99.5**	**2/C1**	Weston and Lydy (2014) cited in Bower JC (2017)	endpoint immobilization selected
insect	Hexagenia sp.	mortality	96 h	LC50	0.44	mm	R	99.7	2/C1	Putt A.E. 2003 (A.7.4.1.2/02) cited in (BASF, 2011) Document IIIA 7.4 page 146 Section 7.4.1.2, Annex Point IIA, VII.7.2, Aquatic toxicity to invertebrates	
insect	Hexagenia sp.	mortality	96 h	LC50	1.231	m	S	99.5	2/C1	Weston and Lydy (2014) cited in Bower JC (2017)	
insect	**Hexagenia sp.**	**immobilisation**	**96 h**	**EC50**	**0.48**	**m**	**S**	**99.5**	**2/C1**	Weston and Lydy (2014) cited in Bower JC (2017)	endpoint immobilization selected as the most sensitive
insect	Hydropsyche sp.	mortality	96 h	LC50	2.107	m	S	99.5	2/C1	Weston and Lydy (2014) cited in Bower JC (2017)	
insect	**Hydropsyche sp.**	**immobilisation**	**96 h**	**EC50**	**0.602**	**m**	**S**	**99.5**	**2/C1**	Weston and Lydy (2014) cited in Bower JC (2017)	endpoint immobilization selected as the most sensitive
insect	Isoperla quinquepunctata	mortality	96 h	LC50	0.113	m	S	99.5	2/C1	Weston and Lydy (2014) cited in Bower JC (2017)	
insect	**Isoperla quinquepunctata**	**immobilisation**	**96 h**	**EC50**	**0.101**	**m**	**S**	**99.5**	**2/C1**	Weston and Lydy (2014) cited in Bower JC (2017)	endpoint immobilization selected as the most sensitive
insect	Nectopsyche sp.	mortality	96 h	LC50	> 2.947	m	S	99.5	2/C2	Weston and Lydy (2014) cited in Bower JC (2017)	Supportive
insect	**Nectopsyche sp.**	**immobilisation**	**96 h**	**EC50**	**0.634**	**m**	**S**	**99.5**	**2/C1**	Weston and Lydy (2014) cited in Bower JC (2017)	endpoint immobilization selected as the most sensitive
insect	Serratella micheneri	mortality	48 h	LC50	> 0.722	m	S	99.5	2/C2	Weston and Lydy (2014) cited in Bower JC (2017)	supportive
insect	**Serratella micheneri**	**immobilisation**	**48 h**	**EC50**	**0.589**	**m**	**S**	**99.5**	**2/C1**	Weston and Lydy (2014) cited in Bower JC (2017)	endpoint immobilization selected as the most sensitive
insect	Sympetrum frequens	mortality (larvae)	48 h	LC50	2775	n-m	R	> 99	R2/C1	Jinguji, Ohtsu, Ueda, and Goka (2018b)	
insect	**Sympetrum frequens**	**feeding behavior**	**48 h**	**EC50**	**2.9**	**n-m**	**R**	**> 99**	**R2/C1**	Jinguji et al. (2018b)	endpoint feeding behaviour selected as the most sensitive
insect	Sympetrum infuscatum	mortality (larvae)	48 h	LC50	1020	n-m	R	> 99	R2/C1	Jinguji et al. (2018b)	

Group	Species	Endpoint	Duration	Parameter	value (µg/L)	Analytics	Exposure	Purity (%)	Validity	Reference	Notes
insect	**Sympetrum infuscatum**	**feeding behavior**	**48 h**	**EC50**	**29.3**	**n-m**	**R**	**> 99**	**R2/C1**	Jinguji et al. (2018b)	endpoint feeding behaviour selected as the most sensitive
insect	Taenionema sp.	mortality	96 h	LC50	> 0.184	m	S	99.5	2/C2	Weston and Lydy (2014) cited in Bower JC (2017)	
insect	Taenionema sp.	immobilisation	96 h	EC50	> 0.184	m	S	99.5	2/C2	Weston and Lydy (2014) cited in Bower JC (2017)	
insect	Tricorythodes sp.	mortality	48 h	LC50	> 1.229	m	S	99.5	2/C2	Weston and Lydy (2014) cited in Bower JC (2017)	
insect	Tricorythodes sp.	immobilisation	48 h	EC50	> 1.229	m	S	99.5	2/C2	Weston and Lydy (2014) cited in Bower JC (2017)	
bivalve	Corbicula fluminea	mortality	96 h	LC50	> 2000	m-gm	R	99.7	2/C2	Putt (2003a) cited in U. EPA (2006)	supportive
bivalve	Elliptio complanata	mortality	24 h	EC50	> 2000	m	R	99.7	2/C2	Bringolf et al. (2007) cited in Bower JC (2017)	supportive, glochidia
bivalve	Lampsilis fasciola	mortality	24 h	EC50	> 2000	m	R	99.7	2/C2	Bringolf et al. (2007) cited in Bower JC (2017)	supportive, glochidia
bivalve	Lampsilis fasciola	mortality	48 h	EC50	> 2000	m	R	99.7	2/C2	Bringolf et al. (2007) cited in Bower JC (2017)	supportive, glochidia
bivalve	Lampsilis fasciola	mortality	96 h	EC50	> 2000	m	R	99.7	2/C2	Bringolf et al. (2007) cited in Bower JC (2017)	supportive, juveniles
bivalve	Lampsilis siliquoidea	mortality	96 h	EC50	> 2000	m	R	99.7	2/C2	Bringolf et al. (2007) cited in Bower JC (2017)	supportive, juveniles
bivalve	Lampsilis siliquoidea	mortality	24 h	EC50	> 2000	m	R	99.7	2/C2	Bringolf et al. (2007) cited in Bower JC (2017)	supportive, glochidia
bivalve	Lampsilis siliquoidea	mortality	96 h	EC50	> 2000	m	R	99.7	2/C2	Bringolf et al. (2007) cited in Bower JC (2017)	supportive, glochidia
bivalve	Villosa constricta	mortality	24 h	EC50	> 2000	m	R	99.7	2/C2	Bringolf et al. (2007) cited in Bower JC (2017)	supportive, glochidia
bivalve	Villosa constricta	mortality	48 h	EC50	> 2000	m	R	99.7	2/C2	Bringolf et al. (2007) cited in Bower JC (2017)	supportive, glochidia

Group	Species	Endpoint	Duration	Parameter	value (µg/L)	Analytics	Exposure	Purity (%)	Validity	Reference	Notes
clitellata	Lumbriculus variegatus	mortality	96 h	LC50	> 1900	m-gm	R	99.7	2/C2	Putt (2003b) cited in U. EPA (2006)	supportive
crustacean	Ceriodaphnia dubia	mortality (larvae)	24 h	LC50	33.3	nom-m	S	98	2/C1	Wilson (2008). cited in Bower JC (2017),	Racemate
crustacean	Ceriodaphnia dubia	mortality (larvae)	24 h	LC50	18.1	nom-m	S	97.3	2/C1	Wilson (2008). cited in Bower JC (2017),	(+/S) enantiomer
crustacean	Ceriodaphnia dubia	mortality (larvae)	24 h	LC50	65.2	nom-m	S	98.1	2/C1	Wilson (2008). cited in Bower JC (2017),	(-/R) enantiomer
crustacean	Ceriodaphnia dubia	mortality (larvae)	48 h	LC50	30.3	nom-m	S	98	2/C1	Wilson (2008). cited in Bower JC (2017),	Racemate
crustacean	Ceriodaphnia dubia	mortality (larvae)	48 h	LC50	10.3	nom-m	S	97.3	2/C1	Wilson (2008). cited in Bower JC (2017),	(+/S) enantiomer
crustacean	Ceriodaphnia dubia	mortality (larvae)	48 h	LC50	50.1	nom-m	S	98.1	2/C1	Wilson (2008). cited in Bower JC (2017),	(-/R) enantiomer
mean (racemate and the most toxic enantiomer (+/S))					**17.7**	**µg/L**					
crustacean	**Daphnia magna**	**behaviour**	**48 h**	**EC50**	**190**	**m-am**	**T**	**100**	**2/C1**	MCNamara P.C. 1990 cited in EC (2004) Vol.3 B9 p.568	
crustacean	Hyalella azteca	mortality	96 h	LC50	1.593	m	S	99.5	2/C1	Weston and Lydy (2014) cited in Bower JC (2017)	
crustacean	Hyalella azteca	mortality	96 h	LC50	1.725	m	S	99.5	2/C1	Weston and Lydy (2014) cited in Bower JC (2017)	
crustacean	Hyalella azteca	immobilisation	96 h	EC50	0.729	m	S	99.5	2/C1	Weston and Lydy (2014) cited in Bower JC (2017)	endpoint immobilization selected as the most sensitive
crustacean	Hyalella azteca	immobilisation	96 h	EC50	0.727	m	S	99.5	2/C1	Weston and Lydy (2014) cited in Bower JC (2017)	endpoint immobilization selected as the most sensitive
mean (immobilization)					**0.728**	**µg/L**					

Group	Species	Endpoint	Duration	Parameter	value (µg/L)	Analytics	Exposure	Purity (%)	Validity	Reference	Notes
crustacean	Procambarus clarkii	mortality	96 h	LC50	124.89	nom-m	S	98	2/C1	Overmyer et al. (2007) cited in Bower JC (2017)	Racemate
crustacean	Procambarus clarkii	mortality	96 h	LC50	81.7	nom-m	S	98	2/C1	Overmyer et al. (2007) cited in Bower JC (2017)	(+/S) enantiomer
crustacean	Procambarus clarkii	mortality	96 h	LC50	163.5	nom-m	S	98	2/C1	Overmyer et al. (2007) cited in Bower JC (2017)	(-/R) enantiomer
mean (racemate and the most toxic enantiomer (+/S))					**101.01**	**µg/L**					
fish	Cyprinus carpio	mortality	96 h	LC50	430	mm	T	> 95	2/C1	Handley et al., (1991) cited in EC (2004) Vol.3 B9 p.551	
fish	Cyprinus carpio	mortality	96 h	LC50	428	m	S	99.1	R2/C1	S. K. Gupta et al. (2014)	
mean					**429**	**µg/L**					
fish	**Ictalurus punctatus**	**mortality**	**96 h**	**LC50**	**560**	**m-am**	**T**	**97.08**	**2/C1**	Dionne (1997) cited in EC (2004) Vol.3 B9 p.552	
fish	**Lepomis macrochirus**	**mortality**	**96 h**	**LC50**	**85.2**	**m-am**	**T**	**95.4**	**2/C1**	Ward (1991) cited in EC (2004) Vol.3 B9 p.551	
fish	**Oncorhynchus mykiss (Salmo gairdneri)**	**mortality**	**96 h**	**LC50**	**248**	**m-am**	**T**	**95.4**	**2/C1**	Ward (1991) cited in EC (2004) Vol.3 B9 p.551	
fish	Oryzias latipes	mortality	96 h	LC50	94.2	n-m	S	98.9	R2/C1	Nillos, Lin, Gan, Bondarenko, and Schlenk (2009)	Racemate
fish	Oryzias latipes	mortality	96 h	LC50	95.4	n-m	S	> 97	R2/C1	Nillos et al. (2009)	(+/S) enantiomer
fish	Oryzias latipes	mortality	96 h	LC50	98.3	n-m	S	> 97	R2/C1	Nillos et al. (2009)	(-/R) enantiomer
mean (racemate and the most toxic enantiomer (+/S))					**94.8**	**µg/L**					

Group	Species	Endpoint	Duration	Parameter	value (µg/L)	Analytics	Exposure	Purity (%)	Validity	Reference	Notes
fish	Pimephales promelas	mortality (larvae)	7 d	LC50	208	nom-m	R	97.8	2/C2	Baird et al. (2013) cited in Bower JC (2017)	Racemate
fish	Pimephales promelas	mortality (larvae)	7 d	LC50	227	nom-m	R	97.8	2/C2	Baird et al. (2013) cited in Bower JC (2017)	(+/S) enantiomer
fish	Pimephales promelas	mortality (larvae)	7 d	LC50	365	nom-m	R	97.8	2/C2	Baird et al. (2013) cited in Bower JC (2017)	(-/R) enantiomer
mean (racemate and the most toxic enantiomer (+/S))					**217**	**µg/L**					
amphibian	Xenopus laevis	mortality	96 h	LC50	850	nom-m	S	98	2/C1	Overmyer et al. (2007) cited in Bower JC (2017)	Racemate
amphibian	Xenopus laevis	mortality	96 h	LC50	910	nom-m	S	98	2/C1	Overmyer et al. (2007) cited in Bower JC (2017)	(+/S) enantiomer
amphibian	Xenopus laevis	mortality	96 h	LC50	1140	nom-m	S	98	2/C1	Overmyer et al. (2007) cited in Bower JC (2017)	(-/R) enantiomer
mean (racemate and the most toxic enantiomer (+/S))					**879**	**µg/L**					

Acute saltwater effect data											
Group	Species	Endpoint	Duration	Parameter	value (µg/L)	Analytics	Exposure	Purity (%)	Validity	Reference	Notes
algae	Skeletonema costatum	growth rate	120 h	EC50	> 140	mm	S	96.1	2/C2	Hoberg (1993) cited in EC (2004) Vol.3 B9 p.586	supportive, single concentration tested
algae	**Dunaliella tertiolecta**	**cell number**	**96 h**	**EC50**	**631.2**	**nom-m**	**S**	**98**	**2/C1**	Overmyer et al. (2007) cited in Bower JC (2017)	
bivalve	**Crassostrea virginica**	**growth rate**	**96 h**	**EC50**	**770**	**mm**	**T**	**96.1**	**2/C1**	Dionne (1993) cited in EC (2004) Vol.3 B9 p.569	
bivalve	Mercenaria mercenaria	mortality	96 h	EC50	177	nom-m	S	98	2/C1	Overmyer et al. (2007) cited in Bower JC (2017)	Racemate
bivalve	Mercenaria mercenaria	mortality	96 h	EC50	208	nom-m	S	98	2/C1	Overmyer et al. (2007) cited in Bower JC (2017)	(+/S) enantiomer
bivalve	Mercenaria mercenaria	mortality	96 h	EC50	187	nom-m	S	98	2/C1	Overmyer et al. (2007) cited in Bower JC (2017)	(-/R) enantiomer
mean (mortality, racemate and the most toxic enantiomer (+/S))					**182**	**µg/L**					
crustacean	Americamysis bahia (Mysidopsis bahia)	mortality (cummulative)	96 h	LC50	0.14	mm	S	96.1	2/C1	Machado (1993) cited in EC (2004) Vol.3 B9 p.553	
crustacean	**Americamysis bahia (Mysidopsis bahia)**	**immobilisation**	**96 h**	**EC50**	**0.067**	**m-gm**	**R**	**>= 98**	**R2/C1**	Hano et al. (2019).	endpoint immobilization selected as the most sensitive
crustacean	Americamysis bahia (Mysidopsis bahia)	mortality	96 h	LC50	0.086	m-gm	R	>= 98	R2/C1	Hano et al. (2019).	

Group	Species	Endpoint	Duration	Parameter	value (µg/L)	Analytics	Exposure	Purity (%)	Validity	Reference	Notes
crustacean	Amphiascus tenuiremis	mortality	96 h	LC50	6.8	m	S	98	2/C1	Chandler et al. (2004) cited in Bower JC (2017)	adult organisms (male and female combined)
crustacean	**Amphiascus tenuiremis**	**mortality**	**96 h**	**LC50**	**3.5**	**m**	**S**	**98**	**2/C1**	Chandler et al. (2004	male adults tested, endpoint selected as males are more sensitive than females
crustacean	Amphiascus tenuiremis	mortality	96 h	LC50	13	m	S	98	2/C1	Chandler et al. (2004	female adults tested
crustacean	**Palaemonetes pugio**	**mortality (adult/parent)**	**96 h**	**LC50**	**0.32**	**nom-m**	**S**	**98**	**2/C1**	Overmyer et al. (2007) cited in Bower JC (2017)	Racemate
crustacean	Palaemonetes pugio	mortality (larvae)	96 h	LC50	0.68	nom-m	S	98	2/C1	Overmyer et al. (2007) cited in Bower JC (2017)	Racemate
crustacean	**Penaeus japonicus**	**immobilisation**	**96 h**	**EC50**	**0.17**	**m-gm**	**R**	**>= 98**	**R2/C1**	Hano et al. (2019),	endpoint immobilization selected as the most sensitive
crustacean	Penaeus japonicus	mortality	96 h	LC50	0.21	m-gm	R	>= 98	R2/C1	Hano et al. (2019),	
fish	**Cyprinodon variegatus**	**mortality**	**96 h**	**LC50**	**130**	**mm**	**T**	**96.1**	**2/C1**	Machado (1993) cited in EC (2004) Vol.3 B9 p.553	

Chronic freshwater effect data											
Group	Species	Endpoint	Duration	Parameter	value (µg/L)	Analytics	Exposure	Purity (%)	Validity	Reference	Notes
cyanobacterium	Anabaena flos-aquae	growth rate	120 h	NOEC	170	mm	S	96.1	2/C1	Hoberg J.R. 1993 cited in EC (2004) Vol.3 B9 p.585	supportive
algae	Navicula pelliculosa	growth rate	120 h	NOEC	120	mm	S	96.1	2/C1	Hoberg J.R. 1993 cited in EC (2004) Vol.3 B9 p.586	supportive
algae	**Scenedesmus subspicatus**	**growth rate**	**96 h**	**NOEC**	**40**	**nom-m**	**S**	**> 95**	**2/C1**	Handley J.W., Mead C., Bartlett A.J. 1991 cited in EC (2004) Vol.3 B9 p.584	
algae	Selenastrum capricornutum	growth rate	120 h	NOEC	140	mm	S	96.1	2/C1	Hoberg J.R. 1993 cited in EC (2004) Vol.3 B9 p.585	supportive
higher plant	**Lemna gibba**	**biomass**	**14 d**	**NOEC**	**81**	**mm**	**S**	**96.1**	**2/C1**	Han Hoberg, J. R. (A.7.4.3.5.2/01) cited in (BASF, 2011) Document IIIA 7.4 page 240 Section 7.4.3.5, Annex Point IIIA, XIII.3.4, Aquatic plant toxicity	
insect	**Chironomus riparius**	**development**	**28 d**	**NOEC**	**0.1168**	**m-i**	**S**	**99.14**	**2/C2**	Funk M (A7.4.3.4./02) 2004 cited in (BASF, 2011) Document IIIA 7.4 page 191 Section 7.4.3.4, Annex Point IIIA, XIII.2.4, Effects on reproduction and growth rate with an appropriate invertebrate species	supportive, OECD guideline 219
crustacean	Daphnia magna	length	21 d	NOEC	9.8	mm	T	100	2/C1	MCNamara P.C. 1990 cited in EC (2004) Vol.3 B9 p.577	endpoint length selected as the most sensitive
crustacean	Daphnia magna	mortality	21 d	NOEC	20	mm	T	100	2/C1	MCNamara P.C. 1990 cited in EC (2004) Vol.3 B9 p.577	
fish	Oncorhynchus mykiss (Salmo gairdneri)	mortality (larvae)	90 d	NOEC	15	mm	T	96.7	2/C1	Machado M.W. 1992 cited in EC (2004) Vol.3 B9 p.561	

Chronic saltwater effect data											
Group	Species	Endpoint	Duration	Parameter	value (µg/L)	Analytics	Exposure	Purity (%)	Validity	Reference	Notes
algae	Skeletonema costatum	growth rate	120 h	NOEC	140	mm	S	96.1	2/C1	Hoberg J.R. 1993 cited in EC (2004) Vol.3 B9 p.586	supportive
algae	Dunaliella tertiolecta	cell number	96 h	NOEC	250	nom-m	S	98	2/C1	Overmyer et al. (2007) cited in Bower JC (2017)	supportive
crustacean	**Americamysis bahia (Mysidopsis bahia)**	**length**	**28 d**	**NOEC**	**0.0077**	**mm**	**T**	**97.7**	**2/C1**	Machado M 1995 cited in EC (2004) Vol.3 B9 p.578	**crustacean**
fish	**Cyprinodon variegatus**	**multiple endpoints**	**34 d**	**NOEC**	**2.9**	**mm**	**T**	**97.08**	**2/C1**	Sousa JV 1998 cited in EC (2004) Vol.3 B9 p.563	endpoint selected as the most sensitive
fish	Cyprinodon variegatus	hatching rate	5 d	NOEC	6	mm	T	98	2/C1	Dionne E. 2000 cited in EC (2004) Vol.3 B9 p.565	
fish	Cyprinodon variegatus	fertility	59 d	NOEC	6	mm	T	98	2/C1	Dionne E. 2000 cited in EC (2004) Vol.3 B9 p.565	
fish	Cyprinodon variegatus	length	28 d	NOEC	6	mm	T	98	2/C1	Dionne E. 2000 cited in EC (2004) Vol.3 B9 p.565	
fish	Cyprinodon variegatus	length	28 d	NOEC	6	mm	T	98	2/C1	Dionne E. 2000 cited in EC (2004) Vol.3 B9 p.565	
fish	Cyprinodon variegatus	length	59 d	NOEC	6	mm	T	98	2/C1	Dionne E. 2000 cited in EC (2004) Vol.3 B9 p.565	
fish	Cyprinodon variegatus	length	110 d	NOEC	6	mm	T	98	2/C1	Dionne E. 2000 cited in EC (2004) Vol.3 B9 p.565	
fish	Cyprinodon variegatus	mortality (adult/parent)	59 d	NOEC	13	mm	T	98	2/C1	Dionne E. 2000 cited in EC (2004) Vol.3 B9 p.565	
fish	Cyprinodon variegatus	mortality (adult/parent)	110 d	NOEC	13	mm	T	98	2/C1	Dionne E. 2000 cited in EC (2004) Vol.3 B9 p.565	
fish	Cyprinodon variegatus	mortality (larvae)	28 d	NOEC	13	mm	T	98	2/C1	Dionne E. 2000 cited in EC (2004) Vol.3 B9 p.565	
fish	Cyprinodon variegatus	mortality (larvae)	28 d	NOEC	13	mm	T	98	2/C1	Dionne E. 2000 cited in EC (2004) Vol.3 B9 p.565	
fish	Cyprinodon variegatus	weight	28 d	NOEC	13	mm	T	98	2/C1	Dionne E. 2000 cited in EC (2004) Vol.3 B9 p.565	

Group	Species	Endpoint	Duration	Parameter	value (µg/L)	Analytics	Exposure	Purity (%)	Validity	Reference	Notes
fish	Cyprinodon variegatus	weight	28 d	NOEC	13	mm	T	98	2/C1	Dionne E. 2000 cited in EC (2004) Vol.3 B9 p.565	
fish	Cyprinodon variegatus	weight	110 d	NOEC	13	mm	T	98	2/C1	Dionne E. 2000 cited in EC (2004) Vol.3 B9 p.565	

Legend

Chemical analitycs

n: based on nominal concentrations
m: based on measured concentrations
m-gm based on mean measured concentrations (geometric mean)
mm based on mean measured concentrations
mm-i based on mean measured start concentration
m-i based on measured start concentration
m-twa: based on measured concentrations («time-weighted average»)
nom-i based on nominal concentrations; recovery at the start was determined. In case recovery was 80-120 %, nominal effect concentrations are regarded as valid. In case recovery was < 80 %, effect values are regarded as invalid.
nom-m based on nominal concentrations; recovery at the start was determined. In case recovery was 80-120 %, nominal effect concentrations are regarded as valid. In case recovery was < 80 %, effect values are regarded as invalid or, if possible, calculated (e.g. «time-weighted average»).

Exposure
S static
R semi-static
T flow-through

Relevance/Reliability
Klimisch: 1 Reliable without restriction, 2 Reliable with restriction, 3 Not reliable, 4 Not assignable
Cred: R1 Reliable without restriction, R2 Reliable with restriction, R3 Not reliable, R4 Not assignable
C1 Relevant without restriction, C2 Relevant with restriction, C3 Not reliable, C4 Not assignable

4.1 Graphic representation of effect data

All available data have been plotted independently of their relevance and reliability in **Figure 1**, A (acute data) and B (chronic data). The mean water-solubility limit of fipronil is at 3.3 mg/L and is indicated in the **Figure 1** as a dotted line. Some effect data lie above the solubility threshold and thus are considered unreliable. In **Figure 1**, C (acute data) and D (chronic data), the dataset is limited to only relevant and reliable data which has been used in EQS derivation.

Through graphical representation of the acute data, (**Figure 1**, **A, C**), two groups, insects and crustaceans, emerge as the most sensitive organisms towards fipronil. Also in the chronic data set, the lowest effect data are from studies with insects and crustaceans (**Figure 1**, **B, D**). However, the data set contains only one relevant and reliable entry for insects (*Chironomus riparius*), which was derived in a water-sediment test (**Figure 1**, **D**). Moreover, chronic effect concentrations for (freshwater and saltwater) fish are in the similar range as those for freshwater crustaceans.

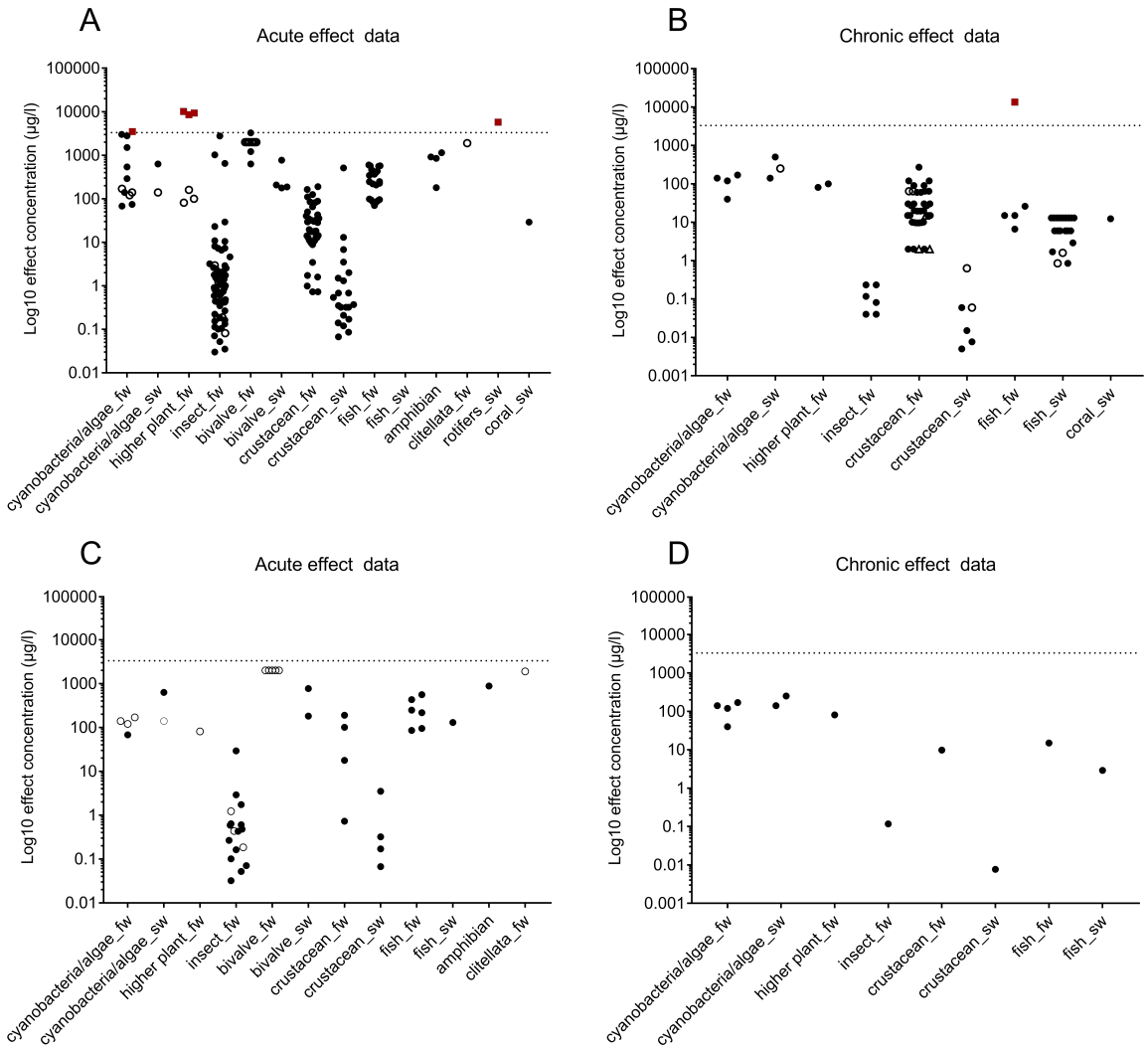


Figure 1: Graphical representation of acute and chronic effect data from toxicity tests with fipronil. Data are not normalized for OC. All data for acute and chronic toxicity, irrespective of reliability and relevance are depicted in A and B, respectively. The reliable and relevant, data is presented in C and D for acute and chronic studies, respectively, with one data point per organism, . Freshwater data: fw.Saltwater data: sw.. Not filled symbols represent unbound data (>), (≥), (<), (≤). The meansolubility limit of fipronil (3.3 mg/L) is indicated as dotted line.

Both, acute and chronic reliable and relevant data are available for one insect species (*Chironomus riparius*) with an acute to chronic ratio[11] of 14.9. For the crustaceans *Daphnia magna* and *Americamysis bahia (Mysidopsis bahia)* the acute to chronic ratio is 8.5 and 8.7, respectively. For fish, the acute to chronic ratio was 16.5 for *Oncorhynchus mykiss (Salmo gairdneri)* and 44.8 for *Cyprinodon variegatus*. Additionally, acute to chronic ratios are available for two algae species (*Scenedesmus subspicatus* and *Dunaliella tertiolecta*) with acute to chronic ratios of 1.7 and 2.5, respectively.

[11] The acute to chronic ratio was calculated by dividing the L(E)C50 from acute test by the NOEC from a chronic study performed with the same species.

4.2 Comparison between marine and freshwater species

As suggested by the EU TGD for EQS (EC, 2018), for statistical comparison of marine and freshwater species, one value per species is selected, all effect data are log-transformed, and the two datasets are compared for significant differences.

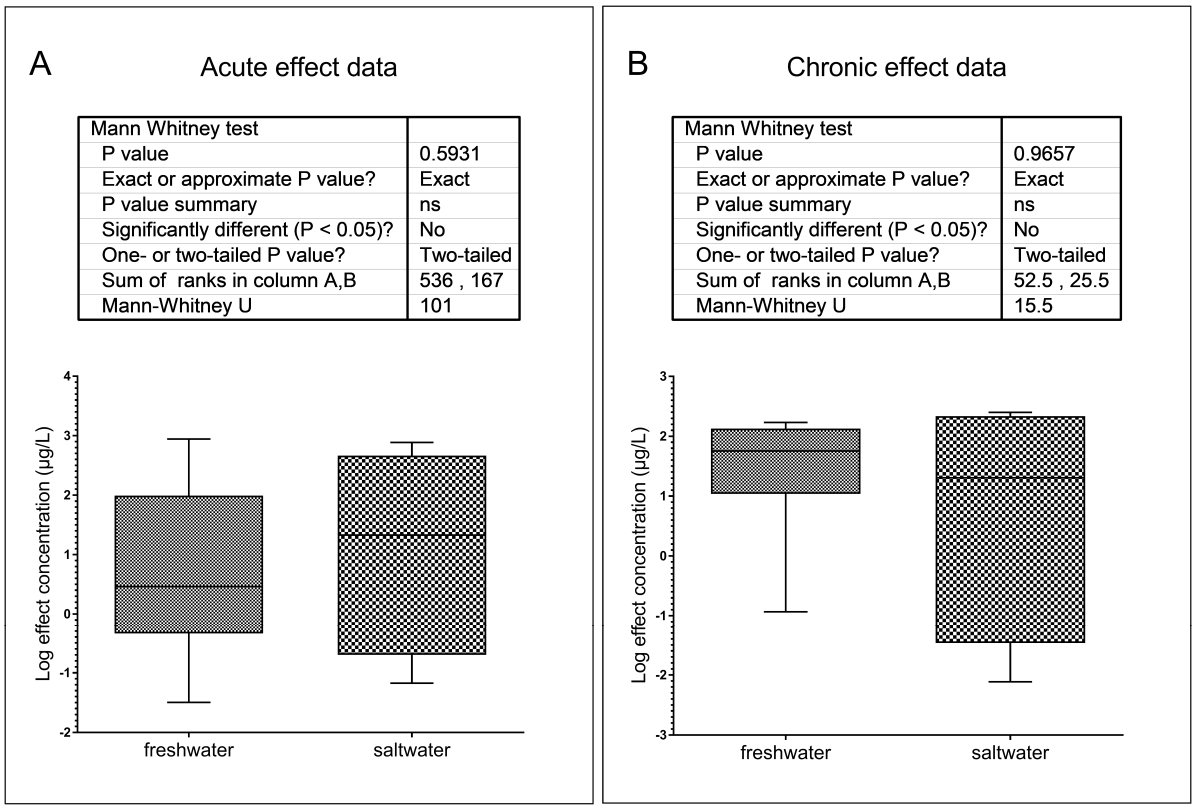


Figure 2: Statistical test for differences between acute (A) and chronic (B) data for freshwater and saltwater organisms. Data is depicted as a boxplot in addition to the summary of the unpaired t-test (non-parametric, Mann Withney test).

The analysis of the complete dataset shows, that overall, there is no significant difference between freshwater and saltwater species in acute and chronic studies (**Figure 2**). However, among all organism groups, insects and crustaceans can be identified as the most sensitive organisms towards fipronil in acute studies. In chronic studies, fish show a similar sensitivity towards fipronil as freshwater crustaceans and are therefore also considered among the sensitive groups.

Although some data for marine crustaceans and fish is available, data on insects in the marine environments is missing. By isolating the data for freshwater and saltwater crustaceans and fish, the test for differences was repeated (**Figure 3** A). The isolated crustacean and fish dataset for acute effect data indicates that the saltwater species are significantly (α 0.05) more sensitive towards fipronil as compared to the freshwater organisms. However, the acute crustacean freshwater and saltwater

datasets have a different representation of organisms. Water fleas dominate the freshwater dataset[12] whereas shrimps/prawns[13] dominate the saltwater dataset (**Figure 3** B). By considering the different representation of organisms in the crustacean dataset, it becomes clear that shrimp/prawns (Decapoda) are the most sensitive organisms within this group (**Figure 3** B). As there is not enough freshwater data on shrimps available, a statistical analysis of difference between freshwater and saltwater shrimp is not possible. In addition, the chronic crustacean dataset does not allow a statistical analysis of difference between freshwater and saltwater species. Therefore, in order not to underrepresent the most sensitive crustaceans, freshwater and saltwater datasets are combined.

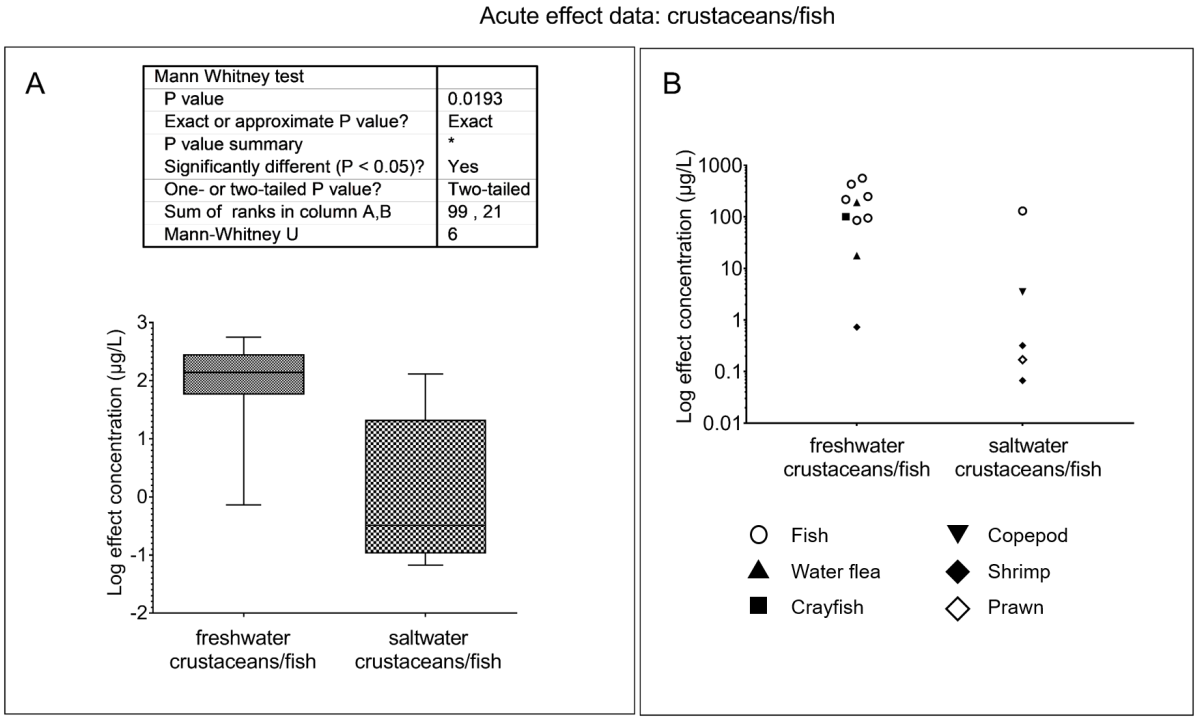


Figure 3: Statistical test for differences between acute crustacean and fish data for freshwater and saltwater organisms. A: data is depicted as a boxplot in addition to the summary of the unpaired t-test (non-parametric, Mann Whitney test). B: data is shown as scatter plot and the different organism groups are represented with distinct symbols.

5 Chronic toxicity

5.1 Derivation of CQC (AA-EQS) using the Assessment Factor (AF) method

The CQC_{AF} ($AA\text{-}EQS_{AF}$) is determined using assessment factors (AFs) applied to the lowest credible datum from long-term toxicity tests. The lowest long-term effect datum available for fipronil is the NOEC of 0.0077 µg/L (**Table 7**) for the crustacean *Americamysis bahia* (*Mysidopsis bahia*). This value originates from a study by Machado (1995) cited in EC (2004) p. 578 and mentioned in Bower JC (2017) p. A149. The study was performed under GLP with the use of radiolabelled fipronil. During a 28-day

[12] Freshwater data crustaceans: two water fleas, one shrimp, one crayfish
[13] Saltwater data crustaceans: two shrimp, one prawn, one copepod

flow-through exposure, multiple endpoints were measured. The 28-d NOEC 7.7 ng a.i./L was based on the effect length of male mysids.

Table 8 Most sensitive relevant and reliable chronic data summarized from **Table 7**

Group	Species	Duration	Effect concentration	Value [µg/L]	Reference
Basic data					
Algae	*Scenedesmus subspicatus*	96 h	NOEC	40	Handley (1991) cited in EC (2004) Vol.3 B9 p.584
Crustaceans	*Americamysis bahia (Mysidopsis bahia)*	28 d	NOEC	0.0077	Machado (1995) cited in EC (2004) Vol.3 B9 p.578
Fish	*Cyprinodon variegatus*	34 d	NOEC	2.9	Sousa (1998) cited in EC (2004) Vol.3 B9 p.563
Additional data					
Insect	*Chironomus riparius*	28 d	NOEC	0.1168*	Funk (2004) cited in BASF (2011) Document IIIA 7.4 p. 191
Higher plant	*Lemna gibba*	14 d	NOEC	81	Anonymos, cited in BASF (2011) Document IIIA 7.4 p. 240
* the value for *Chironomus riparius* was obtained in a water-sediment test and is considered supportive					

In cases where long term data (NOEC or EC_{10}) is being available for three species representing different living and feeding conditions, the EU TGD for EQS recommends the application of an assessment factor of 10 on the lowest credible datum (Table 3 in EC (2018)).

The dataset for fipronil contains chronic effect data for ≥ three species representing three trophic levels (crustaceans, fish, and algae). Additionally, one chronic insect study is available (**Table 8**).

The suggested assessment factor is thus 10 in accordance with EU TGD for EQS:

$$\mathrm{CQC}_{AF}\ (\mathrm{AA}-\mathrm{EQS}_{AF}) = \frac{lowest\ EC_{10}\ or\ NOEC}{AF}$$

$$\mathrm{CQC}_{AF}\ (\mathrm{AA}-\mathrm{EQS}_{AF}) = \frac{0.0077\ \left(\frac{\mu g}{L}\right)}{10} = 0.00077\ \left(\frac{\mu g}{L}\right)$$

The application of an AF of 10 to the lowest credible chronic datum results in a **CQC_{AF} ($AA\text{-}EQS_{AF}$) = 0.00077 µg/L**.

5.2 Derivation of CQC (AA-EQS) using the species sensitivity distribution (SSD) method

The minimum data requirements recommended for the application of the SSD approach for EQS water derivation is preferably more than 15, but at least 10 NOEC/EC_{10}, from different species covering at

least eight taxonomic groups (EC (2018), p. 43). In case of fipronil, chronic effect data are available for 12 species. The least sensitive organism group (primary producers, **Figure 1**) is represented by seven species. The remaining data covers two families of the phylum Chordata (Cyprinodontidae and Salmonidae), two species of crustaceans and one insect species. Conclusively, not enough data are available for applying the SSD approach.

5.3 Determination of CQC (AA-EQS) according to mesocosm/field data

Several chronic mesocosm studies are available for fipronil. The majority of the mesocosm studies focuses on the adverse effects of fipronil formulations on the aquatic community following the recommended application on rice paddy fields. Koya Hashimoto, Kasai, Hayasaka, Goka, and Hayashi (2020) performed a mesocosm experiment over the duration of three successive years. Each year, fipronil was applied once as the product Prince® (1.0 % fipronil) in nursery boxes of rice seedlings. The study showed that five taxa (belonging to cladocerans and insects) were affected, with dragonflies (Odonata) being the most sensitive taxon. One study by Hayasaka, Korenaga, Suzuki, Saito, et al. (2012) applied the same product (Prince®) in a paddy mesocosm for two successive years and reported changes in mesocosm community and structure. Further studies (K. Hashimoto et al., 2019; Kasai et al., 2016) used a similar setup (Prince®, applied once in nursery boxes, rice seedlings) and indicated a strong adverse effect on predatory insects, with dragonflies being again the most effected taxon. A further study used a different formulation, Regent® 800 WG (a.i. fipronil) and exposed amphipods *in situ* up to 89 days following the product application (Pinto et al., 2021). The formulation was highly toxic to *Hyalella meinerti* causing 100 % mortality. Additionally, the authors exposed amphipods in the laboratory to water that was collected from the mesocosm at different time points (Pinto et al., 2021). Water from the treated mesocosm was also toxic to *Hyalella meinerti*, even 89 days post contamination.

Within the mesocosms, where fipronil formulations were applied as recommended for nursery boxes of rice seedlings, the studies report a rapid decline of fipronil concentrations with time (K. Hashimoto et al., 2019; Hayasaka, Korenaga, Suzuki, Saito, et al., 2012; Kasai et al., 2016; Pinto et al., 2021). The fipronil half-lives (DT_{50}) in the water phase differ in between studies and years of sampling with reported DT_{50} =5.4 d. (year 2010) and 1.1 d. (year 2011) (Hayasaka, Korenaga, Suzuki, Saito, et al. (2012)), ca. 4 d. (Pinto et al., 2021) and 16.9 d. (Kasai et al., 2016). (K. Hashimoto et al., 2019; Kasai et al., 2016) Due to the unstable fipronil concentration in the aqueous phase, the application of formulations in seedling nursery boxes and missing information on the presence of transformation products, the described paddy field mesocosms cannot be used for CQC derivation.

In addition to the paddy field mesocosms, two further mesocosm studies are available for fipronil (study 1: described in (S.S. Walse, Pennington, Scott, & Ferry, 2004; Wirth et al., 2004), study 2: described in (Miller et al., 2020)). One study was performed in an estuarine mesocosm with a semi-diurnal tidal cycle (S.S. Walse et al., 2004; Wirth et al., 2004). The mesocosm contained marsh cores as sediment and was inhabited by the following organisms: the hard clam (*Mercenaria mercenaria*), the American oyster (*Crassostrea virginica*), the grass shrimp (*Palaemonetes pugio*) and the sheepshead minnow (*Cyprinidon variegatus*). Fipronil as active substance was spiked at three different concentrations (150, 355, and 5000 ng/L) at the start of the experiment and the effect of fipronil on the organisms as well as the chemicals fate within the mesocosm, were monitored during 28 days (S.S. Walse et al., 2004; Wirth et al., 2004). No significant effects on fish (*Cyprinodon variegatus*), clams (*Mercenaria mercenaria*), or oysters (*Crassostrea virginica*) were observed. Only the grass shrimp (*Palaemonetes pugio*) was adversely affected by fipronil. The study reports a 28-day LC_{50} of 357 ng/L for *Palaemonetes pugio.* However, it is not stated if the LC_{50} was derived based on nominal or measured concentration. Nonetheless, the toxicity data and fipronil concentrations over the entire duration of the experiment are provided, which allows a recalculation of the toxicity values. In all three mesocosm replicates, a two-phased decrease of fipronil concentration could be observed with a fast decrease in the first 96 h and a slow decrease thereafter (S.S. Walse et al., 2004). Within the first seven days, > 40 % of the initial fipronil concentration declined in all treatments (Wirth et al., 2004). For the nominal fipronil concentrations of 150, 355, and 5000 ng/L time-weighted average concentrations of 60, 116 and 1979 ng/L were calculated based on the data provided in (Wirth et al., 2004). The corresponding 28 d TWA LC_{50} is then 92.62 ng/L (95% CI: 83.61 to 102.6) and 28 d TWA LC_{10} is then 26.67 ng/L. *Palaemonetes pugio* belongs to one of the organism groups that are especially sensitive to fipronil (Crustaceans). However, due to the decline of fipronil concentration over the course of the experiment, a stable chronic exposure cannot be assured. Additionally, the authors report the formation of transformation products in the water-sediment system, which can also induce adverse effects. For these reasons, the study by (S.S. Walse et al., 2004; Wirth et al., 2004) cannot be used for CQC derivation.

One further study (Miller et al., 2020) investigated the effect of the active substance fipronil and its transformation products in a 30-day freshwater mesocosm experiment with invertebrates and algae. In this study, 36 mesocosms (30 treatments and 6 controls) were equipped with recirculating water tanks mimicking streams. The water and sediment used were collected from a non-contaminated site and screened for contaminants. The water parameters and chemical concentrations were analyzed frequently and reported to be stable throughout the experimental duration. In mesocosms spiked with fipronil, transformation products were present. However, as the fipronil transformation products were

below the LOD in the lowest fipronil treatment and at higher fipronil concentrations, the amount of transformation products remained below the level which is expected to cause adverse effects on benthic organisms, the authors stated "the effect of these nontarget compounds on analysis was concluded to be minimal". The mesocosm data by (Miller et al., 2020) contains values for 14 insect species belonging to the orders ephemeroptera, plecoptera, trichoptera and diptera. The study reports effect concentrations (EC_{20} and EC_{50}) as TWA at which the abundance of larval invertebrates was reduced by 20 or 50 % relative to the controls, with the most sensitive species being *Drunella grandis* (28 d EC_{20}= 0.002 µg/L). Additionally, a decline in taxa richness and structural changes in the benthic community were observed upon exposure to fipronil. As no NOEC/EC_{10} values are provided by (Miller et al., 2020), this study cannot be used for CQC derivation.

6 Acute toxicity

6.1 Derivation of AQC (MAC-EQS) using the Assessment Factor (AF) method

The AQC_{AF} (MAC-EQS_{AF}) is determined using assessment factors (AFs) applied to the lowest credible datum from short-term toxicity tests. The lowest short-term effect datum for fipronil is the EC_{50} of 0.032 µg/L (**Table 7**) for the the insect *Chironomus dilutus*. The datum originates from the study by Weston and Lydy (2014), where the effect of fipronil was tested based on the sublethal endpoint 'ability of the midge to thrash when gently prodded', which corresponds to the endpoint immobility. The EC_{50} value is the geometrical mean of two repetitions of the same test, with EC_{50} values of 30 and 35 ng/L identified in the first and second test respectively (Weston & Lydy, 2014). The study is cited in (Bower JC, 2017) p. A22.

Table 9 Most sensitive relevant and reliable acute data for fipronil summarized from **Table 7**

Group	Species	Duration	Effect concentration	Value [µg/L]	Reference
Basic data					
Algae	*Scenedesmus subspicatus*	96 h	EbC50	68	Handley (1991) cited in EC (2004) Vol.3 B9 p. 584
Crustaceans	*Americamysis bahia (Mysidopsis bahia)*	96 h	EC50	0.067	Hano et al. (2019)
Fish	*Lepomis macrochirus*	96 h	LC50	85.2	Ward (1991) cited in EC (2004) Vol.3 B9 p. 551
Additional data					
Insect	*Chironomus dilutus*	96 h	EC50	0.032	Weston and Lydy (2014) cited in Bower JC (2017)
Bivalve	*Mercenaria mercenaria*	96 h	EC50	182	(Overmyer et al., 2007) cited in Bower JC (2017)
Amphibian	*Xenopus laevis*	96 h	LC50	879	(Overmyer et al., 2007) cited in Bower JC (2017)
Higher plant	*Lemna gibba*	14 d	EC50	> 160	Hoberg (1993) cited in EC (2004) Vol.3 B9 p. 596
Clitellata	*Lumbriculus variegatus*	96 h	LC50	> 1900	Putt (2003b) cited in U. EPA (2006)

The generic assessment factor in case of at least one short-term L(E)C50 from each of three trophic levels of the base set (fish, crustaceans and algae) being available is 100. This factor can be lowered to 10 when acute toxicity data for different species do not have a higher standard deviation than a factor of three in both directions or known mode of toxic action and representative species for the most sensitive taxonomic group included in the data set (Table 5 in EC (2018)). The base set (three trophic levels) for fipronil is complete (**Table 9**). Based on the acute data available, crustaceans are the most sensitive organism group to fipronil. Additionally, insects are very sensitive to fipronil, as compared to other taxa (**Table 7**). Fipronil is an insecticide with a known mode-of-action. The compound interacts with GABA-gated chloride channels and glutamate-gated chloride (GluCl) channels of insects and disrupts their central nervous system. Therefore, adverse effects on behavior are expected to be the most sensitive endpoint, which is also supported by the fipronil dataset (**Table 7**). Both sensitive organism groups (crustaceans and insects), as well as data on movement impairment, are represented in the acute dataset. Therefore, the suggested assessment factor is 10 in accordance with EU TGD for EQS:

$$\mathrm{AQC}_{AF}\ (\mathrm{MAC}-\mathrm{EQS}_{AF}) = \frac{lowest\ EC_{10}\ or\ NOEC}{AF}$$

$$\mathrm{AQC}_{AF}\ (\mathrm{MAC}-\mathrm{EQS}_{AF}) = \frac{0.032\left(\frac{\mu g}{L}\right)}{10} = 0.0032\ \left(\frac{\mu g}{L}\right)$$

The application of an AF of 10 to the lowest credible acute datum results in a **MAC-EQS$_{AF}$ = 0.0032 µg/L**.

6.2 Derivation of AQC (MAC-EQS) using the species sensitivity distribution (SSD) method

The minimum data requirements recommended for the application of the SSD approach for EQS water derivation is preferably more than 15, but at least 10 NOEC/EC$_{10}$, from different species covering at least eight taxonomic groups (EC (2018), p. 43). In total, the acute dataset for fipronil contains effect data for 48 species. The phylum chordata is represented by eight species (one amphibian and seven fish species) belonging to seven families. The subphylum crustacea (phylum arthropoda) is represented by eight species from seven families. The dataset of the class insects (phylum arthropoda) contains 17 species belonging to 11 families. The phylum mollusca is represented by seven species from four families. The primary producers are represented by the spermatophyta *Lemna gibba*, three species of

chlorophyte from three different families, two diatoms from the phylum ochrophyta and bacillariophyta and one cyanobacteria. Additionally, data for one annelida species (*Lumbriculus variegatus*) is available in the acute dataset for fipronil. Conclusively, all data requirements for the derivation of AQC (MAC-EQS) using the SSD method (EC (2018), p. 43) are met.

However, for several species and taxonomic groups only unbounded right-censored effect values ($L(E)C_{50}$ > the highest tested concentration) are available (total unbound data n=14). This concerns all diatoms, the cyanobacteria, the spermatophyta, the annelida, five molluscs, three insects and one alga. Nonetheless, those values provide valuable information on species sensitivity. For the species sensitivity distribution (SSD) of the acute effect concentrations of all species, unbounded right-censored values were included to cover the full range of sensitivities and to identify the most sensitive taxa. For the species sensitivity distribution (SSD) of the acute effect concentrations of the most sensitive species, unbounded values were discarded to prevent potential bias in HC_5 estimates.

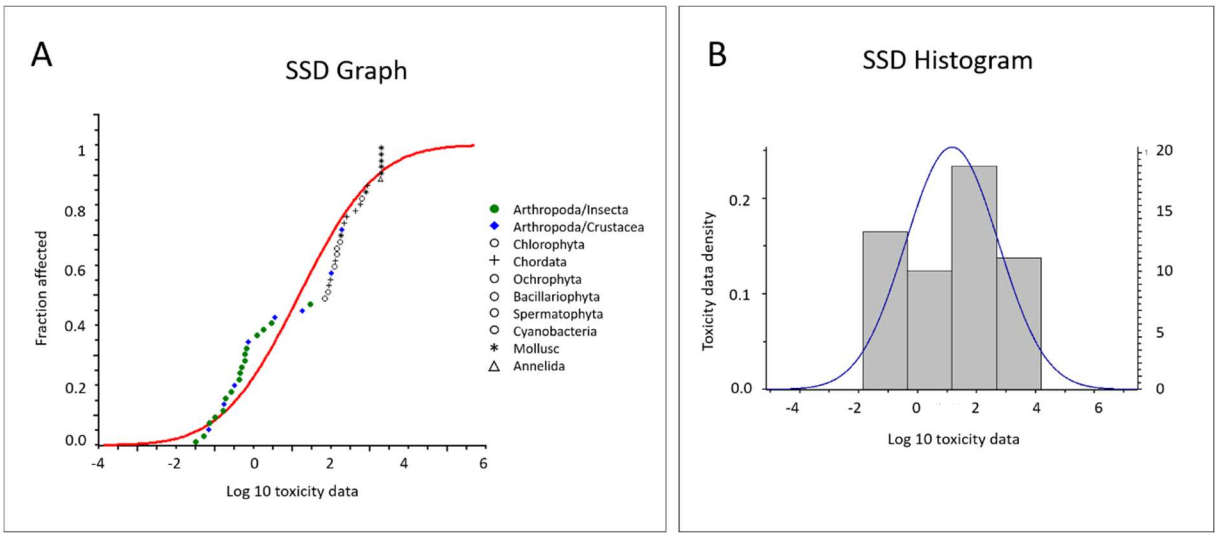


Figure 4 Species sensitivity distribution (SSD) of the acute effect concentrations of all species based on relevant and reliable studies (**Table 7**) generated with ETX 2.2 (van Vlaardingen, Traas, Wintersen, & Aldenberg, 2005) . Number of data points (n) = 48; requirements for normal distribution (B) according to ETX 2.2 (van Vlaardingen et al., 2005) were not met. SSD histogram is shown in B.

The SSD graph based on available data for all organism groups (generated with ETX 2.2 (van Vlaardingen et al., 2005)) is shown in ***Figure 4*** A. When considering all available organism data, the SSD does not meet requirements for normal distribution (B) according to ETX 2.2 (van Vlaardingen et al., 2005) and is unsuitable for the calculation of HC_5 due to a possible bias resulting from the inclusion of unbound data. Nonetheless, the visualization of all data shows that the SSD is divided in two, with the Arthropoda (crustacea and insects) showing a higher sensitivity towards fipronil as compared to other taxonomic groups.

According to the EU TGD for EQS, an SSD should be based on the most sensitive groups of species (EC, 2018). In case of fipronil acute data, there is a clear difference in sensitivity between arthropoda (crustaceans and insects)[14] as compared to other organisms (***Figure 4*** A), which can be explained by the insecticidal mode-of-action. The insecticide adversely affects the central nervous system by interacting with GABA-gated chloride channels and glutamate-gated chloride (GluCl) channels (1.4 Mode of action). The glutamate receptors are specific for insects (Narahashi, Zhao, Ikeda, Nagata, & Yeh, 2007). Additionally, GABA receptors differ between organism groups, with fipronil showing higher affinity towards the receptor structure of invertebrates (Ratra & Casida, 2001).

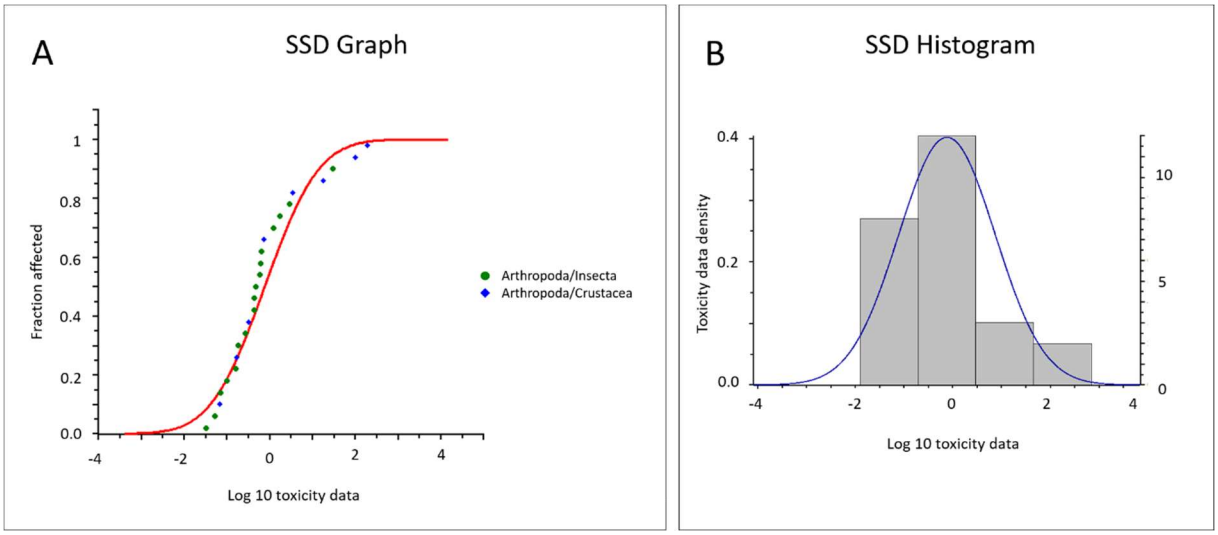


Figure 5 Species sensitivity distribution (SSD) of the acute effect concentrations of most sensitive species (A) based on relevant and reliable studies (**Table 7**) generated with ETX 2.2 (van Vlaardingen et al. 2005). Number of data points (n) =5; requirements for normal distribution (B) according to ETX 2.2 (van Vlaardingen et al. 2005) were not met. SSD histogram is shown in B.

The SSD graph based on available data for insects and crustaceans (generated with ETX 2.2 (van Vlaardingen et al., 2005) is shown in **Figure 5** A. However, the SSD of most sensitive species does, again, not meet the requirements for normal distribution (**Figure** B) according to ETX 2.2 (van Vlaardingen et al., 2005). In conclusion, the quality of the SSD is not sufficient to serve as base for the MAC-EQS.

6.3 Determination of AQC (MAC-EQS) according to mesocosm/field data

No field or mesocosm studies that provide effect concentrations of fipronil are available, thus, no AQC (AA-EQS) based on field data or mesocosm data has been derived.

[14] In the chronc data-set, fish show a similar sensitivity towards fipronil as freshwater crustaceans.

7 Derivation of a biota standard to protect wildlife from secondary poisoning ($QS_{biota, sec pois, fw}$)

Based on the reported BCF and log K_{ow} values for fipronil, a $QS_{biota, sec pois, fw}$ needs to be derived (see section 2.3). A relevant food chain for the trophic transfer of fipronil in Swiss surface waters would be:

Algae – invertebrate (– fish) – fish/mammal/bird

The EU TGD for EQS states that the "food item that will determine the final value for the quality standard in biota is not only dependent on the energy contents of the food items, but also on the bioaccumulation characteristics of the substance through the food chain. Thus, a "critical food item" needs to be identified based on these properties.

Bioconcentration data for fish (*Lepomis macrochirus* and *Oreochromis niloticus*) indicated an initial uptake and concentration of fipronil in fish tissue. However, the parent compound was reported to be eliminated from fish tissue *via* biotransformation and excretion. In addition, it was shown that Bivalves (*Anodonta woodiana*) are capable of biotransforming fipronil (Qu, Ma, Liu, Jing, et al., 2016), indicating biotransformation processes also at lower trophic levels. The estimation of fipronil bioaccumulation (BCFBAF calculator (U. EPA, 2020) at different trophic levels, suggested the highest BAF for the lower trophic level. In agreement, a food web analysis reported the highest biomagnification factor (BMF of 5.31) at the base of the food web (producer vs. consumer I) (Roche et al., 2009). The available data thus indicates that fipronil is subject to bio-dilution within the food web. According to EU TGD for EQS, invertebrates should be selected as the critical food item for substances where bio-dilution occurs. Against this background, the critical food item is invertebrates (e.g. bivalves).

For derivation of $QS_{biota, sec pois, fw}$, BAF is preferred. If reliable experimental bioaccumulation data are not available, the BAF might also be estimated by QSAR (EC, 2018). The BCFBAF tool of EPISuite (U. EPA, 2020) suggests a BAF of 241.8 L/kg wet-wt for lower trophic level including bio-transformation (based on a lipid content of 5.98% and a logKow of 4.0). The assumed rate constants are 0.18/d and 0.10/d for 10 g and 100 g fish, respectively. Normalized to the lipid content of Bivalves (1%), the corresponding BAF would be 40.4 L/kg wet-wt.

Table 10 lists mammalian and avian oral toxicity data relevant for the assessment of secondary poisoning. Effect data for wildlife species was not available, thus, the assessment is limited to laboratory test species. If available, long-term effect data are to be preferred over acute effect data.

Bird toxicity data on dietary and oral exposure indicate that members of the Order Galliformes are especially sensitive to fipronil (EC, 2004). A 28-days oral toxicity and reproduction study with one

member of that Order, the Bobwhite Quail (*Colinus virginianus*), reports a NOEC of 0.8 mg/kg bodyweight per day (corresponding to 10 mg/kg diet) (**Table 10**). In mammalian toxicity studies, the rat (*Rattus norvegicus*) was the most sensitive among the tested organisms (rat, mouse, rabbit and dog) (EC, 2004). The lowest NOEL of 0.5 ppm was identified in a long-term toxicity and carcinogenicity study. This corresponds to 0.019 mg/kg bodyweight per day of fipronil in males and 0.025 mg/kg bodyweight per day in females (**Table 10**). The study was intended to last for 104-weeks between October 1990 and October 1992, but was shortened to 89 weeks for males and 91 weeks for females (EC, 2004). As male rats appear to be generally more sensitive to fipronil (**Table 10**), the NOEL of 0.019 mg/kg bodyweight per day (males) is selected.

For the derivation of a $QS_{biota,\ sec\ pois,\ fw}$, the NOEL of 0.019 mg/kg bodyweight per day in rats is selected.

The data is provided as daily dose normalized to bodyweight. The median bodyweight of the male rats at the beginning of the experiment was 190.5 g (189 to 192 g).

Thus, the value can be expressed as concentration in the diet normalized to energy content of the food (EU TGD, 4.4.5.1 – Method A) and the daily energy expenditure (DEE) of the rat (mammal) can then be estimated as follows:

$$logDEE\ \left[\frac{kJ}{d}\right] = 0.8136 + 0.7149 * \log bw[g]$$

$$logDEE\ \left[\frac{kJ}{d}\right] = 0.8136 + 0.7149 * 2.28$$

This results in a DEE of 277.65 kJ/d (logDEE = 2.44 kJ/d)

The diet concentration on an energy basis (mg/kJ) can now be calculated as:

$$c_{energy\ normalized}\ \left[\frac{mg}{kJ}\right] = dose\ \left[\frac{mg}{kgbw * d}\right] * \frac{bw\ [kg]}{DEE\ \left[\frac{kJ}{d}\right]}$$

$$c_{energy\ normalized}\ \left[\frac{mg}{kJ}\right] = 0.019\ \left[\frac{mg}{kgbw * d}\right] * \frac{0.1905\ [kg]}{2.44\ \left[\frac{kJ}{d}\right]}$$

This results in an energy content normalized fipronil concentration of 0.001481 mg/kJ.

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

$$c_{food\ item}\left[\frac{mg}{kg_{ww}}\right] = c_{energy\ nomralized}\left[\frac{mg}{kJ}\right] * \ energy\ content_{food\ item,dw} * \ (1 - moisture\ fraction_{food\ item})$$

According to Table 7 of EU TGD for EQS, standard moisture content and energy content of bivalves are 92% and 19 kJ/g_{dw} (19000 kJ/kg_{dw}), respectively.

$$c_{food\ item}\left[\frac{mg}{kg_{ww}}\right] = 0.001481\left[\frac{mg}{kJ}\right] * \ 19000 * \ (1 - 0.92)$$

The resulting fipronil concentration in mussels is 2.25 mg/kg_{ww}.

As the calculations are based on a NOEL from a chronic mammal study, it is not necessary to account for limited exposure time.

The oral and dietary toxicity dataset for fipronil contains studies with four mammalian species belonging to three distinct Orders (rodentia, carnivora and lagomorpha). The birds are represented by seven species from four Orders (anseriformes, Cclumbiformes, galliformes and passeriformes). Some additional unbound data and data obtained with fipronil formulations is available for birds and one reptile (*Acanthodactylus dumerili*). Hoverer, this data cannot be included in the SSD. For the derivation of an SSD, data for minimum of 10 species is required. Additionally, to conduct a SSD, the dataset should contain data for wildlife-relevant predatory species. In case of fipronil, the data set in not sufficient to perform an SSD.

The estimated, lipid normalized BAF is 40.4 L/kg wet-wt (low trophic level, 1% lipid content, (U. EPA, 2020)).

According to Table 10 EU TGD for EQS, an assessment factor of 10 should be applied to an effect concentration based on the lowest long-term datum available. The suggested assessment factor is thus 10 in accordance with EU TGD for EQS:

$$QS_{\text{biota,sec pois,fw}}\ [\text{mg/kg}] = \frac{\text{lowest chronic value}\ [\frac{\text{mg}}{\text{kg}_{\text{ww}}}]}{AF}$$

$$QS_{\text{biota,sec pois,fw}}\ [\text{mg/kg}] = \frac{2.25\ \text{mg/kg}_{\text{ww}}}{10}$$

$$QS_{\text{biota,sec pois,fw}}\left[\frac{\text{mg}}{\text{kg}}\right] = 0.225$$

$$QS_{\text{biota,sec pois,fw}}\left[\frac{\mu\text{g}}{\text{kg}}\right] = 225.16$$

To converting the biota standard into an equivalent concentration in water, the BAF of 40.4 L/kg wet-wt is used:

$$QS_{\text{biota,sec pois,fw}}\ [\ \mu\text{g/L}] = \frac{QSbiota}{BAF}$$

$$QS_{\text{biota,sec pois,fw}}\ \left[\frac{\mu\text{g}}{\text{L}}\right] = \frac{225.16}{40.4} = 5.57$$

The application of an AF of 10 to the lowest credible chronic datum results in a **$QS_{\text{Biota, sec pois, fw}}$ = 0.225 mg/kg** or **5.56 µg/L/L**.

-EQS) for Fipronil

g of top predators

Endpoint	Effect concentration			Comment	Reference
Acute toxicity to birds					
Median LD50		39.19	mg/kg		Mineau (2001) cited in *Weir, Suski, and Salice (2010)*
NA	>	500	mg/kg	feeding activity not affected up to >500 mg/kg	*Avery, Primus, Mihaich, Decker, and Humphrey (1998)*
LD50	>	2000	md/kg bw	begin of treatment April 10 end of treatment April 20	*Lopez-Antia, Ortiz-Santaliestra, Camarero, Mougeot, and Mateo (2015)*
NOED		16	mg/kg bw	Substance administered in a single dose by oral intubation, initial mean bodyweight 435 g/bird	Hakin and Rodgers (1992) cited in EC (2004) p. 509
LD50		34	mg/kg bw	Substance administered in a single dose by oral intubation, initial mean bodyweight 435 g/bird	Hakin and Rodgers (1992) cited in EC (2004) p. 509
LD50		34	mg/kg		Stavola (1994b) cited in Lopez-Antia et al. (2015)
NOEL		2150	mg/kg bw	Substance administered in gelatine capsules, initial mean bodyweight 1172 g/bird, two test doses : 1470 and 2150 mg a.i./kg bw, NOEL is the highest dose tested	Pedersen (1990) cited in EC (2004) p. 507
LD50	>	2150	mg/kg	Substance administered in gelatine capsules, initial mean bodyweight 1172 g/bird, two test doses : 1470 and 2150 mg a.i./kg bw	Pedersen (1990) cited in EC (2004) p. 507
LD50	>	2500	mg/kg		Goodyear(1994b) cited in Lopez-Antia et al. (2015)
NOED		1	mg/kg bw	Substance administered in gelatine capsules, initial mean bodyweight 215 g/bird	Pedersen (1990) cited in EC (2004) p. 506
LD50		11.3	mg/kg		Goodyear(1994a) cited in Lopez-Antia et al. (2015)
LD50		11.3	mg/kg bw	Substance administered in gelatine capsules, initial mean bodyweight 215 g/bird	Pedersen (1990) cited in EC (2004) p. 506
LD50		1065	mg/kg		PED (Pesticide Ecotoxicity Database) (2010) cited in Weir et al. (2010)
LD50	>	2000	mg/kg bw	Substance administered in a single dose by oral intubation, mean bodyweight 445 g/bird	Hakin and Rodgers (1991) cited in EC (2004) p. 510
LD50	>	500	mg/kg		Stavola (1994c) cited in Kitulagodage (2011)

Passer domesticus	Oral	NA	LD50		1000	mg/kg		Goodyear (1994c) cited in Kitulagodage (2011)
Perdix perdix	Oral	14 d	LD80		30	mg/kg bw	Substance administered in a single dose in a gelatine capsule, initial mean bodyweight 364 g/bird	Grolleau (1993) cited in EC (2004) p. 510
Phasianus colchicus	Oral	14 d	LD50		31	mg/kg		Stavola (1994) cited in Kitulagodage (2011)
Phasianus colchicus	Oral	35 d	NOEL		10	mg/kg	Substance administered in a single dose by oral intubation, mean bodyweight 1257 g/bird	Hakin and Rodgers (1992) cited in EC (2004) p. 508
Phasianus colchicus	Oral	35 d	LD50		31	mg/kg bw	Substance administered in a single dose by oral intubation, mean bodyweight 1257 g/bird	Hakin and Rodgers (1992) cited in EC (2004) p. 508
Quiscalus major	Oral	4 d	NA	>	500	mg/kg	feeding activity not affected up to >500 mg/kg	Avery et al. (1998)
Taeniopygia guttata	Oral	28 d	LD50		45.41	mg/kg	formulation study, estimated LD50 (eLD50)	*Kitulagodage, Astheimer, and Buttemer (2008)*
Short-term toxicity to birds								
Colinus virginianus	Oral	5 d	NOEL		3.77	mg/kg bw/day	Substance mixed into standard bird diet, initial mean bodyweights 26-31 g/bird, NOEL: 19.5 mg/kg diet	Pedersen (1990) cited in EC (2004) p. 514
Anas platyrhynchos	Oral	5 d	NOEL		554	mg/kg bw/day	Substance mixed into standard bird diet, initial mean bodyweights 90 – 110 g/bird, NOEL: 1250 mg/kg diet	Pedersen (1990) cited in EC (2004) p. 515
Subchronic toxicity and reproductive toxicity to birds								
Colinus virginianus	Oral	28 d	NOEC		0.8	mg/kg bw/day	Substance mixed into standard bird diet, initial mean bodyweights 168 - 247 g/bird, NOEC: 10 mg a.i./kg diet	Pedersen (1993) cited in EC (2004) p. 520
Colinus virginianus	Oral	28 d	NOEC		0.8	mg/kg bw/day	Substance mixed into standard bird diet, initial mean bodyweights 205 – 225 g/bird, NOEC: 10 mg a.i./kg diet	Pedersen and DuCharme (1992) cited in EC (2004) p. 520
Anas platyrhynchos	Oral	28 d	NOEC		96	mg/kg bw/day	Substance mixed into standard bird diet, initial mean bodyweights 871 - 1297 g/bird, NOEC: 1000 mg a.i./kg diet	Pedersen (1993) cited in EC (2004) p. 522
Anas platyrhynchos	Oral	28 d	NOEC		91	mg/kg bw/day	Substance mixed into standard bird diet, initial mean bodyweights 828 – 1393 g/bird, NOEC: 1000 mg a.i./kg diet	Pedersen and Lesar (1993) cited in EC (2004) p. 522
Toxicity to reptiles								
Acanthodactylus dumerili	Oral	4 weeks	LD50		30	mg/kg bw	formulation study, LD50 based on a.i.	Peveling and Demba (2003)

Species	Exposure	Duration	Endpoint	Effect concentration	Comment		Reference
Mammals acute oral toxicity							
Rattus norvegicus	Oral	14 d	LD50	92 mg/kg bw	(males)	Substance administered in a single dose by oral intubation, body weight 110-138 g	Gardener (1988a) cited in EC (2004) p. 102
Rattus norvegicus	Oral	14 d	LD50	97 mg/kg bw	(males and females combined)	Substance administered in a single dose by oral intubation, body weight 110-138 g	Gardener (1988a) cited in EC (2004) p. 102
Rattus norvegicus	Oral	14 d	LD50	103 mg/kg bw	(females)	Substance administered in a single dose by oral intubation, body weight 110-138 g	Gardener (1988a) cited in EC (2004) p. 102
Mus musculus	Oral	14 d	LD50	91 mg/kg bw	(females)	Substance administered in a single dose by oral intubation, body weight 20-24 g	Mondot and Dange (1995) cited in EC (2004) p. 103
Mus musculus	Oral	14 d	LD50	95 mg/kg bw	(males and females combined)	Substance administered in a single dose by oral intubation, body weight 20-30 g	Mondot and Dange (1995) cited in EC (2004) p. 103
Mus musculus	Oral	14 d	LD50	98 mg/kg bw	(males)	Substance administered in a single dose by oral intubation, body weight 26-30 g	Mondot and Dange (1995) cited in EC (2004) p. 103
Rattus norvegicus	Oral	28 d	NOAEL	1.43 mg/kg bw	(males)	based on molecular endpoints, repeated exposure, formulation study, Substance administered via oral gavage, bw males (28-32 g), also states that NOAEL is <1.87 mg/kg bw	Abouelghar, El-Bermawy, and Salman (2020)
Rattus norvegicus	Oral	24 h	LD50	143.5 mg/kg bw	(males)	based on molecular endpoints, repeated exposure, formulation study, LD50 is based on a.i., substance administered via oral	Abouelghar et al. (2020)

						gavage, bw males (28-32 g)	
Mammals oral 28-day toxicity							
Canis familiaris	Oral	28 d	NOEL	1 mg/kg/day		Substance administered trought capsules, body weight 8.85-10.75 kg	Holmes (1991d) cited in EC (2004) p. 120
Rattus norvegicus	Oral	28 d	NOEL	< 3.4 mg/kg bw /day	(males)	Substance administered trought diet, body weight 187-234 g	Peters et al., (1990a) cited in EC (2004) p. 117
Rattus norvegicus	Oral	28 d	NOEL	< 3.5 mg/kg bw /day	(females)	Substance administered trought diet, body weight 156-193 g	Peters et al., (1990a) cited in EC (2004) p. 117
Rattus norvegicus	Oral	28 d	NOEL	< 25 ppm		Substance administered trought diet, body weight 156-234 g	Peters et al., (1990a) cited in EC (2004) p. 117
Mammals oral 90-day toxicity							
Canis familiaris	Oral	90 d	NOAEL	0.5 mg/kg bw/day		Substance administered trought capsule, body weight 7.3-9.8 kg	Holmes (1991a) cited in EC (2004) p. 126
Rattus norvegicus	Oral	90 d	NOEL	0.07 mg/kg/day		Substance administered trought diet, body weight 135-203 g, NOEL:1ppm	Holmes (1991c) cited in EC (2004) p.122
Rattus norvegicus	Oral	90 d	NOAEL	0.33 mg/kg bw/day	(males)	Substance administered trought diet, body weight 154-203 g,	Holmes (1991c) cited in EC (2004) p. 122
Rattus norvegicus	Oral	90 d	NOAEL	0.37 mg/kg bw/day	(females)	Substance administered trought diet, body weight 135-180 g	Holmes (1991c) cited in EC (2004) p. 122
Rattus norvegicus	Oral	90 d	NOAEL	0.35 mg/kg bw/day	(males and females combined)	Substance administered trought diet, body weight 135-180 g	Holmes (1991c) cited in EC (2004) p. 122
Rattus norvegicus	Oral	90 d	NOAEL	5 ppm		Substance administered trought diet, body weight 135-203 g	Holmes (1991c) cited in EC (2004) p. 122
Mammals oral 1-year toxicity							
Canis familiaris	Oral	364 d	NOAEL	0.2 mg/kg bw/day		Substance administered trought capsule, body weight 6.3-9.1 kg	Holmes (1992a) cited in EC (2004) p. 128

Canis familiaris	Oral	364 d	NOEL	0.3	mg/kg bw/day		Substance administered trought diet, body weight 7.3-9.1 kg	Holmes (1993a) cited in EC (2004) p. 131
Mammals long-term toxicity and carcinogenicity								
Rattus norvegicus	Oral	104 weeks	NOAEL	0.5	ppm		Substance administered via diet, study terminatedat different timepoints for male and female (week 88-91), 189 to 192 g (males) and 158 to 162 g (females)	Aughton (1992b) cited in EC (2004) p. 146
Rattus norvegicus	**Oral**	**104 weeks**	**NOAEL**	**0.019**	**mg/kg bw/day**	**(males)**	Substance administered via diet, study terminatedat different timepoints for male and female (week 88-91), 189 to 192 g (males) and 158 to 162 g (females)	Aughton (1992b) cited in EC (2004) p. 146
Rattus norvegicus	**Oral**	**104 weeks**	**NOAEL**	**0.025**	**mg/kg bw/day**	**(females)**	Substance administered via diet, study terminatedat different timepoints for male and female (week 88-91), 189 to 192 g (males) and 158 to 162 g (females)	Aughton (1992b) cited in EC (2004) p. 146
Mus musculus	Oral	78 weeks	NOAEL	0.5	ppm		Substance administered via diet, mean bw male (24-30 g) and female (19 - 28 g)	Broadmeadow (1992a) cited in EC (2004) p. 156
Mus musculus	Oral	78 weeks	NOAEL	0.055	mg/kg bw/day	(males)	Substance administered via diet, mean bw male (24-30 g) and female (19 - 28 g)	Broadmeadow (1992a) cited in EC (2004) p. 156
Mus musculus	Oral	78 weeks	NOAEL	0.063	mg/kg bw/day	(females)	Substance administered via diet, mean bw male (24-30 g) and female (19 - 28 g)	Broadmeadow (1992a) cited in EC (2004) p. 156
Mammals multi-generation reproduction study in the rat								
Rattus norvegicus	Oral		NOAEL	3	ppm	(general toxic effects)	Substance administered via diet, mean bw male	King (1992a) cited in EC (2004) p. 160

						(115-168 g) and female (99-140 g)	
Rattus norvegicus	Oral		NOAEL	0.25 mg/kg bw/day	(males) (general toxic effects)	Substance administered via diet, mean bw male (115-168 g) and female (99-140 g)	King (1992a) cited in EC (2004) p. 160
Rattus norvegicus	Oral		NOAEL	0.27 mg/kg bw/day	(females) (general toxic effects)	Substance administered via diet, mean bw male (115-168 g) and female (99-140 g)	King (1992a) cited in EC (2004) p. 160
Rattus norvegicus	Oral		NOEL	30 ppm	(reproductive effects)	Substance administered via diet, mean bw male (115-168 g) and female (99-140 g)	King (1992a) cited in EC (2004) p. 160
Rattus norvegicus	Oral		NOEL	2.53 mg/kg bw/day	(males) (reproductive effects)	Substance administered via diet, mean bw male (115-168 g) and female (99-140 g)	King (1992a) cited in EC (2004) p. 160
Rattus norvegicus	Oral		NOEL	2.74 mg/kg bw/day	(females) (reproductive effects)	Substance administered via diet, mean bw male (115-168 g) and female (99-140 g)	King (1992a) cited in EC (2004) p. 160
Mammals teratogenicity study							
Rattus norvegicus	Oral		NOAEL	4 mg/kg/day	(maternal toxicity)	Substance administered via daily oral gavage, mated rats bw 207 and 280g	Brooker and John (1991a) cited in EC (2004) p. 168
Rattus norvegicus	Oral		NOAEL	20 mg/kg/day	(developmental toxicity)	Substance administered via daily oral gavage, mated rats bw 207 and 280g	Brooker and John (1991a) cited in EC (2004) p. 168
Oryctolagus cuniculus	Oral		NOAEL	0.2 mg/kg bw/day	(maternal toxicity)	Substance administered via daily oral gavage, bw 3.27 and 4.95 kg, days 6 to 19 post coitum	King (1990c) cited in EC (2004) p. 170
Oryctolagus cuniculus	Oral		NOEL	1 mg/kg bw/day	(developmental toxicity)	Substance administered via daily oral gavage, bw 3.27 and 4.95 kg, days 6 to 19 post coitum	King (1990c) cited in EC (2004) p. 170

Oryctolagus cuniculus	Oral	14 d	NOAEL	0.2	mg/kg bw/day	(maternal toxc effect)	Substance administered via daily oral gavage, bw. (3.31 – 4.82 kg) NOAEL = highest concentration tested	Anonymos cited in BASF (2011) Document IIIA 6.8 p. 7
Oryctolagus cuniculus	Oral	14 d	NOEL	>= 0.1	mg/kg bw/day	embryotoxic/teratogenic effect	Substance administered via daily oral gavage, bw. (3.31 – 4.82 kg) NOAEL = highest concentration tested	Anonymos cited in BASF (2011) Document IIIA 6.8 p. 7
Mammals acute neurotoxicity								
Rattus norvegicus	Oral	14 d	NOEL	0.5	mg/kg bw	(neurotoxicity and general toxicity)	Substance administered via oral gavage, bw males (221.8 - 291.0 g) and females (144.5-186.3 g), 14 days post-treatment	Gill et al., (1993a) cited in EC (2004) p. 173
Rattus norvegicus	Oral	14 d	NOEL	2.5	mg/kg bw	(neurotoxicity and general toxicity)	Substance administered via oral gavage, bw males (248-329 g) and females (181-229 g)	Hughes (1997a) cited in EC (2004) p. 177
Mammals 90-day neurotoxicity								
Rattus norvegicus	Oral	90 d	NOEL	>= 150	ppm	(neurotoxicity)	Substance administered via diet, bw males (251.2-279.9 g) and females (162.6 - 192.3 g)	Driscoll and Hurley (1993a) cited in EC (2004) p. 183
Rattus norvegicus	Oral	90 d	NOEL	8.9	mg/kg bw/day	(males) (neurotoxicity)	Substance administered via diet, bw males (251.2-279.9 g) and females (162.6 - 192.3 g)	Driscoll and Hurley (1993a) cited in EC (2004) p. 183
Rattus norvegicus	Oral	90 d	NOEL	10.8	mg/kg bw/day	(females) (neurotoxicity)	Substance administered via diet, bw males (251.2-279.9 g) and females (162.6 - 192.3 g)	Driscoll and Hurley (1993a) cited in EC (2004) p. 183
Rattus norvegicus	Oral	90 d	NOEL	5	ppm	(general toxicity)	Substance administered via diet, bw males (251.2-279.9 g) and females (162.6 - 192.3 g)	Driscoll and Hurley (1993a) cited in EC (2004) p. 183
Rattus norvegicus	Oral	90 d	NOEL	0.3	mg/kg bw/day	(males) (general toxicity)	Substance administered via diet, bw males (251.2-	Driscoll and Hurley (1993a) cited in EC (2004) p. 183

							279.9 g) and females (162.6 - 192.3 g)	
Rattus norvegicus	Oral	90 d	NOEL	0.4	mg/kg bw/day	(females) (general toxicity)	Substance administered via diet, bw males (251.2-279.9 g) and females (162.6 - 192.3 g)	Driscoll and Hurley (1993a) cited in EC (2004) p. 183
Mammals developmental neurotoxicity								
Rattus norvegicus	Oral		NOAEL	10	ppm	(developmental toxicity)	Substance administered via diet, bw females (208.3-320.6 g)	Mandella (1995a) cited in EC (2004) p. 185
Rattus norvegicus	Oral		NOAEL	0.91	mg/kg/day	(developmental toxicity)	Substance administered via diet, bw females (208.3-320.6 g)	Mandella (1995a) cited in EC (2004) p. 185
Rattus norvegicus	Oral		NOEL	5	ppm	(general toxicity)	Substance administered via diet, bw females (208.3-320.6 g)	Mandella (1995a) cited in EC (2004) p. 185
Rattus norvegicus	Oral		NOEL	0.05	mg/kg/day	(general toxicity)	Substance administered via diet, bw females (208.3-320.6 g)	Mandella (1995a) cited in EC (2004) p. 185
Mammals reproductive toxicity								
Rattus norvegicus	Oral	9 d	NOAEL	4	mg/kg bw/d	maternal, toxic effect	Substance administered via oral gavage, females (170 and 228 g), exposure from days 6 to 15 post coitum (p.c.)	Anonymos cited in BASF (2011) Document IIIA 6.8, p. 1
Rattus norvegicus	Oral	9 d	NOEL	20	mg/kg bw/d	maternal embryotoxic /teratogeenic effects,	Substance administered via oral gavage, females (170 and 228 g), exposure from days 6 to 15 post coitum (p.c.), NOAEL=highest concentration tested	Anonymos cited in BASF (2011) Document IIIA 6.8, p. 1

8 Toxicity of transformation products

Fipronil is transformed in the environment to a broad variety of transformation products summarized in ***Table 4***. Toxicity data are available for fipronil sulfone (MB 46136), fipronil sulfide (MB 45950), fipronil desulfinyl (MB 46513), fipronil amide (RPA 200766), RPA104615 and RPA200761 (**Effect data** on transformation products of fipronil

Table 16). In order to facilitate data interpretation, all valid toxicity data for the transformation products and, as comparison, for fipronil are visualized in ***Figure 6***.

The acute (***Figure 6*** A) and chronic (***Figure 6*** B) toxicity data of fipronil transformation products indicate that, among all, fipronil sulfone (MB 46136), fipronil sulfide (MB 45950) and fipronil desulfinyl (MB 46513) are the most toxic products. By isolating the most sensitive taxa (crustaceans, insects and fish) for those transformation products (***Figure 6*** C, D), it becomes clear that those taxa are more sensitive to fipronil sulfone (MB 46136) and fipronil sulfide (MB 45950) as compared to the parent compound.

The lowest long-term effect data available for fipronil sulfone (MB 46136) and fipronil sulfide (MB 45950) originate from the studies with the saltwater crustacean *Americamysis bahia* (*Mysidopsis bahia*), with NOEC of 0.0051 µg/L and 0.0046 µg/L for fipronil sulfone (MB 46136) and fipronil sulfide (MB 45950), respectively (**Table 11**). Similarly, in studies with the parent compound, *Americamysis bahia* (*Mysidopsis bahia*) was the most sensitive species in chronic studies (NOEC of 0.0077 µg/L, **Table *8***). In case of fipronil desulfinyl (MB 46513) however, no data is available on this saltwater crustacean. Instead, the lowest long-term endpoint is NOEC of 41 µg/L for *Daphnia magna* (**Table 11**). In long-term toxicity studies with fipronil, fipronil sulfone (MB 46136) and fipronil sulfide (MB 45950), *Daphnia magna* was ~1000 times, 3000 times and 90 times less sensitive as compared to *Americamysis bahia (Mysidopsis bahia)*, respectively (**Effect data** on transformation products of fipronil

Table 16, **Table 7**).

The lowest short-term effect data available for fipronil sulfone (MB 46136) and fipronil sulfide (MB 45950) originate from the study by Weston and Lydy (2014) with the insect species *Chironomus dilutus* (**Effect data** on transformation products of fipronil

Table 16). The effect of the transformation products was tested on the ability of the midge to thrash when prodded, with the resulting EC_{50} values of 0.0077 µg/L and 0.0099 µg/L for fipronil sulfone (MB 46136) and fipronil sulfide (MB 45950), respectively (**Table 11**). Similarly, the lowest acute effect data for the parent compound originated from the same study with the reported EC_{50} for *Chironomus*

dilutus of 0.032 µg/L, **Table 9**). In case of fipronil desulfinyl (MB 46513) however, no data is available on any insect species. Instead, the lowest short-term endpoint is LC_{50} of 1.5 µg/L for the crustacean *Americamysis bahia (Mysidopsis bahia)* (**Table 11**). In short-term toxicity studies with fipronil, fipronil sulfone (MB 46136) and fipronil sulfide (MB 45950), *Americamysis bahia (Mysidopsis bahia)* was two to eight times less sensitive as compared to *Chironomus dilutus* (**Effect data** on transformation products of fipronil

Table 16, **Table 7**).

Based on the analysis of available effect data and the data-gaps regarding fipronil desulfinyl (MB 46513), fipronil sulfone (MB 46136) and fipronil sulfide (MB 45950) are considered transformation products of concern.

Fipronil sulfone (MB 46136), fipronil sulfide (MB 45950) and fipronil desulfinyl (MB 46513) have the potential to interfere with chloride channels and are thus toxicologically relevant (EC, 2004). Available data regarding the oral toxicity of transformation products for mammals and birds is summarized in **Data for** fipronil transformation products on secondary poisoning of top predators

Table 17. Based on acute data fipronil sulfone (MB 46136) showed lower toxicity, fipronil sulfide (MB 45950) had similar effects and fipronil desulfinyl (MB 46513) showed higher toxicity as compared to fipronil. Unfortunately, data on developmental or reproductive toxicity for fipronil sulfone (MB 46136) and fipronil sulfide (MB 45950) are missing.

Within biota, fipronil sulfone was reported to be the main biotransformation product of fipronil and to be more persistent in biota as compared to the parent compound (Chapleoalnd Hall (1992) cited in (EC, 2004)). In view of the biotransformation of fipronil to fipronil sulfone (MB 46136) within the food chain, long-term oral toxicity data on fipronil sulfone (MB 46136) are needed for a profound assessment of secondary poisoning.

Conclusively, the transformation products fipronil sulfone (MB 46136), fipronil sulfide (MB 45950) and fipronil desulfinyl (MB 46513) are toxicologically relevant based on their effects on the aquatic environment and mammalian/bird toxicity data. Additionally, the transfer of fipronil sulfone (MB 46136) within the trophic chain is a factor worth considering. Miller et al. (2020) reported a similar distribution pattern for the transformation products as compared to the parent compound. The parent compound and Fipronil sulfone (MB 46136) were detected in ~22 % of the urban sampling sites, whereas fipronil sulfide (MB 45950) and fipronil desulfinyl (MB 46513) were detected less often (11-13 %). Further information on the transformation products regarding their mode or action,

bioaccumulation and environmental fate is provided in Annex II (Section 13.3). However, the derivation of acute and chronic quality criteria for the transformation products is beyond the scope of this dossier.

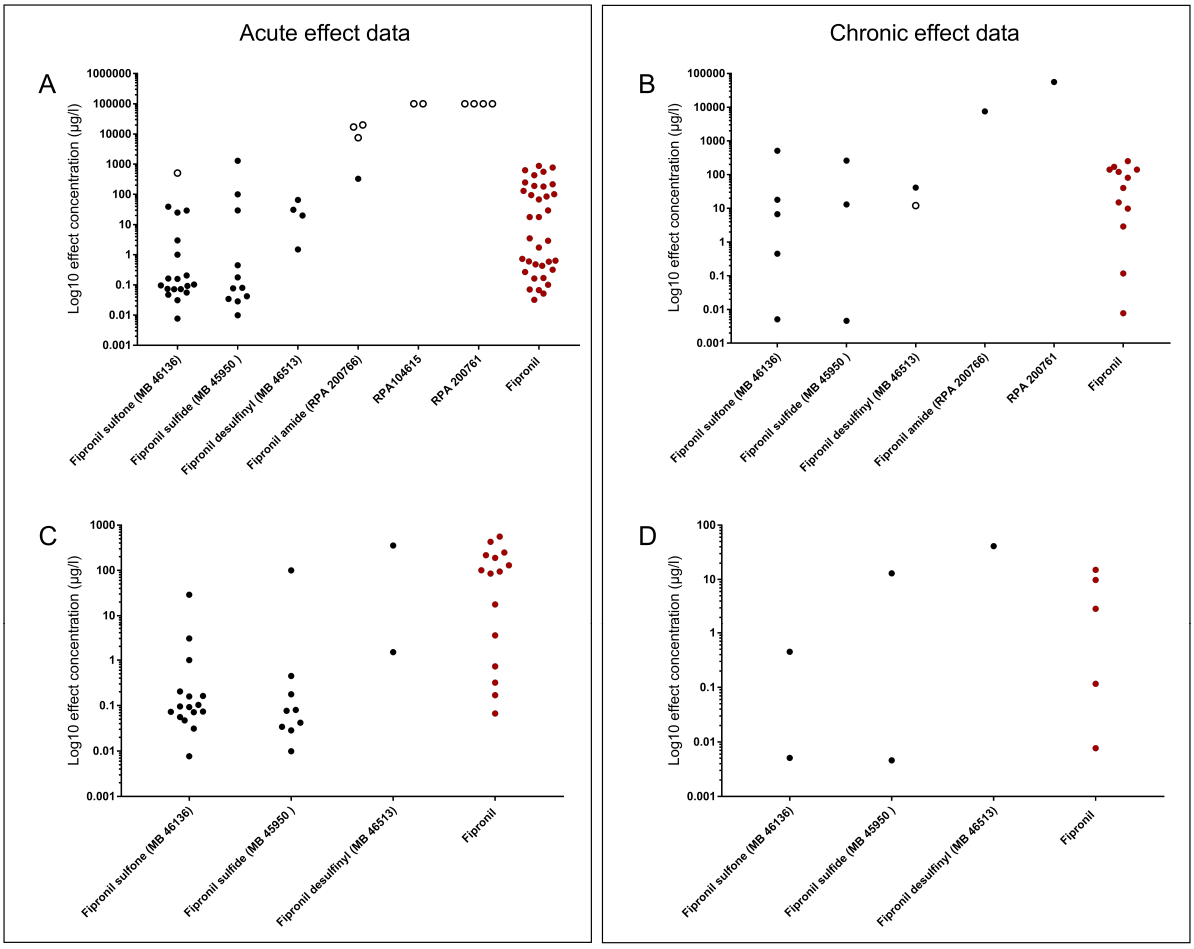


Figure 6: acute (A, C) and chronic (B, D) effect data for fipronil (red) and the fipronil transformation products (black). In A and B data for Fipronil sulfone (MB 46136), Fipronil sulfide (MB 45950), Fipronil desulfinyl (MB 46513), Fipronil amide (RPA 200766), RPA104615 and RPA200761 are depicted. For a better vizualisation, the effect data are limited to the most sensitive taxa (crustaceans, insects and fish) and Fipronil, Fipronil sulfone (MB 46136), Fipronil sulfide (MB 45950) and Fipronil desulfinyl (MB 46513) in C and D.

Table 11: lowest acue and chronic effect data for Fipronil (as a comparison) and its transformation products Fipronil sulfone (MB 46136), Fipronil sulfide (MB 45950) and Fipronil desulfinyl (MB 46513). Further data and references are provided in **Table 16**

Substance	Acute effect data		Chronic effect data	
fipronil	EC50=0.032 µg/L	*Chironomus dilutus*	NOEC=0.0077 µg/L	*Americamysis bahia (Mysidopsis bahia)*
Fipronil sulfone (MB 46136)	EC50=0.0077 µg/L	*Chironomus dilutus*	NOEC=0.0051 µg/L	*Americamysis bahia (Mysidopsis bahia)*
Fipronil sulphide (MB 45950)	EC50=0.0099	*Chironomus dilutus*	NOEC=0.0046 µg/L	*Americamysis bahia (Mysidopsis bahia)*
Fipronil desulfinyl (MB 46513)	LC50=1.5 µg/L	*Americamysis bahia (Mysidopsis bahia)*	NOEC=41 µg/L	*Daphnia magna*

9 Proposed CQC (AA-EQS) and AQC (MAC-EQS) to protect aquatic species

The different QS values for each derivation method included in the EU TGD for EQS are summarized in **Table 12**. According to the EU TGD for EQS, the most reliable extrapolation method for each substance should be used (EC, 2018).

For highly hydrophobic compounds, the finally derived EQS (which is an $EQS_{water,\ dissolved}$) should be corrected using the default concentration of suspended matter (C_{SPM}) and the partition coefficient to suspended matter ($K_{p,susp}$) (EC 2018). As discussed in Section 2.2, correction based on OC content is not indicated for fipronil.

Table 12 QS derived according to the methodologies stipulated in the EU TGD for EQS and their corresponding assessment factors (AF). All concentrations expressed as µg/L. Proposed EQS are in bold letters/numbers.

	Value (*µg/L*)	AF
CQC_{AF} ($AA\text{-}EQS_{AF}$)	**0.00077**	**10**
AQC_{AF} ($MAC\text{-}EQS_{AF}$)	**0.0032**	**10**
$QS_{biota,\ sec\ pois,\ fw}$	5.56	10

For CQC_{AF} ($AA\text{-}EQS_{AF}$) the value of 0.00077 µg/L based on laboratory data (Section 6.1) was derived. For AQC (MAC-EQS) the quality of the SSD was insufficient for EQS derivation and therefore AQC (MAC-EQS) was based on the assessment factor method with the value of 0.0032 µg/L.

The suggested $QS_{Biota,\ sec\ pois,\ fw}$ (5.56 µg/L) is higher than the suggested CQC_{AF} ($AA\text{-}EQS_{AF}$) (0.00077 µg/L). Thus, it can be assumed that application of the suggested CQC_{AF} ($AA\text{-}EQS_{AF}$) will be protective of secondary poisoning of predators.

A **CQC (AA-EQS) of 0.00077 µg/L** and an **AQC (MAC-EQS) of 0.0032 µg/L for fipronil** including the application of an AF of 10 in both cases are thus suggested.

10 Protection of aquatic organisms and uncertainty analysis

A large dataset is available for fipronil, which allowed the derivation of CQC and AQC based on the assessment factor method (AF of 10). The most sensitive organism groups for fipronil are crustaceans and insects. Additionally, fish show a compareable sensitivity to freshwater crustaceans in chronic studies. Representatives of these three taxa (insects, crustaceans and fish) are included in chronic and acute datasets. However, some insect species require the application of sediment, especially in long-term studies. The necessity of sediment addition to the system however, adds a factor of uncertainty to the study due to sorption of fipronil to sediment particles.

The majority of acute insect data originate from the study by Weston and Lydy (2014). The authors included well-characterized, standard species, but also field-collected organisms, for which standardized test-protocols do not yet exist. Despite this drawback, the study by Weston and Lydy (2014) provides valuable information on species sensitivities and is relevant and reliable.

Fipronil is applied as a racemic mixture (1:1) with the (+/S) and (-/R) showing different effects depending on the organism. As the degradation and uptake of fipronil by biota is also enantioselective, this might result in shifted racemic ratio and changes in toxic potential as compared to the racemate.

Within the environment and biota, fipronil is transformed to a range of transformation products, some of which have a higher toxicity as compared to the parent compound. Although the toxic transformation products dissipate from water into sediment, they need to be considered.

The derived QC can be regarded as protective of aquatic organisms. However, the lowest mesocosm EC_{20} value (28 d EC_{20}= 0.002 µg/L, *Drunella grandis*) reported by (Miller et al., 2020) is only a factor of ~2.6 higher than CQC (AA-EQS). In view of the high sensitivity of insects to fipronil, a larger insect dataset and more information on the environmental fate of fipronil enantiomers and transformation products as described above would make the QC more robust.

Based on the reported LOQ-range for fipronil with the use of ESI(-)-LC-MS/MS (0.0005 – 0.006 µg/l, Spycher et al. (2018)), the detectability of fipronil around the suggested CQC_{AF} and AQC_{AF} will depend on surface water matrix.

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12 Annex I: Effect data

Table 13: Effect data collection of all effect data for fipronil. An evaluation of validity[15] was performed according to the CRED criteria (Moermond et al., 2016). Study evaluations from the DAR **EC (2004)** were adopted as "face value" according to the TGD for EQS. Grey="not robust" (validity 3 or 4) study or valid study not used, study in bold in black = most relevant study for the species; underlined values = critical toxicity values for the assessment factor method. h= hours, d=days, n.r. = not reported.

Acute freshwater effect data											
Group	Species	Endpoint	Duration	Parameter	value (µg/L)	Analytics	Exposure	Purity (%)	Validity	Reference	Notes
cyanobacterium	Anabaena flos-aquae	growth rate	120 h	EC50	> 170	m-am	S	96.1	2/C2	Hoberg J.R. 1993 cited in EC (2004) Vol.3 B9 p.585	supportive
algae	Chlorella pyrenoidosa	growth rate	48 h	EC50	3500	n.r.	S	98	R4/C1	Gao, Wang, Jiang, Han, et al. (2020)	
algae	Chlorella pyrenoidosa	growth rate	72 h	EC50	3000	n.r.	S	98	R4/C1	Gao, Wang, Jiang, Han, et al. (2020)	
algae	Chlorella pyrenoidosa	growth rate	96 h	EC50	2800	n.r.	S	98	R4/C1	Gao, Wang, Jiang, Han, et al. (2020)	
algae	Navicula pelliculosa	growth rate	120 h	EC50	> 120	m-am	S	96.1	2/C2	Hoberg J.R. 1993 cited in EC (2004) Vol.3 B9 p.586	
algae	Pseudokirchnerella subcapitata (Raphidocelis subcapitata/Selenastrum capricornutum)	population abundace	120 h	EC50	140	n.r.	S	96.1	R4/C4	U. EPA (1992)	
algae	Scenedesmus obliquus	cell number	72 h	EC50	540	nom	S	96.5	R3/C1	Qu et al. (2014)	
algae	Scenedesmus obliquus	cell number	72 h	EC50	1500	nom	S	96.5	R3/C1	Qu et al. (2014)	
algae	Scenedesmus obliquus	cell number	72 h	EC50	290	nom	S	96.5	R3/C1	Qu et al. (2014)	
algae	**Scenedesmus subspicatus**	**biomass**	**96 h**	**EbC50**	**68**	**nom-m**	**S**	**> 95**	**2/C1**	Handley J.W., Mead C., Bartlett A.J. 1991 cited in EC (2004) Vol.3 B9 p.584	the 96 h EbC50 is selected since an ErC50 is only available for the endpoint growth rate for the time interval 24 - 48 h.

[15] According to ((Moermond et al., 2016), validity is divided into reliability (R) and relevance (C), with the classes to be assigned (1-4) corresponding to the Klimisch classes ((Klimisch et al., 1997)). An evaluation of reliability was not performed if a study was rated as not relevant (C3). The studies evaluated according to Klimisch are not marked with a letter.

Group	Species	Endpoint	Duration	Parameter	value (µg/L)	Analytics	Exposure	Purity (%)	Validity	Reference	Notes
algae	Scenedesmus subspicatus	growth rate	96 h	ErC50	74	nom-m	S	> 95	3/C1	Handley et al., (1991) cited in EC (2004) Vol.3 B9 p.584	RMS: With the performed calculation of growth rate and at the sight of the absorbance values results, the ErC50 is most likely underestimated
algae	Selenastrum capricornutum	growth rate	120 h	EC50	> 140	mm	S	96.1	2/C2	Hoberg J.R. 1993 cited in EC (2004) Vol.3 B9 p.585	
higher plant	Lemna minor	growth rate	7 d	EC50	9360	n.r.	S	96.5	R3/C1	Qu, Ma, Liu, Gao, et al. (2016)	Racemate,
higher plant	Lemna minor	growth rate	7 d	EC50	10140	n.r.	S	99.4	R3/C1	Qu, Ma, Liu, Gao, et al. (2016)	(+/S) fipronil
higher plant	Lemna minor	growth rate	7 d	EC50	8510	n.r.	S	99.5	R3/C1	Qu, Ma, Liu, Gao, et al. (2016)	(-/R) fipronil
higher plant	**Lemna gibba**	**growth rate**	**14 d**	**EC50**	**> 160**	**m-i**	**S**	**96.1**	**2/C2**	Hoberg, J.R. 1993 cited in EC (2004) Vol.3 B9 p.596	
higher plant	Lemna gibba	biomass	14 d	EC50	> 81	m-gm	S	96.1	2/C2	Han Hoberg, J. R. (A.7.4.3.5.2/01) cited in (BASF, 2011) Document IIIA 7.4 page 240 Section 7.4.3.5, Annex Point IIIA, XIII.3.4, Aquatic plant toxicity	
higher plant	Lemna gibba	growth rate	120 h	EC50	> 100	n.r.	S	96.1	R4/C4	U. EPA (1992)	
insect	Aedes aegypti	mortality (larvae)	24 h	LC50	10.49	n.r.	S	97.5	R4/C1	Tsikolia et al. (2013)	
insect	Aedes aegypti	mortality	48 h	LC50	1.54	n.r.	S	97.1	R3/C1	Ali et al., (1998) cited in Bower JC (2017)	
insect	Aedes aegypti	mortality	48 h	LC50	6.601	n	S	99.9	R4/C1	Chaton, Ravanel, Meyran, and Tissut (2001) cited in Bower JC (2017)	
insect	Aedes aegypti	mortality	24 h	LC50	10.841	n	S	99.9	R4/C1	Chaton et al. (2001) cited in Bower JC (2017)	
insect	Aedes aegypti	mortality	48 h	LC50	2.5355	n	S	99.9	R4/C1	Chaton et al. (2001) cited in Bower JC (2017)	
insect	Aedes aegypti	mortality	24 h	LC50	3.1912	n	S	99.9	R4/C1	Chaton et al. (2001) cited in Bower JC (2017)	
insect	Aedes albopictus	mortality	48 h	LC50	8.1	n.r.	S	97.1	R3/C1	Ali et al., (1998) cited in Bower JC (2017)	
insect	Aedes albopictus	mortality	48 h	LC50	23	n.r.	S	97.1	R3/C1	Ali et al., (1998) cited in Bower JC (2017)	
insect	Aedes taeniorhynchus	mortality	48 h	LC50	0.43	n.r.	S	97.1	R3/C1	Ali et al., (1998) cited in Bower JC (2017)	
insect	Aedes taeniorhynchus	mortality	24 h	LC50	1.4	n.r.	S	97.1	R3/C1	Ali et al., (1998) cited in Bower JC (2017)	

Group	Species	Endpoint	Duration	Parameter	value (µg/L)	Analytics	Exposure	Purity (%)	Validity	Reference	Notes
insect	Anopheles quadrimaculatus	mortality	48 h	LC50	0.43	n.r.	S	97.1	R3/C1	Ali et al., (1998) cited in Bower JC (2017)	
insect	Baetis tricaudatus	mortality	48 h	LC50	0.105	m	S	99.5	2/C1	Weston and Lydy (2014) cited in Bower JC (2017)	
insect	**Baetis tricaudatus**	**immobilisation**	**48 h**	**EC50**	**0.0519**	**m**	**S**	**99.5**	**2/C1**	Weston and Lydy (2014) cited in Bower JC (2017)	endpoint immobilization selected as the most sensitive
insect	Chaoborus crystallinus	mortality	48 h	LC50	646.33	n.r.	S	99.9	3/C1	Chaton, Ravanel, Tissut, and Meyran (2002) cited in Bower JC (2017)	
insect	Cheumatopsyche brevilineata	immobilisation	48 h	EC50	0.133	m	S	> 98	3/C2	Iwafune, Yokoyama, Nagai, and Horio (2011) cited in Bower JC (2017)	
insect	Cheumatopsyche brevilineata	mortality	48 h	LC50	0.153	nom	S	98	R4/C1	Yokoyama et al. (2009) cited in Bower JC (2017)	
insect	Chironomus annularius	mortality	48 h	LC50	2.448	n.r.	S	99.9	3/C1	Chaton et al. (2002) cited in Bower JC (2017)	
insect	Chironomus crassicaudatus	mortality	48 h	LC50	0.42	n.r.	S	97.1	R3/C1	Ali et al., (1998) cited in Bower JC (2017)	
insect	Chironomus dilutus	mortality	96 h	LC50	> 0.0815	m	S	99.5	2/C2	Weston and Lydy (2014) cited in Bower JC (2017)	
insect	Chironomus dilutus	immobilisation	96 h	EC50	0.035	m	S	99.5	2/C1	Weston and Lydy (2014) cited in Bower JC (2017)	endpoint immobilization selected as the most sensitive
insect	Chironomus dilutus	immobilisation	96 h	EC50	0.03	m	S	99.5	2/C1	Weston and Lydy (2014) cited in Bower JC (2017)	endpoint immobilization selected as the most sensitive
mean (immobilization)					**0.032**	**µg/L**					
insect	Chironomus riparius	mortality (larvae)	48 h	LC50	1.74	n-m	S	>= 97	R2/C1	Monteiro et al. (2019).	OECD guideline 235

Group	Species	Endpoint	Duration	Parameter	value (µg/L)	Analytics	Exposure	Purity (%)	Validity	Reference	Notes
insect	Chironomus tentans	mortality	10 d	LC50	0.43	mm	R	98.3	2/C2	Putt A.E (A.7.4.3.5.1/01) 2003 cited in (BASF, 2011) Document IIIA 7.4 page 220 Section 7.4.3.5, Annex Point IIIA, XIII.3.4, Effects on sediment dwelling organisms	Supportive short-term data. Sediment/water system. Sediment from: Glen Charlie Pond, Massachusetts, 2.8% organic carbon 94% sand 6% silt % clay, pH 5.7, exposure stage is L3 larvae.
insect	Chironomus tentans	growth	10 d	LC50	0.73	mm	R	98.3	2/C2	Putt A.E (A.7.4.3.5.1/01) 2003 cited in (BASF, 2011) Document IIIA 7.4 page 220 Section 7.4.3.5, Annex Point IIIA, XIII.3.4, Effects on sediment dwelling organisms	Supportive short-term data. Sediment/water system. Sediment from: Glen Charlie Pond, Massachusetts, 2.8% organic carbon 94% sand 6% silt % clay, pH 5.7, exposure stage is L3 larvae.
insect	Cricotopus lebetis	mortality	96 h	LC50	1.06	n.r.	S	99	R4/C3	Stratman, Wilson, Overholt, Cuda, and Netherland (2013) cited in Bower JC (2017)	
insect	Cricotopus lebetis	mortality	24 h	LC50	7.26	n.r.	S	99	R4/C3	Stratman et al. (2013) cited in Bower JC (2017)	
insect	Cricotopus lebetis	mortality	48 h	LC50	2.61	n.r.	S	99	R4/C3	Stratman et al. (2013) cited in Bower JC (2017)	
insect	Cricotopus lebetis	mortality	72 h	LC50	1.78	n.r.	S	99	R4/C3	Stratman et al. (2013) cited in Bower JC (2017)	
insect	Culex nigripalpus	mortality	48 h	LC50	0.87	n.r.	S	97.1	R3/C1	Ali et al., (1998) cited in Bower JC (2017)	
insect	Culex nigripalpus	mortality	24 h	LC50	1.4	n.r.	S	97.1	R3/C1	Ali et al., (1998) cited in Bower JC (2017)	

Group	Species	Endpoint	Duration	Parameter	value (µg/L)	Analytics	Exposure	Purity (%)	Validity	Reference	Notes
insect	Culex quinquefasciatus	mortality	48 h	LC50	4.6	n.r.	S	97.1	R3/C1	Ali et al., (1998) cited in Bower JC (2017)	
insect	Culex quinquefasciatus	mortality	48 h	LC50	7.3	n.r.	S	97.1	R3/C1	Ali et al., (1998) cited in Bower JC (2017)	
insect	Diphetor hageni	mortality	48 h	LC50	0.347	m	S	99.5	2/C1	Weston and Lydy (2014) cited in Bower JC (2017)	
insect	**Diphetor hageni**	**immobilisation**	**48 h**	**EC50**	**0.163**	**m**	**S**	**99.5**	**2/C1**	Weston and Lydy (2014) cited in Bower JC (2017)	endpoint immobilization selected as the most sensitive
insect	Ephemeralla excrucians	mortality	48 h	LC50	> 0.436	m	S	99.5	2/C2	Weston and Lydy (2014) cited in Bower JC (2017)	supportive
insect	Ephemeralla excrucians	immobilisation	48 h	EC50	> 0.436	m	S	99.5	2/C2	Weston and Lydy (2014) cited in Bower JC (2017)	supportive
insect	Fallceon quilleri	mortality	48 h	LC50	> 0.187	m	S	99.5	2/C2	Weston and Lydy (2014) cited in Bower JC (2017)	supportive
insect	**Fallceon quilleri**	**immobilisation**	**48 h**	**EC50**	**0.0707**	**m**	**S**	**99.5**	**2/C1**	Weston and Lydy (2014) cited in Bower JC (2017)	endpoint immobilization selected as the most sensitive
insect	Glyptotendipes paripes	mortality	48 h	LC50	0.42	n.r.	S	97.1	R3/C1	Ali et al., (1998) cited in Bower JC (2017)	
insect	Glyptotendipes paripes	mortality	24 h	LC50	0.91	n.r.	S	97.1	R3/C1	Ali et al., (1998) cited in Bower JC (2017)	
insect	Helicopsyche sp.	mortality	96 h	LC50	> 0.842	m	S	99.5	2/C2	Weston and Lydy (2014) cited in Bower JC (2017)	supportive
insect	**Helicopsyche sp.**	**immobilisation**	**96 h**	**EC50**	**0.267**	**m**	**S**	**99.5**	**2/C1**	Weston and Lydy (2014) cited in Bower JC (2017)	endpoint immobilization selected
insect	Hexagenia sp.	mortality	96 h	LC50	0.44	mm	R	99.7	2/C1	Putt A.E. 2003 (A.7.4.1.2/02) cited in (BASF, 2011) Document IIIA 7.4 page 146 Section 7.4.1.2, Annex Point IIA, VII.7.2, Aquatic toxicity to invertebrates	
insect	Hexagenia sp.	mortality	96 h	LC50	1.231	m	S	99.5	2/C1	Weston and Lydy (2014) cited in Bower JC (2017)	
insect	**Hexagenia sp.**	**immobilisation**	**96 h**	**EC50**	**0.48**	**m**	**S**	**99.5**	**2/C1**	Weston and Lydy (2014) cited in Bower JC (2017)	endpoint immobilization selected as the most sensitive

Group	Species	Endpoint	Duration	Parameter	value (µg/L)	Analytics	Exposure	Purity (%)	Validity	Reference	Notes
insect	Hydropsyche sp.	mortality	96 h	LC50	2.107	m	S	99.5	2/C1	Weston and Lydy (2014) cited in Bower JC (2017)	
insect	**Hydropsyche sp.**	**immobilisation**	**96 h**	**EC50**	**0.602**	**m**	**S**	**99.5**	**2/C1**	Weston and Lydy (2014) cited in Bower JC (2017)	endpoint immobilization selected as the most sensitive
insect	Ischnura senegalensis	immobilisation	24 h	EC50	1.835	n	S	98.1	R4/C3	Sugita, Agemori, and Goka (2018)	
insect	Isoperla quinquepunctata	mortality	96 h	LC50	0.113	m	S	99.5	2/C1	Weston and Lydy (2014) cited in Bower JC (2017)	
insect	**Isoperla quinquepunctata**	**immobilisation**	**96 h**	**EC50**	**0.101**	**m**	**S**	**99.5**	**2/C1**	Weston and Lydy (2014) cited in Bower JC (2017)	endpoint immobilization selected as the most sensitive
insect	Nectopsyche sp.	mortality	96 h	LC50	> 2.947	m	S	99.5	2/C2	Weston and Lydy (2014) cited in Bower JC (2017)	Supportive
insect	**Nectopsyche sp.**	**immobilisation**	**96 h**	**EC50**	**0.634**	**m**	**S**	**99.5**	**2/C1**	Weston and Lydy (2014) cited in Bower JC (2017)	endpoint immobilization selected as the most sensitive
insect	Polypedilum nubiferum	mortality	48 h	LC50	1	n.r.	S	95	R4/C1	Stevens, Burdett, Mudford, Helliwell, and Doran (2011a) cited in Bower JC (2017)	
insect	Polypedilum nubiferum	mortality	48 h	LC50	2.18	n.r.	S	95	R4/C1	Stevens et al. (2011a) cited in Bower JC (2017)	
insect	Serratella micheneri	mortality	48 h	LC50	> 0.722	m	S	99.5	2/C2	Weston and Lydy (2014) cited in Bower JC (2017)	supportive
insect	**Serratella micheneri**	**immobilisation**	**48 h**	**EC50**	**0.589**	**m**	**S**	**99.5**	**2/C1**	Weston and Lydy (2014) cited in Bower JC (2017)	endpoint immobilization selected as the most sensitive
insect	Simulium vittatum	mortality	48 h	LC50	0.223	m	S	> 98	3/C1	Overmyer, Mason, and Armbrust (2005)	
insect	Simulium vittatum	mortality	48 h	LC50	0.65	m	S	98	3/C1	Overmyer et al. (2007) cited in Bower JC (2017)	
insect	Simulium vittatum	mortality	48 h	LC50	0.72	m	S	98	3/C1	Overmyer et al. (2007) cited in Bower JC (2017)	
insect	Simulium vittatum	mortality	48 h	LC50	0.74	m	S	98	3/C1	Overmyer et al. (2007) cited in Bower JC (2017)	
insect	Sympetrum frequens	mortality (larvae)	48 h	LC50	2775	n-m	R	> 99	R2/C1	Jinguji et al. (2018b)	
insect	**Sympetrum frequens**	**feeding behavior**	**48 h**	**EC50**	**2.9**	**n-m**	**R**	**> 99**	**R2/C1**	Jinguji et al. (2018b)	endpoint feeding behaviour selected as the most sensitive

Group	Species	Endpoint	Duration	Parameter	value (µg/L)	Analytics	Exposure	Purity (%)	Validity	Reference	Notes
insect	Sympetrum infuscatum	mortality (larvae)	48 h	LC50	1020	n-m	R	> 99	R2/C1	Jinguji et al. (2018b)	
insect	**Sympetrum infuscatum**	**feeding behavior**	**48 h**	**EC50**	**29.3**	**n-m**	**R**	**> 99**	**R2/C1**	Jinguji et al. (2018b)	endpoint feeding behaviour selected as the most sensitive
insect	Taenionema sp.	mortality	96 h	LC50	> 0.184	m	S	99.5	2/C2	Weston and Lydy (2014) cited in Bower JC (2017)	
insect	Taenionema sp.	immobilisation	96 h	EC50	> 0.184	m	S	99.5	2/C2	Weston and Lydy (2014) cited in Bower JC (2017)	
insect	Tricorythodes sp.	mortality	48 h	LC50	> 1.229	m	S	99.5	2/C2	Weston and Lydy (2014) cited in Bower JC (2017)	
insect	Tricorythodes sp.	immobilisation	48 h	EC50	> 1.229	m	S	99.5	2/C2	Weston and Lydy (2014) cited in Bower JC (2017)	
bivalve	Anodonta woodiana	mortality	72 h	LC50	1210	n.r.	S	96.5	R3/C1	Qu, Ma, Liu, Gao, et al. (2016) and (Qu, Ma, Liu, Jing, et al., 2016)	Data published twice, racemate
bivalve	Anodonta woodiana	mortality	72 h	LC50	630	n.r.	S	99.4	R3/C1	Qu, Ma, Liu, Gao, et al. (2016) and (Qu, Ma, Liu, Jing, et al., 2016)	Data published twice, (+/S) fipronil
bivalve	Anodonta woodiana	mortality	72 h	LC50	3270	n.r.	S	99.5	R3/C1	Qu, Ma, Liu, Gao, et al. (2016) and (Qu, Ma, Liu, Jing, et al., 2016)	Data published twice, (-/R) fipronil
bivalve	Corbicula fluminea	mortality	96 h	LC50	> 2000	m-gm	R	99.7	2/C2	Putt (2003a) cited in U. EPA (2006)	supportive
bivalve	Elliptio complanata	mortality	24 h	EC50	> 2000	m	R	99.7	2/C2	Bringolf et al. (2007) cited in Bower JC (2017)	supportive, glochidia
bivalve	Lampsilis fasciola	mortality	24 h	EC50	> 2000	m	R	99.7	2/C2	Bringolf et al. (2007) cited in Bower JC (2017)	supportive, glochidia
bivalve	Lampsilis fasciola	mortality	48 h	EC50	> 2000	m	R	99.7	2/C2	Bringolf et al. (2007) cited in Bower JC (2017)	supportive, glochidia

Group	Species	Endpoint	Duration	Parameter	value (µg/L)	Analytics	Exposure	Purity (%)	Validity	Reference	Notes
bivalve	Lampsilis fasciola	mortality	96 h	EC50	> 2000	m	R	99.7	2/C2	Bringolf et al. (2007) cited in Bower JC (2017)	supportive, juveniles
bivalve	Lampsilis siliquoidea	mortality	96 h	EC50	> 2000	m	R	99.7	2/C2	Bringolf et al. (2007) cited in Bower JC (2017)	supportive, juveniles
bivalve	Lampsilis siliquoidea	mortality	24 h	EC50	> 2000	m	R	99.7	2/C2	Bringolf et al. (2007) cited in Bower JC (2017)	supportive, glochidia
bivalve	Lampsilis siliquoidea	mortality	96 h	EC50	> 2000	m	R	99.7	2/C2	Bringolf et al. (2007) cited in Bower JC (2017)	supportive, glochidia
bivalve	Villosa constricta	mortality	24 h	EC50	> 2000	m	R	99.7	2/C2	Bringolf et al. (2007) cited in Bower JC (2017)	supportive, glochidia
bivalve	Villosa constricta	mortality	48 h	EC50	> 2000	m	R	99.7	2/C2	Bringolf et al. (2007) cited in Bower JC (2017)	supportive, glochidia
clitellata	Lumbriculus variegatus	mortality	96 h	LC50	> 1900	m-gm	R	99.7	2/C2	Putt (2003b) cited in U. EPA (2006)	supportive
crustacean	Acanthocyclops robustus	mortality	48 h	LC50	84.895	n.r.	S	99.9	3/C1	Chaton et al. (2002) cited in Bower JC (2017)	
crustacean	Ceriodaphnia dubia	immobilisation	48 h	EC50	0.99	n	R	95	R4/C1	Hayasaka, Korenaga, Suzuki, Sanchez-Bayo, and Goka (2012)	
crustacean	Ceriodaphnia dubia	immobilisation	48 h	EC50	17.9	m	S	98	3/C1	Konwick et al. (2005) cited in Bower JC (2017)	Racemate, exposure under light
crustacean	Ceriodaphnia dubia	immobilisation	48 h	EC50	17.5	m	S	98	3/C1	Konwick et al. (2005) cited in Bower JC (2017)	Racemate, exposure in the dark
crustacean	Ceriodaphnia dubia	immobilisation	48 h	EC50	17.7	m	S	98	3/C1	Konwick et al. (2005) cited in Bower JC (2017)	Racemate, light and dark exposure cobined
crustacean	Ceriodaphnia dubia	immobilisation	48 h	EC50	11.3	m	S	98	3/C1	Konwick et al. (2005) cited in Bower JC (2017)	(+/S) fipronil, exposure under light

Group	Species	Endpoint	Duration	Parameter	value (µg/L)	Analytics	Exposure	Purity (%)	Validity	Reference	Notes
crustacean	Ceriodaphnia dubia	immobilisation	48 h	EC50	9.4	m	S	98	3/C1	Konwick et al. (2005) cited in Bower JC (2017)	(+/S) fipronil, exposure in the dark, only highest/lowest concentrations measured. Other concentrations calculated by adjustment using standard deviations of those measured.
crustacean	Ceriodaphnia dubia	immobilisation	48 h	EC50	10.3	m	S	98	3/C1	Konwick et al. (2005) cited in Bower JC (2017)	(+/S) fipronil, light and dark exposure combined
crustacean	Ceriodaphnia dubia	immobilisation	48 h	EC50	35.4	m	S	98	3/C1	Konwick et al. (2005) cited in Bower JC (2017)	(-/R) fipronil, exposure under light
crustacean	Ceriodaphnia dubia	immobilisation	48 h	EC50	28.4	m	S	98	3/C1	Konwick et al. (2005) cited in Bower JC (2017)	(-/R) fipronil, exposure in the dark
crustacean	Ceriodaphnia dubia	immobilisation	48 h	EC50	31.9	m	S	98	3/C1	Konwick et al. (2005) cited in Bower JC (2017)	(-/R) fipronil, light and dark exposure combined
crustacean	Ceriodaphnia dubia	mortality (larvae)	24 h	LC50	33.3	nom-m	S	98	2/C1	Wilson (2008). cited in Bower JC (2017),	Racemate
crustacean	Ceriodaphnia dubia	mortality (larvae)	24 h	LC50	18.1	nom-m	S	97.3	2/C1	Wilson (2008). cited in Bower JC (2017),	(+/S) enantiomer
crustacean	Ceriodaphnia dubia	mortality (larvae)	24 h	LC50	65.2	nom-m	S	98.1	2/C1	Wilson (2008). cited in Bower JC (2017),	(-/R) enantiomer

Group	Species	Endpoint	Duration	Parameter	value (µg/L)	Analytics	Exposure	Purity (%)	Validity	Reference	Notes
crustacean	Ceriodaphnia dubia	mortality (larvae)	48 h	LC50	30.3	nom-m	S	98	2/C1	Wilson (2008). cited in Bower JC (2017),	Racemate
crustacean	Ceriodaphnia dubia	mortality (larvae)	48 h	LC50	10.3	nom-m	S	97.3	2/C1	Wilson (2008). cited in Bower JC (2017),	(+/S) enantiomer
crustacean	Ceriodaphnia dubia	mortality (larvae)	48 h	LC50	50.1	nom-m	S	98.1	2/C1	Wilson (2008). cited in Bower JC (2017),	(-/R) enantiomer
mean (racemate and the most toxic enantiomer (+/S))					**17.7**	**µg/L**					
crustacean	Ceriodaphnia reticulata	immobilisation	48 h	EC50	8.83	n	R	95	R4/C1	Hayasaka, Korenaga, Suzuki, Sanchez-Bayo, et al. (2012)	
crustacean	Daphnia magna	immobilisation	48 h	EC50	88.3	n	R	95	R4/C1	Hayasaka, Korenaga, Suzuki, Sanchez-Bayo, et al. (2012)	
crustacean	Daphnia magna	immobilisation	48 h	EC50	110	n.r.	n.r.	98	R4/C1	Gao, Wang, Jiang, Miao, et al. (2020)	
crustacean	**Daphnia magna**	**behaviour**	**48 h**	**EC50**	**190**	**m-am**	**T**	**100**	**2/C1**	MCNamara P.C. 1990 cited in EC (2004) Vol.3 B9 p.568	
crustacean	Daphnia magna	n.r.	96 h	EC50	12.9	n.r.	T	n.r.	2/C4	Ward G.S. and Rabe B.A. 1989 cited in EC (2004) Vol.3 B9 p.569	
crustacean	Daphnia magna	n.r.	72 h	EC50	12.9	n.r.	T	n.r.	2/C4	Ward G.S. and Rabe B.A. 1989 cited in EC (2004) Vol.3 B9 p.569	
crustacean	Daphnia magna	n.r.	48 h	EC50	12.9	n.r.	T	n.r.	2/C4	Ward G.S. and Rabe B.A. 1989 cited in EC (2004) Vol.3 B9 p.569	
crustacean	Daphnia magna	immobilisation	48 h	EC50	42.9	m	S	> 98	3/C2	Iwafune et al. (2011)	
crustacean	Daphnia magna	immobilisation	48 h	EC50	34.8	n	S	99	R4/C1	Chevalier et al. (2015)	
crustacean	Daphnia pulex	immobilisation	48 h	EC50	40.392	n	R	95	R4/C1	Hayasaka, Korenaga, Suzuki, Sanchez-Bayo, et al. (2012)	
crustacean	Diaptomus castor	mortality	48 h	LC50	3.4535	n.r.	S	99.9	3/C1	Chaton et al. (2002)	

Group	Species	Endpoint	Duration	Parameter	value (µg/L)	Analytics	Exposure	Purity (%)	Validity	Reference	Notes
crustacean	Hyalella azteca	mortality	96 h	LC50	1.593	m	S	99.5	2/C1	Weston and Lydy (2014) cited in Bower JC (2017)	
crustacean	Hyalella azteca	mortality	96 h	LC50	1.725	m	S	99.5	2/C1	Weston and Lydy (2014) cited in Bower JC (2017)	
crustacean	Hyalella azteca	immobilisation	96 h	EC50	0.729	m	S	99.5	2/C1	Weston and Lydy (2014) cited in Bower JC (2017)	endpoint immobilization selected as the most sensitive
crustacean	Hyalella azteca	immobilisation	96 h	EC50	0.727	m	S	99.5	2/C1	Weston and Lydy (2014) cited in Bower JC (2017)	endpoint immobilization selected as the most sensitive
mean (immobilization)					**0.728**	**µg/L**					
crustacean	Moina macrocopa	immobilisation	48 h	EC50	29.57	n	R	95	R4/C1	Hayasaka, Korenaga, Suzuki, Sanchez-Bayo, et al. (2012)	
crustacean	Procambarus clarkii	mortality	96 h	LC50	14.3	m	S	98	3/C2	Schlenk et al. (2001)	
crustacean	Procambarus clarkii	mortality	96 h	LC50	124.89	nom-m	S	98	2/C1	Overmyer et al. (2007) cited in Bower JC (2017)	Racemate
crustacean	Procambarus clarkii	mortality	96 h	LC50	81.7	nom-m	S	98	2/C1	Overmyer et al. (2007) cited in Bower JC (2017)	(+/S) enantiomer
crustacean	Procambarus clarkii	mortality	96 h	LC50	163.5	nom-m	S	98	2/C1	Overmyer et al. (2007) cited in Bower JC (2017)	(-/R) enantiomer
mean (racemate and the most toxic enantiomer (+/S))					**101.01**	**µg/L**					
crustacean	Procambarus zonangulus	mortality	96 h	LC50	19.5	m	S	98	3/C2	Schlenk et al. (2001)	
crustacean	Simocephalus elizabethae	mortality	48 h	LC50	11.13	n.r.	S	95	R4/C1	Stevens, Burdett, Mudford, Helliwell, and Doran (2011b)	
crustacean	Simocephalus elizabethae	mortality	48 h	LC50	14.11	n.r.	S	95	R4/C1	Stevens et al. (2011b)	
fish	Carassius gibelio	mortality	96 h	LC50	70	n.r.	R	98	R4/C1	Gao, Wang, Jiang, Miao, et al. (2020)	

Group	Species	Endpoint	Duration	Parameter	value (µg/L)	Analytics	Exposure	Purity (%)	Validity	Reference	Notes
fish	Cyprinus carpio	mortality	96 h	LC50	430	mm	T	> 95	2/C1	Handley et al., (1991) cited in EC (2004) Vol.3 B9 p.551	
fish	Cyprinus carpio	mortality	96 h	LC50	428	m	S	99.1	R2/C1	S. K. Gupta et al. (2014)	
mean					**429**	**µg/L**					
fish	Danio rerio	mortality (larvae)	96 h	LC50	459	n	R	98	R4/C1	H. Xu et al. (2018)	
fish	Danio rerio	larval development	96 h	EC50	344	n	R	98	R4/C1	H. Xu et al. (2018)	
fish	Danio rerio	mortality (larvae)	114 h	LC50	597	n	R	> 97.6	R4/C1	Yan et al. (2016)	
fish	Danio rerio	larval development	114 h	EC50	573	n	R	> 97.6	R4/C1	Yan et al. (2016)	
fish	Danio rerio	mortality (juvenile)	24 h	LC50	220.4	n	S	97.6	R4/C1	Wu et al. (2014)	
fish	Epomis macrochirus	mortality	96 h	LC50	83	n.r.	T	100	R4/C4	U. EPA (1992)	
fish	**Ictalurus punctatus**	**mortality**	**96 h**	**LC50**	**560**	**m-am**	**T**	**97.08**	**2/C1**	Dionne (1997) cited in EC (2004) Vol.3 B9 p.552	
fish	**Lepomis macrochirus**	**mortality**	**96 h**	**LC50**	**85.2**	**m-am**	**T**	**95.4**	**2/C1**	Ward (1991) cited in EC (2004) Vol.3 B9 p.551	
fish	Misgurnus anguillicaudatus	mortality	96 h	LC50	90	n.r.	R	98	R4/C1	Gao, Wang, Jiang, Miao, et al. (2020)	
fish	**Oncorhynchus mykiss (Salmo gairdneri)**	**mortality**	**96 h**	**LC50**	**248**	**m-am**	**T**	**95.4**	**2/C1**	Ward (1991) cited in EC (2004) Vol.3 B9 p.551	
fish	Oncorhynchus mykiss (Salmo gairdneri)	mortality	96 h	LC50	246	n.r.	T	100	R4/C4	U. EPA (1992)	
fish	Oryzias latipes	mortality	96 h	LC50	94.2	n-m	S	98.9	R2/C1	Nillos et al. (2009)	Racemate
fish	Oryzias latipes	mortality	96 h	LC50	95.4	n-m	S	> 97	R2/C1	Nillos et al. (2009)	(+/S) enantiomer
fish	Oryzias latipes	mortality	96 h	LC50	98.3	n-m	S	> 97	R2/C1	Nillos et al. (2009)	(-/R) enantiomer
mean (racemate and the most toxic enantiomer (+/S))					**94.8**	**µg/L**					
fish	Pimephales promelas	mortality (larvae)	24 h	LC50	398.29	n.r.	S	98.5	R4/C1	Beggel, Werner, Connon, and Geist (2010) cited in Bower JC (2017).	

Group	Species	Endpoint	Duration	Parameter	value (µg/L)	Analytics	Exposure	Purity (%)	Validity	Reference	Notes
fish	Pimephales promelas	mortality (larvae)	7 d	LC50	227	nom-m	R	97.8	2/C2	Baird et al. (2013) cited in Bower JC (2017)	(+/S) enantiomer
fish	Pimephales promelas	mortality (larvae)	7 d	LC50	365	nom-m	R	97.8	2/C2	Baird et al. (2013) cited in Bower JC (2017)	(-/R) enantiomer
mean (racemate and the most toxic enantiomer (+/S))					**217**	**µg/L**					
fish	Rutilus frisii	mortality (adult/parent)	96 h	LC50	572	n	S	98	R4/C1	Ardeshir, Zolgharnein, Movahedinia, Salamat, and Zabihi (2017)	
amphibian	Xenopus laevis	mortality	96 h	LC50	850	nom-m	S	98	2/C1	Overmyer et al. (2007) cited in Bower JC (2017)	Racemate
amphibian	Xenopus laevis	mortality	96 h	LC50	910	nom-m	S	98	2/C1	Overmyer et al. (2007) cited in Bower JC (2017)	(+/S) enantiomer
amphibian	Xenopus laevis	mortality	96 h	LC50	1140	nom-m	S	98	2/C1	Overmyer et al. (2007) cited in Bower JC (2017)	(-/R) enantiomer
mean (racemate and the most toxic enantiomer (+/S))					**879**	**µg/L**					
amphibian	Pelophylax nigromaculatus	mortality	96 h	LC50	180	n.r.	R	98	R4/C4	Gao, Wang, Jiang, Miao, et al. (2020)	

Acute saltwater effect data											
Group	Species	Endpoint	Duration	Parameter	value (µg/L)	Analytics	Exposure	Purity (%)	Validity	Reference	Notes
algae	Skeletonema costatum	growth rate	120 h	EC50	> 140	mm	S	96.1	2/C2	Hoberg (1993) cited in EC (2004) Vol.3 B9 p.586	supportive, single concentration tested
algae	**Dunaliella tertiolecta**	**cell number**	**96 h**	**EC50**	**631.2**	**nom-m**	**S**	**98**	**2/C1**	Overmyer et al. (2007) cited in Bower JC (2017)	
rotifers	Brachionus plicatilis	mortality	24 h	LC50	5735	n	S	n.r.	R3/C1	Lee, Park, Hwang, Lee, and Han (2018)	
coral	Acropora tenuis	larval development	48 h	EC50	29.1	m-twa	S	> 98	R2/C3	Flores, Kaserzon, Elisei, Ricardo, and Negri (2020)	
bivalve	**Crassostrea virginica**	**growth rate**	**96 h**	**EC50**	**770**	**mm**	**T**	**96.1**	**2/C1**	Dionne (1993) cited in EC (2004) Vol.3 B9 p.569	
bivalve	Mercenaria mercenaria	mortality	96 h	EC50	177	nom-m	S	98	2/C1	Overmyer et al. (2007) cited in Bower JC (2017)	Racemate
bivalve	Mercenaria mercenaria	mortality	96 h	EC50	208	nom-m	S	98	2/C1	Overmyer et al. (2007) cited in Bower JC (2017)	(+/S) enantiomer
bivalve	Mercenaria mercenaria	mortality	96 h	EC50	187	nom-m	S	98	2/C1	Overmyer et al. (2007) cited in Bower JC (2017)	(-/R) enantiomer
mean (mortality, racemate and the most toxic enantiomer (+/S))					**182**	**µg/L**					
crustacean	Americamysis bahia (Mysidopsis bahia)	mortality (cummulative)	96 h	LC50	0.14	mm	S	96.1	2/C1	Machado (1993) cited in EC (2004) Vol.3 B9 p.553	
crustacean	**Americamysis bahia (Mysidopsis bahia)**	**immobilisation**	**96 h**	**EC50**	**0.067**	**m-gm**	**R**	**>= 98**	**R2/C1**	Hano et al. (2019).	endpoint immobilization selected as the most sensitive
crustacean	Americamysis bahia (Mysidopsis bahia)	mortality	96 h	LC50	0.086	m-gm	R	>= 98	R2/C1	Hano et al. (2019).	
crustacean	Amphiascus tenuiremis	mortality	96 h	LC50	6.8	m	S	98	2/C1	Chandler et al. (2004) cited in Bower JC (2017)	adult organisms (male and female combined)
crustacean	**Amphiascus tenuiremis**	**mortality**	**96 h**	**LC50**	**3.5**	**m**	**S**	**98**	**2/C1**	Chandler et al. (2004	male adults tested, endpoint selected as males are more sensitive than females
crustacean	Amphiascus tenuiremis	mortality	96 h	LC50	13	m	S	98	2/C1	Chandler et al. (2004	female adults tested
crustacean	Crangon uritai	immobilisation	96 h	EC50	1.5	m-gm	R	>= 98	R3/C2	Hano et al. (2019) cited in Bower JC (2017)	
crustacean	Crangon uritai	mortality	96 h	LC50	2	m-gm	R	>= 98	R3/C2	Hano et al. (2019) cited in Bower JC (2017)	
crustacean	Farfantepenaeus aztecus	mortality	96 h	LC50	0.12	n	R	>= 97	R3/C2	Al-Badran, Fujiwara, and Mora (2019)	

Group	Species	Endpoint	Duration	Parameter	value (µg/L)	Analytics	Exposure	Purity (%)	Validity	Reference	Notes
crustacean	Farfantepenaeus aztecus	mortality	96 h	LC50	1.3	n	R	>= 97	R4/C1	(Al-Badran, Fujiwara, Gatlin, & Mora, 2018)	
crustacean	**Palaemonetes pugio**	**mortality (adult/parent)**	**96 h**	**LC50**	**0.32**	**nom-m**	**S**	**98**	**2/C1**	Overmyer et al. (2007) cited in Bower JC (2017)	Racemate
crustacean	Palaemonetes pugio	mortality (larvae)	96 h	LC50	0.68	nom-m	S	98	2/C1	Overmyer et al. (2007) cited in Bower JC (2017)	Racemate
crustacean	Palaemonetes pugio	mortality (adult/parent)	96 h	LC50	0.37	nom-m	S	98	3/C1	Overmyer et al. (2007) cited in Bower JC (2017)	(+/S) fipronil
crustacean	Palaemonetes pugio	mortality (larvae)	96 h	LC50	0.54	nom-m	S	98	3/C1	Overmyer et al. (2007) cited in Bower JC (2017)	(+/S) fipronil
crustacean	Palaemonetes pugio	mortality (adult/parent)	96 h	LC50	0.32	nom-m	S	98	3/C1	Overmyer et al. (2007) cited in Bower JC (2017)	(-/R) fipronil
crustacean	Palaemonetes pugio	mortality (larvae)	96 h	LC50	0.35	nom-m	S	98	3/C1	Overmyer et al. (2007) cited in Bower JC (2017)	(-/R) fipronil
crustacean	Palaemonetes pugio	mortality (adult/parent)	96 h	LC50	0.32	n.r.	R	n.r.	3/C2	Key, Chung, Opatkiewicz, Wirth, and Fulton (2003)	
crustacean	Palaemonetes pugio	mortality (larvae)	96 h	LC50	0.68	n.r.	R	n.r.	3/C2	Key et al. (2003)	
crustacean	Palaemonetes pugio	mortality (embryo)	96 h	LC50	512	n.r.	R	n.r.	3/C2	Key et al. (2003)	
crustacean	**Penaeus japonicus**	**immobilisation**	**96 h**	**EC50**	**0.17**	**m-gm**	**R**	**>= 98**	**R2/C1**	Hano et al. (2019),	endpoint immobilization selected as the most sensitive
crustacean	Penaeus japonicus	mortality	96 h	LC50	0.21	m-gm	R	>= 98	R2/C1	Hano et al. (2019),	
fish	**Cyprinodon variegatus**	**mortality**	**96 h**	**LC50**	**130**	**mm**	**T**	**96.1**	**2/C1**	Machado (1993) cited in EC (2004) Vol.3 B9 p.553	

Chronic freshwater effect data											
Group	Species	Endpoint	Duration	Parameter	value (µg/L)	Analytics	Exposure	Purity (%)	Validity	Reference	Notes
cyanobacterium	Anabaena flos-aquae	growth rate	120 h	NOEC	170	mm	S	96.1	2/C1	Hoberg J.R. 1993 cited in EC (2004) Vol.3 B9 p.585	supportive
algae	Navicula pelliculosa	growth rate	120 h	NOEC	120	mm	S	96.1	2/C1	Hoberg J.R. 1993 cited in EC (2004) Vol.3 B9 p.586	supportive
algae	**Scenedesmus subspicatus**	**growth rate**	**96 h**	**NOEC**	**40**	**nom-m**	**S**	**> 95**	**2/C1**	Handley J.W., Mead C., Bartlett A.J. 1991 cited in EC (2004) Vol.3 B9 p.584	
algae	Selenastrum capricornutum	growth rate	120 h	NOEC	140	mm	S	96.1	2/C1	Hoberg J.R. 1993 cited in EC (2004) Vol.3 B9 p.585	supportive
higher plant	**Lemna gibba**	**biomass**	**14 d**	**NOEC**	**81**	**mm**	**S**	**96.1**	**2/C1**	Han Hoberg, J. R. (A.7.4.3.5.2/01) cited in (BASF, 2011) Document IIIA 7.4 page 240 Section 7.4.3.5, Annex Point IIIA, XIII.3.4, Aquatic plant toxicity	
higher plant	Lemna gibba	growth rate	120 h	NOEL	100	n.r.	S	96.1	R4/C4	U. EPA (1992)	
insect	**Chironomus riparius**	**development**	**28 d**	**NOEC**	**0.1168**	**m-i**	**S**	**99.14**	**2/C2**	Funk M (A7.4.3.4./02) 2004 cited in (BASF, 2011) Document IIIA 7.4 page 191 Section 7.4.3.4, Annex Point IIIA, XIII.2.4, Effects on reproduction and growth rate with an appropriate invertebrate species	supportive, OECD guideline 219
insect	Chironomus riparius	development	28 d	LOEC	0.2336	m-i	S	99.14	2/C2	Funk M (A7.4.3.4./02) 2004 cited in (BASF, 2011) page 191 Section 7.4.3.4, Annex Point IIIA, XIII.2.4, Effects on reproduction and growth rate with an appropriate invertebrate species	
insect	Chironomus riparius	emergence	28 d	LOEC	0.2336	m-i	S	99.14	2/C2	Funk M (A7.4.3.4./02) 2004 cited in (BASF, 2011) page 191 Section 7.4.3.4, Annex Point IIIA, XIII.2.4, Effects on reproduction and growth rate with an appropriate invertebrate species	
insect	Chironomus riparius	larvae growth rate	28 d	LOEC	0.081	n-m	S	>= 97	R2/C1	Monteiro et al. (2019)	
insect	Chironomus riparius	adult emergence rate	28 d	LOEC	0.04	n-m	S	>= 97	R2/C1	Monteiro et al. (2019)	
insect	Chironomus riparius	weight	28 d	LOEC	0.04	n-m	S	>= 97	R2/C1	Monteiro et al. (2019)	

Group	Species	Endpoint	Duration	Parameter	value (µg/L)	Analytics	Exposure	Purity (%)	Validity	Reference	Notes
crustacean	Americamysis bahia (Mysidopsis bahia)	survival	28 d	LOEC	0	n.r.	T	97.7	R4/C4	U. EPA (1992)	
crustacean	Ceriodaphnia dubia	multiple endpoints	8 d	LOEC	15	nom-m	S	98	3/C2	Wilson et al. (2008) cited in Bower JC (2017)	
crustacean	Ceriodaphnia dubia	multiple endpoints	8 d	LOEC	2	nom-m	S	97.3	3/C2	Wilson et al. (2008) cited in Bower JC (2017)	
crustacean	Ceriodaphnia dubia	multiple endpoints	8 d	LOEC	30	nom-m	S	98.1	3/C2	Wilson et al. (2008) cited in Bower JC (2017)	
crustacean	Ceriodaphnia dubia	time to first breed	8 d	NOEC	60	nom-m	S	98	3/C1	Wilson et al. (2008) cited in Bower JC (2017)	
crustacean	Ceriodaphnia dubia	time to first breed	8 d	NOEC	64	nom-m	S	97.3	3/C1	Wilson et al. (2008) cited in Bower JC (2017)	
crustacean	Ceriodaphnia dubia	time to first breed	8 d	NOEC	30	nom-m	S	98.1	3/C1	Wilson et al. (2008) cited in Bower JC (2017)	
crustacean	Ceriodaphnia dubia	number offspring per surviving parent	8 d	NOEC	< 15	nom-m	S	98	3/C2	Wilson et al. (2008) cited in Bower JC (2017)	
crustacean	Ceriodaphnia dubia	number offspring per surviving parent	8 d	NOEC	< 2	nom-m	S	97.3	3/C2	Wilson et al. (2008) cited in Bower JC (2017)	
crustacean	Ceriodaphnia dubia	number offspring per surviving parent	8 d	NOEC	10	nom-m	S	98.1	3/C1	Wilson et al. (2008) cited in Bower JC (2017)	
crustacean	Ceriodaphnia dubia	brood size	8 d	NOEC	< 15	nom-m	S	98	3/C2	Wilson et al. (2008) cited in Bower JC (2017)	
crustacean	Ceriodaphnia dubia	brood size	8 d	NOEC	< 2	nom-m	S	97.3	3/C2	Wilson et al. (2008) cited in Bower JC (2017)	
crustacean	Ceriodaphnia dubia	brood size	8 d	NOEC	10	nom-m	S	98.1	3/C1	Wilson et al. (2008) cited in Bower JC (2017)	
crustacean	Ceriodaphnia dubia	mortality (adult/parent)	8 d	NOEC	60	nom-m	S	98	3/C1	Wilson et al. (2008) cited in Bower JC (2017)	
crustacean	Ceriodaphnia dubia	mortality (adult/parent)	8 d	NOEC	64	nom-m	S	97.3	3/C1	Wilson et al. (2008) cited in Bower JC (2017)	
crustacean	Ceriodaphnia dubia	mortality (adult/parent)	8 d	NOEC	90	nom-m	S	98.1	3/C1	Wilson et al. (2008) cited in Bower JC (2017)	
crustacean	Ceriodaphnia dubia	time to first breed	8 d	LOEC	120	nom-m	S	98	3/C2	Wilson et al. (2008) cited in Bower JC (2017)	

Group	Species	Endpoint	Duration	Parameter	value (µg/L)	Analytics	Exposure	Purity (%)	Validity	Reference	Notes
crustacean	Ceriodaphnia dubia	time to first breed	8 d	LOEC	> 64	nom-m	S	97.3	3/C2	Wilson et al. (2008) cited in Bower JC (2017)	
crustacean	Ceriodaphnia dubia	time to first breed	8 d	LOEC	90	nom-m	S	98.1	3/C2	Wilson et al. (2008) cited in Bower JC (2017)	
crustacean	Ceriodaphnia dubia	number offspring per surviving parent	8 d	LOEC	15	nom-m	S	98	3/C2	Wilson et al. (2008) cited in Bower JC (2017)	
crustacean	Ceriodaphnia dubia	number offspring per surviving parent	8 d	LOEC	2	nom-m	S	97.3	3/C2	Wilson et al. (2008) cited in Bower JC (2017)	
crustacean	Ceriodaphnia dubia	number offspring per surviving parent	8 d	LOEC	30	nom-m	S	98.1	3/C2	Wilson et al. (2008) cited in Bower JC (2017)	
crustacean	Ceriodaphnia dubia	brood size	8 d	LOEC	15	nom-m	S	98	3/C2	Wilson et al. (2008) cited in Bower JC (2017)	
crustacean	Ceriodaphnia dubia	brood size	8 d	LOEC	2	nom-m	S	97.3	3/C2	Wilson et al. (2008) cited in Bower JC (2017)	
crustacean	Ceriodaphnia dubia	brood size	8 d	LOEC	30	nom-m	S	98.1	3/C2	Wilson et al. (2008) cited in Bower JC (2017)	
crustacean	Ceriodaphnia dubia	mortality (adult/parent)	8 d	LOEC	120	nom-m	S	98	3/C2	Wilson et al. (2008) cited in Bower JC (2017)	
crustacean	Ceriodaphnia dubia	mortality (adult/parent)	8 d	LOEC	> 64	nom-m	S	97.3	3/C2	Wilson et al. (2008) cited in Bower JC (2017)	
crustacean	Ceriodaphnia dubia	mortality (adult/parent)	8 d	LOEC	270	nom-m	S	98.1	3/C2	Wilson et al. (2008) cited in Bower JC (2017)	
crustacean	Daphnia magna	survival	21 d	LOEC	27	n.r.	T	100	R4/C4	U. EPA (1992)	
crustacean	Daphnia magna	growth rate	21 d	LOEC	19.5	n.r.	T	100	R4/C4	U. EPA (1992)	
crustacean	Daphnia magna	growth rate	21 d	LOEC	9.6	n.r.	T	100	R4/C4	U. EPA (1992)	
crustacean	**Daphnia magna**	**length**	**21 d**	**NOEC**	**9.8**	**mm**	**T**	**100**	**2/C1**	MCNamara P.C. 1990 cited in EC (2004) Vol.3 B9 p.577	endpoint length selected as the most sensitive
crustacean	Daphnia magna	mortality	21 d	NOEC	20	mm	T	100	2/C1	MCNamara P.C. 1990 cited in EC (2004) Vol.3 B9 p.577	

Group	Species	Endpoint	Duration	Parameter	value (µg/L)	Analytics	Exposure	Purity (%)	Validity	Reference	Notes
crustacean	Daphnia magna	survival	21 d	LOEC	27	n.r.	T	100	R4/C4	U. EPA (1992)	
crustacean	Daphnia magna	growth rate	21 d	LOEC	19.5	n.r.	T	100	R4/C4	U. EPA (1992)	
crustacean	Daphnia magna	growth rate	21 d	LOEC	9.6	n.r.	T	100	R4/C4	U. EPA (1992)	
fish	**Oncorhynchus mykiss (Salmo gairdneri)**	**mortality (larvae)**	**90 d**	**NOEC**	**15**	**mm**	**T**	**96.7**	**2/C1**	Machado M.W. 1992 cited in EC (2004) Vol.3 B9 p.561	
fish	Oncorhynchus mykiss (Salmo gairdneri)	growth rate	90 d	LOEC	15	n.r.	T	96.7	R4/C4	U. EPA (1992)	
fish	Oncorhynchus mykiss (Salmo gairdneri)	mortality	90 d	LOEC	26	n.r.	T	96.7	R4/C4	U. EPA (1992)	
fish	Oncorhynchus mykiss (Salmo gairdneri)	growth rate	90 d	NOEC	6.6	n.r.	T	96.7	R4/C4	U. EPA (1992)	
fish	Danio rerio	mortality (embryo)	72 h	LC50	13470	n	R	> 90	R4/C1	Park, Lee, Park, Song, and Lim (2020)	

Chronic saltwater effect data											
Group	Species	Endpoint	Duration	Parameter	value (µg/L)	Analytics	Exposure	Purity (%)	Validity	Reference	Notes
algae	Skeletonema costatum	growth rate	120 h	NOEC	140	mm	S	96.1	2/C1	Hoberg J.R. 1993 cited in EC (2004) Vol.3 B9 p.586	supportive
algae	Dunaliella tertiolecta	cell number	96 h	NOEC	250	nom-m	S	98	2/C1	Overmyer et al. (2007) cited in Bower JC (2017)	supportive
algae	Dunaliella tertiolecta	cell number	96 h	LOEC	500	nom-m	S	98	2/C2	Overmyer et al. (2007) cited in Bower JC (2017)	
coral	Acropora tenuis	larval development	48 h	NOEC	12.3	m-twa	S	> 98	R2/C3	Flores et al. (2020)	
crustacean	Americamysis bahia (Mysidopsis bahia)	length	28 d	LOEC	0.015	mm	T	97.7	2/C1	Machado M 1995 cited in EC (2004) Vol.3 B9 p.578	
crustacean	**Americamysis bahia (Mysidopsis bahia)**	**length**	**28 d**	**NOEC**	**0.0077**	**mm**	**T**	**97.7**	**2/C1**	Machado M 1995 cited in EC (2004) Vol.3 B9 p.578	
crustacean	Americamysis bahia (Mysidopsis bahia)	multiple endpoints	28 d	NOEC	0.06	nom-m	S	99.7	2/C4	Cafarella (2005) cited in BASF (2011) Document IIIA 7.4 page 207	
crustacean	Americamysis bahia (Mysidopsis bahia)	multiple endpoints	28 d	LOEC	> 0.06	nom-m	S	99.7	2/C4	Cafarella (2005) cited in BASF (2011) Document IIIA 7.4 page 207	
crustacean	Americamysis bahia (Mysidopsis bahia)	mortality	28 d	LOEC	0.005	n.r.	T	97.7	R4/C4	U. EPA (1992)	
crustacean	Amphiascus tenuiremis	mortality	24 d	LC50	> 0.63	n.r.	R	98	4/C3	Cary, Chandler, Volz, Walse, and Ferry (2004)	
crustacean	Amphiascus tenuiremis	reproduction rate	24 d	EC50	n.r	n.r.	R	98	4/C3	Cary et al. (2004)	

Group	Species	Endpoint	Duration	Parameter	value (µg/L)	Analytics	Exposure	Purity (%)	Validity	Reference	Notes
crustacean	Amphiascus tenuiremis	mortality	21 d	LC50	n.r	m	S	98	4/C4	Chandler et al. (2004) cited in Bower JC (2017)	
fish	Cyprinodon variegatus	multiple endpoints	34 d	NOEC	< 1.6	mm	T	97.08	3/C2	Sousa JV 1998 cited in EC (2004) Vol.3 B9 p.563	
fish	**Cyprinodon variegatus**	**multiple endpoints**	**34 d**	**NOEC**	**2.9**	**mm**	**T**	**97.08**	**2/C1**	Sousa JV 1998 cited in EC (2004) Vol.3 B9 p.563	endpoint selected as the most sensitive
fish	Cyprinodon variegatus	hatching rate	5 d	NOEC	6	mm	T	98	2/C1	Dionne E. 2000 cited in EC (2004) Vol.3 B9 p.565	
fish	Cyprinodon variegatus	fertility	59 d	NOEC	6	mm	T	98	2/C1	Dionne E. 2000 cited in EC (2004) Vol.3 B9 p.565	
fish	Cyprinodon variegatus	length	28 d	NOEC	6	mm	T	98	2/C1	Dionne E. 2000 cited in EC (2004) Vol.3 B9 p.565	
fish	Cyprinodon variegatus	length	28 d	NOEC	6	mm	T	98	2/C1	Dionne E. 2000 cited in EC (2004) Vol.3 B9 p.565	
fish	Cyprinodon variegatus	length	59 d	NOEC	6	mm	T	98	2/C1	Dionne E. 2000 cited in EC (2004) Vol.3 B9 p.565	
fish	Cyprinodon variegatus	length	110 d	NOEC	6	mm	T	98	2/C1	Dionne E. 2000 cited in EC (2004) Vol.3 B9 p.565	
fish	Cyprinodon variegatus	mortality (adult/parent)	59 d	NOEC	13	mm	T	98	2/C1	Dionne E. 2000 cited in EC (2004) Vol.3 B9 p.565	
fish	Cyprinodon variegatus	mortality (adult/parent)	110 d	NOEC	13	mm	T	98	2/C1	Dionne E. 2000 cited in EC (2004) Vol.3 B9 p.565	
fish	Cyprinodon variegatus	mortality (larvae)	28 d	NOEC	13	mm	T	98	2/C1	Dionne E. 2000 cited in EC (2004) Vol.3 B9 p.565	
fish	Cyprinodon variegatus	mortality (larvae)	28 d	NOEC	13	mm	T	98	2/C1	Dionne E. 2000 cited in EC (2004) Vol.3 B9 p.565	
fish	Cyprinodon variegatus	weight	28 d	NOEC	13	mm	T	98	2/C1	Dionne E. 2000 cited in EC (2004) Vol.3 B9 p.565	
fish	Cyprinodon variegatus	weight	110 d	NOEC	13	mm	T	98	2/C1	Dionne E. 2000 cited in EC (2004) Vol.3 B9 p.565	
fish	Cyprinodon variegatus	hatching rate	5 d	LOEC	13	mm	T	98	2/C2	Dionne E. 2000 cited in EC (2004) Vol.3 B9 p.565	
fish	Cyprinodon variegatus	multiple endpoints	n.r.	LOEC	13	mm	T	98	R4/C4	Dionne E. 2000 cited in EC (2004) Vol.3 B9 p.565	

Group	Species	Endpoint	Duration	Parameter	value (µg/L)	Analytics	Exposure	Purity (%)	Validity	Reference	Notes
fish	Cyprinodon variegatus	hatching rate	110 d	LOEC	0.85	n.r.	T	98	R4/C4	U. EPA (1992)	
fish	Cyprinodon variegatus	growth rate	110 d	LOEC	1.7	n.r.	T	98	R4/C4	U. EPA (1992)	
fish	Cyprinodon variegatus	hatching rate	110 d	NOEC	< 0.85	n.r.	T	98	R4/C4	U. EPA (1992)	

Legend

Chemical analitycs

n: based on nominal concentrations
m: based on measured concentrations
m-gm based on mean measured concentrations (geometric mean)
mm based on mean measured concentrations
mm-i based on mean measured start concentration
m-i based on measured start concentration
m-twa: based on measured concentrations («time-weighted average»)
nom-i based on nominal concentrations; recovery at the start was determined. In case recovery was 80-120 %, nominal effect concentrations are regarded as valid. In case recovery was < 80 %, effect values are regarded as invalid.
nom-m based on nominal concentrations; recovery at the start was determined. In case recovery was 80-120 %, nominal effect concentrations are regarded as valid. In case recovery was < 80 %, effect values are regarded as invalid or, if possible, calculated (e.g. «time-weighted average»).

Exposure
S static
R semi-static
T flow-through

Relevance/Reliability
Klimisch: 1 Reliable without restriction, 2 Reliable with restriction, 3 Not reliable, 4 Not assignable
Cred: R1 Reliable without restriction, R2 Reliable with restriction, R3 Not reliable, R4 Not assignable
C1 Relevant without restriction, C2 Relevant with restriction, C3 Not reliable, C4 Not assignable

13 Annex II

13.1 Enantioselective fipronil toxicity

Some organisms show a higher sensetivitytowards one fipronil enantiomer as compared to the other or the racemate (Baird et al., 2013; Konwick et al., 2005; Overmyer et al., 2007; Qu, Ma, Liu, Gao, et al., 2016; Qu et al., 2014; Wilson et al., 2008).

Daphnid (*Ceriodaphnia dubia*), crayfish (*Procambarus clarkii*) and mussel (*Anodonta woodiana*) were more sensitive towards the (+/S) enantiomer than the (-/R) enantiomer, while the racemate showed intermediate toxicity (Konwick et al., 2005; Overmyer et al., 2007; Wilson et al., 2008). In contrast, larval grass shrimp (*Palaemonetes pugio*), african clawed frog (*Xenopus laevis*), duckweed (*Lemna minor*) and green algae (*Scenedesmus obliquus*) displayed the highest toxicity towards the (-/R) enantiomer (Overmyer et al., 2007; Qu, Ma, Liu, Gao, et al., 2016; Qu et al., 2014). In fish larvae (*Pimephales promelas*), enantioselective toxicity was detectable only after seven days of exposure, with racemate and the (+/S) enantiomer being more toxic compared to the (-/R) enantiomer (Baird et al., 2013).

Table 14 summarizes available enantioselective toxicity values (racemate, (+/S and -/R enantiomer) for different organism groups.

Table 14 Toxicity values of fipronil (racemate 1:1) and the two enantiomers (+/S and -/R enantiomer) from studies focused on enantioselective toxicity.

Organism (*scientific name*)	Endpoint	Racemate (1 :1) [µg/L]	+/S [µg/L]	-/R [µg/L]	References
Crustaceans					
Ceriodaphnia dubia	LC_{50} (24 h)	33.3	18.1	65.2	(Wilson et al., 2008)
Ceriodaphnia dubia	LC_{50} (48 h)	30.3	10.3	50.1	(Wilson et al., 2008)
Ceriodaphnia dubia	EC_{50} (48 h)	17.7	10.3	31.9	(Konwick et al., 2005)
Procambarus clarkii	LC_{50} (96 h)	124.89	81.7	163.5	(Overmyer et al., 2007)
Palaemonetes pugio (parent)	LC_{50} (96 h)	0.32	0.54	0.32	(Overmyer et al., 2007)
Palaemonetes pugio(larvae)	LC_{50} (96 h)	0.68	208	0.35	(Overmyer et al., 2007)
Fish					
Pimephales promelas	LC_{50} (7 d)	208	227 L	365	(Baird et al., 2013)
Mussels					
Mercenaria mercenaria	EC_{50} (96 h)	177	208	187	(Overmyer et al., 2007)
Anodonta woodiana	LC_{50} (72 h)	1210	630	3270	(Qu, Ma, Liu, Gao, et al., 2016)
Insects					
Simulium vittatum	LC_{50} (48 h)	0.65	0.72	0.74	(Overmyer et al., 2007)
Amphibians					
Xenopus laevis	LC_{50} (96 h)	850	910	163.5	(Overmyer et al., 2007)
Plants					
Scenedesmus obliquus	EC_{50} (72 h)	540	1500	290	(Qu et al., 2014)
Lemna minor	EC_{50} (7 d)	9360	10140	8510	(Qu, Ma, Liu, Gao, et al., 2016)

13.2 Photolysis, hydrolysis and redox proceses of fipronil

Photolysis: Fipronil is subject to photolytic transformation (first order rate). The main photolysis product is fipronil desulfinyl (MB 46513). In the aqueous environment, photolysis is considered the main transformation route for fipronil, with a half-life of 3.5 h (APVMA 2012; CLH 2014; EFSA 2006). Some fipronil transformation products are also subject to photolysis with DT_{50} of 3.6 h (fipronil sulfide, MB 45950), 13 h (fipronil sulfone, MB 46136) and 38.9 h (fipronil desulfinyl, MB 46513). In waterbodies, photo-transformation of fipronil is reduced through the presence of dissolved organic matter (DOM) due to blocking of the light in addition to energetic quenching (S. S. Walse et al. 2004b) (APVMA 2012; CLH 2014).

Hydrolysis: Fipronil is hydrolytically stable at pH 5 and pH 7. At pH 9, the parent compound is hydrolyzed to fipronil amide (RPA 200766). At alkaline pH, the transformation is best modeled by a pseudo-first order kinetics with a DT_{50} of 28 days (CLH 2014). Similar to the parent compound, the major fipronil transformation products (fipronil sulfone (MB 46136), fipronil desulfinyl (MB 46513) and fipronil sulfide (MB 45950)) are stable at neutral and acidic conditions. At pH 9 they hydrolyze with DT_{50} of 50 d (25°C), 10.9 d (25°C), and 11 d (50°C), respectively (APVMA 2012; EFSA 2006).

Oxidation/reduction: Fipronil can be oxidized to fipronil sulfone (MB 46136) and reduced to fipronil sulfide (MB 45950) (CLH 2014; EFSA 2006).

13.3 Effect data on fipronil formulations

Table 15: Effect data collection of fipronil formulations

Formulation name	Group	Species	Endpoint	Duration	Parameter	Value (µg/L)	Analytics	Exposure	Purity (%)	Validity	Reference	Notes
EXP60720A	fish	*Oncorhynchus mykiss (Salmo gairdneri)*	mortality (juvenile)	96 h	LC50	= 229	mm	S	800 ± 25 g/kg fipronil (certified content 786 g a.i./kg)	2/C3	Suteau P. 1996 cited in EC (2004) Vol.3 B9 p.560	GLP
EXP60720A	crustaceans	*Daphnia magna*	behaviour	48 h	EC50	= 175	mm	S	800 ± 25 g/kg fipronil (certified content 786 g a.i./kg)	2/C3	Suteau P. 1996 cited in EC (2004) Vol.3 B9 p.560	GLP
EXP60720A	algae	*Scenedesmus subspicatus*	biomass	72 h	EbC50	= 166	mm	S	800 ± 25 g/kg fipronil (certified content 786 g a.i./kg)	2/C3	Suteau P. 1996 cited in EC (2004) Vol.3 B9 p.560	GLP
EXP60720A	algae	*Scenedesmus subspicatus*	growth rate	72 h	ErC50	> 211	mm	S	800 ± 25 g/kg fipronil (certified content 786 g a.i./kg)	2/C3	Suteau P. 1996 cited in EC (2004) Vol.3 B9 p.560	GLP
fipronil-2.5% EC	fish	*Clarias gariepinus*	mortality	96 h	LC50	= 6.148	n	S	n.r.	R4/C3	Amaeze et al. (2020)	

Formulation name	Group	Species	Endpoint	Duration	Parameter	Value (µg/L)	Analytics	Exposure	Purity (%)	Validity	Reference	Notes
n.r.	fish	*Oncorhynchus mykiss (Salmo gairdneri)*	mortality	96 h	LC50	>	100	n.r.	R	n.r.	R4/C4	US EPA (1992) Pesticide Ecotoxicity Database (Formerly: Environmental Effects Database (EEDB)), Reference Number 344, Environmental Fate and Effects Division, U.S.EPA, Washington, D.C.
n.r.	crustaceans	*Daphnia magna*	immobilisation	48 h	EC50	>	100	n.r.	S	n.r.	R4/C4	US EPA (1992) Pesticide Ecotoxicity Database (Formerly: Environmental Effects Database (EEDB)), Reference Number 344, Environmental Fate and Effects Division, U.S.EPA, Washington, D.C.
n.r.	fish	*Rhamdia quelen*	mortality	48 h	LC50	=	817.7	n.r.	S	n.r.	R3/C4	Fredianelli et al. (2019)
Regent 4SC, 480 g a.i./L	crustaceans	*Daphnia pulex*	mortality	48 h	LC50	=	15.6	n	S	n.r.	R3/C3	Stark and Vargas (2005)
Regent® 300EC (EXP 61196A)	insects	*Polypedilum nubiferum*	mortality	48 h	LC50	=	0.89	n.r.	S	n.r.	R4/C3	Stevens et al. (2011b)
Regent® 300EC (EXP 61196A)	crustaceans	*Simocephalus elizabethae*	mortality	48 h	LC50	=	19.12	n.r.	S	n.r.	R4/C3	Stevens et al. (2011b)
Regent® 300EC (EXP 61196A)	insects	*Polypedilum nubiferum*	mortality	48 h	LC50	=	1.29	n.r.	S	n.r.	R4/C3	Stevens et al. (2011b)
Regent® 300EC (EXP 61196A)	crustaceans	*Simocephalus elizabethae*	mortality	48 h	LC50	=	15.1	n.r.	S	n.r.	R4/C3	Stevens et al. (2011b)
Regent® 800 WG	fish	*Danio rerio*	mortality	96 h	LC50	=	172	n.r.	n.r.	80% w/v a.i	R4/C4	Moreira et al. (2021)
Regent® 800 WG	crustaceans	*Ceriodaphnia silvestrii*	immobilisation	48 h	EC50	=	3.9	m-i	S	80% w/v a.i	R2/C3	Silva et al. (2020)
Regent® 800 WG	crustaceans	*Ceriodaphnia silvestrii*	immobilisation	48 h	EC10	=	2.1	m-i	S	80% w/v a.i	R2/C3	Silva et al. (2020)
Termidor®, 9.1% a.i.	fish	*Pimephales promelas*	mortality (larvae)	24 h	LC50	=	379.47	n.r.	S	98.5	R4/C3	Beggel et al. (2010) cited in Bower JC (2017)
Turmonil 50 SC	fish	*Cyprinus carpio*	mortality	96 h	LC50	=	665	n.r.	n.r.	n.r.	R4/C4	Qureshi, Bibi, Shahid, and Ghazanfar (2016)

Formulation name	Group	Species	Endpoint	Duration	Parameter	Value (µg/L)	Analytics	Exposure	Purity (%)	Validity	Reference	Notes
Regent® 800 WG	crustaceans	*Ceriodaphnia silvestrii*	time to first breed	8 d	NOEC	= 0.4	m-i	R	80% w/v a.i	R2/C3	Silva et al. (2020)	

Legend

Chemical analitycs

n: based on nominal concentrations
m: based on measured concentrations
m-gm based on mean measured concentrations (geometric mean)
mm based on mean measured concentrations
mm-i based on mean measured start concentration
m-i based on measured start concentration m-twa: based on measured concentrations («time-weighted average»)
nom-i based on nominal concentrations; recovery at the start was determined. In case recovery was 80-120 %, nominal effect concentrations are regarded as valid. In case recovery was < 80 %, effect values are regarded as invalid.
nom-m based on nominal concentrations; recovery at the start was determined. In case recovery was 80-120 %, nominal effect concentrations are regarded as valid. In case recovery was < 80 %, effect values are regarded as invalid or, if possible, calculated (e.g. «time-weighted average»).

Exposure

S static
R semi-static
T flow-through

13.4 Transformation products of fipronil

Mode of action: Fipronil acts by disrupting the chloride flux trough binding to glutamate- and γ-aminobutiric acid (GABA) gated chloride channels (see section 1.4). In chloride-channel binding assays, fipronil sulfone (MB 46136), fipronil sulfide (MB 45950) and fipronil desulfinyl (MB 46513) show channel binding affinity. From a large data set obtained with phenylpyrazoles, a correlation between chloride-channel binding affinity and mammalian toxicity was previously established (EC, 2004). Indeed, all three transformation products induce medical signs of neurotoxicity following oral exposure, and thus are considered toxicologically relevant. Genotoxicity tests were negativ for all transformation products (EC, 2004).

Bioaccumulation and Biomagnification: Fipronil sulfone (MB 46136), fipronil sulfide (MB 45950) and fipronil desulfinyl (MB 46513) can be present in the aquatic environment. Based on their estimated $logK_{ow}$ values of 4.42 (fipronil sulfone), 4.82 (fipronil sulfide) and 4.22 (fipronil desulfinyl) (U. EPA, 2020), uptake and accumulation of the transformation products is a definite possibility. Additionally, fipronil can be biotransformed within organisms, such as fish, to fipronil sulfone (MB 46136) and fipronil sulfide (MB 45950). In a study performed with bluegill fish (*Lepomis macrochirus*), fipronil sulfone (MB 46136) accounted for 43.8 % and fipronil sulfide (MB 45950) accounted for 11.2 % of the initial applied parent compound[16] (Chapleoalnd Hall (1992) cited in (EC, 2004)). Similarly, in tilapia, european eel and rainbow trout, fipronil sulfone (MB 46136) was the main biotransformation product (Konwick et al., 2006; Li, You, & Wang, 2018b; Michel et al., 2016). Also at lower trophic levels, fipronil was predominantly biotransformed to fipronil sulfone (MB 46136) (Qu, Ma, Liu, Jing, et al., 2016). Additionally, fipronil sulfone was shown to persist longer within the organisms as compared to the parent compound (Konwick et al., 2006; Qu, Ma, Liu, Jing, et al., 2016). Considering the efficient biotransformation of fipronil to fipronil sulfone (MB 46136) within different trophic levels, and its slower elimination, fipronil sulfone (MB 46136) appears to be the relevant compound when considering biomagnification.

The BCFBAF tool of EPISuite (U. EPA, 2020) suggests an average BAF of 835.7[17], 1351.7[18] and 460.3[19] L/kg wet-wt for fipronil sulfone (MB 46136), fipronil sulfide (MB 45950) and fipronil desulfinyl (MB

[16] MB 45897 accounted for 26.1 % of the initially applied parent compound, it is a product of biotransformation and does not show binding to chloride receptor nor oral toxicity, thus is not considered toxicologically relevant (EC, 2004).
[17] BAF of 937.5, 799.5 and 770 for the upper, mid and lower trophic level, respectively.
[18] BAF of 1165.5, 1361.5 and 1528 for the upper, mid and lower trophic level, respectively.
[19] BAF of 511.5, 442.15 and 427.25 for the upper, mid and lower trophic level, respectively.

46513), respectively (including biotransformation). Estimated BAF for fipronil is on average 113 L/kg wet-wt, which is 4 to 11 times lower as compared to the transformation products.

Environmental fate: The environmental fate and stability of fipronil transformation products is described in Section 2. Here, a short summary is presented.

Fipronil sulfone (MB 46136) is a product of fipronil oxidation, and is one of the major soil transformation products. In soil, it is considered to be immobile to slightly mobile. However, the soil mobility strongly depends on the type of soil. Nonetheless, contamination of aquatic environments *via* runoff remains a possibility, especially in soil with low organic matter content. Fipronil sulfone is also the major biotransformation product in organisms which can be excreted into the environment. The compound is present in water (2.3 % at 244 d.) and sediment (4.9% in sediment at 244 d.) (EC, 2004, 2011).

Fipronil sulfide (MB 45950) is a product of fipronil reduction. It is present in the water phase (8.9 % at 93 d) but it is mainly sorbed to the sediment (80 % at 120 d.). The compound represents the major transformation product in the sediment (EC, 2004, 2011).

Fipronil desulfinyl (MB 46513) is the main photoproduct of fipronil in water. Its formation strongly depends on light intensity, which varies depending on the latitude and season (EC, 2004, 2011).

Generally, in a water-sediment system, fipronil transformation products are expected to dissipate from water and partition into the sediment (EC, 2004, 2011).

13.5 Effect data on transformation products of fipronil

Table 16: Effect data collection for Fipronil sulfone (MB 46136), Fipronil sulfide (MB 45950), Fipronil desulfinyl (MB 46513) in µg/L. Data were evaluated for relevance and reliability according to the CRED criteria (Moermond et al., 2016).

Fipronil sulfone (MB 46136)											
Group	Species	Endpoint	Duration	Parameter	Value (µg/L)	Analytics	Exposure	Purity (%)	Validity	Reference	Notes
Acute freshwater effect data											
crustacean	*Daphnia magna*	immobilisation	48 h	EC50	= 29	mm	T	100	2/C1	McNamara (1990) cited in EC (2004) Vol.3 B9 p. 572	
crustacean	*Hyalella azteca*	immobilisation	96 h	EC50	= 0.2049512	m	S	99.3	2/C1	Weston and Lydy (2014) in Bower JC (2017)	average value of 2 tests, immobilization is the most sensitive endpoint
fish	*Oncorhynchus mykiss (Salmo gairdneri)*	mortality (juvenile)	96 h	LC50	= 39	mm	T	99.2	2/C1	Bettencourt (1992) cited in EC (2004) Vol.3 B9 p. 556	
fish	*Lepomis macrochirus*	mortality (juvenile)	96 h	LC50	= 25	mm	T	99.2	2/C1	Bettencourt (1992) cited in EC (2004) Vol.3 B9 p. 557	
algae	*Scenedesmus subspicatus*	cell number	72 h	EbC50	> 510	mm	S	99.7	2/C2	Odin-Feurtet (1999) cited in EC (2004) Vol.3 B9 p. 588	EbC50 and ErC50 have the same value
insect	*Baetis tricaudatus*	immobilisation	48 h	EC50	= 0.1035616	m	S	99.3	2/C1	Weston and Lydy (2014) in Bower JC (2017)	average value of 2 tests, immobilization is the most sensitive endpoint
insect	*Chironomus dilutus*	immobilisation	96 h	EC50	= 0.0076974	m	S	99	2/C1	Weston and Lydy (2014) in Bower JC (2017)	average value of 2 tests, immobilization is the most sensitive endpoint
insect	*Diphetor hageni*	immobilisation	48 h	EC50	= 0.0926	m	S	99.3	2/C1	Weston and Lydy (2014) in Bower JC (2017)	immobilization is the most sensitive endpoint
insect	*Fallceon quilleri*	immobilisation	48 h	EC50	= 0.0717	m	S	99.3	2/C1	Weston and Lydy (2014) in Bower JC (2017)	immobilization is the most sensitive endpoint
insect	*Helicopsyche sp.*	immobilisation	96 h	EC50	= 0.0738	m	S	99.3	2/C1	Weston and Lydy (2014) in Bower JC (2017)	immobilization is the most sensitive endpoint

Group	Species	Endpoint	Duration	Parameter	Value (µg/L)	Analytics	Exposure	Purity (%)	Validity	Reference	Notes
insect	*Hexagenia sp.*	immobilisation	96 h	EC50	= 0.163	m	S	99.3	2/C1	Weston and Lydy (2014) in Bower JC (2017)	immobilization is the most sensitive endpoint
insect	*Hydropsyche sp.*	immobilisation	96 h	EC50	= 0.0729	m	S	99.3	2/C1	Weston and Lydy (2014) in Bower JC (2017)	immobilization is the most sensitive endpoint
insect	*Isoperla quinquepunctata*	immobilisation	96 h	EC50	= 0.0474	m	S	99.3	2/C1	Weston and Lydy (2014) in Bower JC (2017)	immobilization is the most sensitive endpoint
insect	*Nectopsyche sp.*	immobilisation	96 h	EC50	= 0.0313	m	S	99.3	2/C1	Weston and Lydy (2014) in Bower JC (2017)	immobilization is the most sensitive endpoint
insect	*Serratella micheneri*	immobilisation	48 h	EC50	= 0.159	m	S	99.3	2/C1	Weston and Lydy (2014) in Bower JC (2017)	immobilization is the most sensitive endpoint
insect	*Taenionema sp.*	immobilisation	96 h	EC50	= 0.0959	m	S	99.3	2/C1	Weston and Lydy (2014) in Bower JC (2017)	immobilization is the most sensitive endpoint
insect	*Sympetrum infuscatum*	feeding behavior	48 h	EC50	= 3	n-m	R	> 99	R2/C1	Jinguji, Ohtsu, Ueda, and Goka (2018a)	behavior is the most sensitive endpoint
insect	*Sympetrum frequens*	feeding behavior	48 h	EC50	= 1	n-m	R	> 99	R2/C1	Jinguji et al. (2018a)	behavior is the most sensitive endpoint
Chronic freshwater effect data											
fish	*Oncorhynchus mykiss (Salmo gairdneri)*	multiple endpoints	96 h	NOEC	= 18	mm	T	99.2	2/C1	Bettencourt (1992) cited in EC (2004) Vol.3 B9 p. 556	
fish	*Lepomis macrochirus*	multiple endpoints	96 h	NOEC	= 6.7	mm	T	99.2	2/C1	Bettencourt (1992) cited in EC (2004) Vol.3 B9 p. 557	
algae	*Scenedesmus subspicatus*	growth rate	72 h	NOEC	= 510	mm	S	99.7	2/C1	Odin-Feurtet (1999) cited in EC (2004) Vol.3 B9 p. 588	
crustacean	*Daphnia magna*	length	21 d	NOEC	= 0.45	mm	R	99.7	2/C1	Janson (2014) cited in Bower JC (2017)	most sensitive endpoint

Group	Species	Endpoint	Duration	Parameter	Value (µg/L)	Analytics	Exposure	Purity (%)	Validity	Reference	Note
Acute saltwater effect data											
crustacean	*Americamysis bahia (Mysidopsis bahia)*	mortality	96 h	LC50	= 0.056	mm	S	> 99	2/C1	Putt (2000) cited in EC (2004) Vol.3 B9 p. 575	
Chronic saltwater effect data											
crustacean	*Americamysis bahia (Mysidopsis bahia)*	weight	28 d	NOEC	= 0.0051	mm	T	>= 99	2/C1	Lima (2000) cited in EC (2004) Vol.3 B9 p. 583	
Fipronil sulfide (MB 45950)											
Group	Species	Endpoint	Duration	Parameter	Value (µg/L)	Analytics	Exposure	Purity (%)	Validity	Reference	Note
Acute freshwater effect data											
crustacean	*Daphnia magna*	immobilisation	48 h	EC50	= 100	mm	T	100	2/C1	MCNamara (1990) cited in EC (2004) Vol.3 B9 p. 571	
crustacean	*Hyalella azteca*	immobilisation	96 h	EC50	= 0.45	m	S	99	2/C1	Weston and Lydy (2014) in Bower JC (2017)	average value of 2 tests, immobilization is the most sensitive endpoint
fish	*Oncorhynchus mykiss (Salmo gairdneri)*	mortality (juvenile)	96 h	LC50	= 29.5	mm	T	95.4	2/C1	Suteau (1996) cited in EC (2004) Vol.3 B9 p. 555	
algae	*Scenedesmus subspicatus*	biomass	72 h	ErC50	= 1300	mm	S	98.8	2/C1	McElligott (1999) cited in EC (2004) Vol.3 B9 p.588	ErC50 is the preferredendpoint
insect	*Baetis tricaudatus*	immobilisation	48 h	EC50	= 0.0803	m	S	99	2/C1	Weston and Lydy (2014) in Bower JC (2017).	
insect	*Chironomus dilutus*	immobilisation	96 h	EC50	= 0.0098818	m	S	99	2/C1	Weston and Lydy (2014) in Bower JC (2017)	average value of 2 tests
insect	*Fallceon quilleri*	immobilisation	48 h	EC50	= 0.0342	m	S	99	2/C1	Weston and Lydy (2014) in Bower JC (2017)	immobilization is the most sensitive endpoint
insect	*Helicopsyche sp.*	immobilisation	96 h	EC50	= 0.177	m	S	99	2/C1	Weston and Lydy (2014) in Bower JC (2017)	immobilization is the most sensitive endpoint

Group	Species	Endpoint	Duration	Parameter	Value (µg/L)	Analytics	Exposure	Purity (%)	Validity	Reference	Note
insect	*Isoperla quinquepunctata*	immobilisation	96 h	EC50	= 0.0422	m	S	99	2/C1	Weston and Lydy (2014) in Bower JC (2017)	immobilization is the most sensitive endpoint
insect	*Nectopsyche sp.*	immobilisation	96 h	EC50	= 0.0285	m	S	99	2/C1	Weston and Lydy (2014) in Bower JC (2017)	
Chronic freshwater effect data											
crustacean	*Daphnia magna*	multiple endpoints	21 d	NOEC	= 13	mm	T	100	2/C1	McNamara (1990) cited in EC (2004) Vol.3 B9 p. 580	
algae	*Scenedesmus subspicatus*	growth	72 h	NOEC	= 260	mm	S	98.8	2/C1	McElligott (1999) cited in EC (2004) Vol.3 B9 p. 588	
Acute saltwater effect data											
crustacean	*Americamysis bahia (Mysidopsis bahia)*	mortality	96 h	LC50	= 0.077	mm	S	99	2/C1	Putt (2000) cited in EC (2004) Vol.3 B9 p. 576	
Chronic saltwater effect data											
crustacean	*Americamysis bahia (Mysidopsis bahia)*	multiple endpoints	28 d	NOEC	= 0.0046	mm	T	>= 99.5	2/C1	Lima (2000) cited in EC (2004) Vol.3 B9 p. 582	
Fipronil desulfinyl (MB 46513)											
Group	Species	Endpoint	Duration	Parameter	Value (µg/L)	Analytics	Exposure	Purity (%)	Validity	Reference	Note
Acute freshwater effect data											
fish	*Oncorhynchus mykiss (Salmo gairdneri)*	mortality (juvenile)	96 h	LC50	= 31	mm	R	98.6	2/C1	Collins (1993) cited in EC (2004) Vol.3 B9 p. 554	
fish	*Lepomis macrochirus*	mortality (juvenile)	96 h	LC50	= 20	mm	T	98.6	2/C1	Collins (1993) cited in EC (2004) Vol.3 B9 p. 554	
algae	*Selenastrum capricornutum*	cell number	120 h	EC50	= 65	mm	S	98.6	2/C1	Hoberg (1993) cited in EC (2004) Vol.3 B9 p. 587	

Group	Species	Endpoint	Duration	Parameter	Value (µg/L)	Analytics	Exposure	Purity (%)	Validity	Reference	Note
Chronic freshwater effect data											
algae	*Selenastrum capricornutum*	cell number	120 h	NOEC	< 12	mm	S	98.6	2/C2	Hoberg (1993) cited in EC (2004) Vol.3 B9 p. 587	
crustacean	*Daphnia magna*	growth	21 d	NOEC	= 41	mm	R	97.81	2/C1	Putt (1992) cited in EC (2004) Vol.3 B9 p. 579	growth as the most senitive endpoint selected
Acute saltwater effect data											
crustacean	*Americamysis bahia (Mysidopsis bahia)*	mortality	96 h	LC50	= 1.5	mm	S	> 99	2/C1	Putt (2000) cited in EC (2004) Vol.3 B9 p. 574	
Fipronil amide (RPA 200766)											
Group	Species	Endpoint	Duration	Parameter	Value (µg/L)	Analytics	Exposure	Purity (%)	Validity	Reference	Notes
Acute freshwater effect data											
crustacean	*Daphnia magna*	immobilisation	48 h	EC50	> 20000	mm	S	99.8	2/C2	Machado (2001) cited in EC (2004) Vol.3 B9 p. 572	EC50 is above the limit of solubility under the conditions of the test
fish	*Oncorhynchus mykiss (Salmo gairdneri)*	mortality	96 h	LC50	> 17000	mm	R	99.8	2/C2	Machado (2001) cited in EC (2004) Vol.3 B9 p. 557	LC50 is above the limit of solubility under the conditions of the test
algae	*Scenedesmus subspicatus*	growth rate	72 h	ErC50	> 7500	mm	S	99.8	2/C2	Hoberg (2001) cited in EC (2004) Vol.3 B9 p. 589	EC50 is above the limit of solubility under the conditions of the test
insect	*Chironomus riparius*	mortality	48 h	LC50	= 327.87	m	S	99.8	2/C1	Funk (2004) cited in Evans (2005) and Funk and Grote (2004) cited in Bower JC (2017)	geomean of 2 entries
Chronic freshwater effect data											
algae	*Scenedesmus subspicatus*	growth rate	72 h	NOEC	= 7500	mm	S	99.8	2/C1	Hoberg (2001) cited in EC (2004) Vol.3 B9 p. 589	

RPA104615											
Group	Species	Endpoint	Duration	Parameter	Value (µg/L)	Analytics	Exposure	Purity (%)	Validity	Reference	Notes
Acute freshwater effect data											
fish	*Oncorhynchus mykiss (Salmo gairdneri)*	mortality (juvenile)	96 h	LC50	> 100000	nom	R	94.7	2/C2	Collins (1993) cited in EC (2004) Vol.3 B9 p. 558	LC50 is above the limit of solubility under the conditions of the test
crustacean	*Daphnia magna*	immobilisation	48 h	EC50	> 100000	nom	S	94.7	2/C2	Collins (1993) cited in EC (2004) Vol.3 B9 p. 573	EC50 is above the limit of solubility under the conditions of the test
RPA200761											
Group	Species	Endpoint	Duration	Parameter	Value (µg/L)	Analytics	Exposure	Purity (%)	Validity	Reference	Notes
Acute freshwater effect data											
fish	*Oncorhynchus mykiss (Salmo gairdneri)*	mortality (juvenile)	96 h	LC50	> 100000	nom-m	R	94.5	2/C2	Wetton (1999) cited in EC (2004) Vol.3 B9 p.559	LC50 is above the limit of solubility under the conditions of the test
crustacean	*Daphnia magna*	immobilisation	48 h	EC50	> 100000	nom-m	S	94.5	2/C2	Wetton & Mullee (1999) cited in EC (2004) Vol.3 B9 p. 574	EC50 is above the limit of solubility under the conditions of the test
algae	*Scenedesmus subspicatus*	growth	72 h	EbC50	> 100000	nom-m	S	94.5	2/C2	Mead and Mullee (1999) cited in EC (2004) Vol.3 B9 p. 591	EC50 is above the limit of solubility under the conditions of the test
algae	*Scenedesmus subspicatus*	growth	72 h	ErC50	> 100000	nom-m	S	94.5	2/C2	Mead and Mullee (1999) cited in EC (2004) Vol.3 B9 p. 591	EC50 is above the limit of solubility under the conditions of the test
Chronic freshwater effect data											
algae	*Scenedesmus subspicatus*	growth	72 h	NOEC	= 56000	nom-m	S	94.5	2/C2	Mead and Mullee (1999) cited in EC (2004) Vol.3 B9 p. 591	

13.6 Data for fipronil transformation products on secondary poisoning of top predators

Table 17: Data for fipronil transformation products on secondary poisoning of top predators

Substance	Mammalian toxicity (oral)			
	Effect concentrtaion	Organism	Duration	Reference
Fipronil sulfone (MB 46136)	LD50 (males) = 218 mg/kg bw LD50 (females) = 184 mg/kg bw LD50 (combined) =257 mg/kg bw	*Rattus norvegicus*	acute	Gardener (1988a) cited in (EC, 2004) Vol.3 B6, p 206
	Avian toxicity (oral)			
	Effect concentrtaion	Organism	Duration	Reference
	LD50 = 41 mg/kg bw	*Colinus virginianus*	acute	Gallagher, Grimes and Beavers (2001) cited in (EC, 2004) Vol.3 B9, p 513
	LD50 > 2000 mg/kg bw	*Anas platyrhynchos*	acute	Gallagher, Grimes and Beavers (2001) cited in (EC, 2004) Vol.3 B9, p514
	LC50 = 84 mg/kg diet	*Colinus virginianus*	short term (5d)	Gallagher et al., (1999) cited in (EC, 2004) Vol.3 B9, p 519
Fipronil sulfide (MB 45950)	Mammalian toxicity (oral)			
	Organism	Duration	Literature	Reference
	LD50 (males) = 69 mg/kg bw LD50 (females) = 100 mg/kg bw LD50 (combined) = 83 mg/kg bw	*Rattus norvegicus*	acute	Dange (1994a) cited in (EC, 2004) Vol.3 B6, p 194
	NOEL = 1 mg/kg bw/day	*Canis familiaris*	28-d	Broadmeadow (1991a) cited in (EC, 2004) Vol.3 B6, p 198
	NOEL = 10 ppm NOEL (males)= 0.69 mg/kg bw/day NOEL (females) = 0.81 mg/kg bw/day	*Rattus norvegicus*	90-d	Broadmeadow (1991b) cited in (EC, 2004) Vol.3 B6, p 200
	Avian toxicity (oral)			
	Effect concentrtaion	Organism	Duration	Reference
	LC50 = 114 mg/kg diet	*Colinus virginianus*	short term (5d)	Gallagher et al., (1999) cited in (EC, 2004) Vol.3 B9, p 518
Fipronil desulfinyl (MB 46513)	Mammalian toxicity (oral)			
	Effect concentrtaion	Organism	Duration	Reference
	LD50 (males) = 18 mg/kg bw LD50 (females) = 15 mg/kg bw LD50 (combined) = 16 mg/kg bw	*Rattus norvegicus*	acute	Dange (1993a) cited in (EC, 2004) Vol.3 B6, p 219
	NOEL = 3 ppm NOEL (males)= 0.23 mg/kg bw/day NOEL (females) = 0.24 mg/kg bw/day	*Rattus norvegicus*	28-d	Dange (1995a) cited in (EC, 2004) Vol.3 B6, p221
	NOEL < 27 ppm NOEL < 1 mg/kg bw/day	*Canis familiaris*	28-d	Dange (1995b) cited in (EC, 2004) Vol.3 B6, p 224
	NOEL = 3 ppm NOEL (males)= 0.177 mg/kg bw/day NOEL (females) = 0.21 mg/kg bw/day	*Rattus norvegicus*	90-d	Dange (1994b) cited in (EC, 2004) Vol.3 B6, p 227
	NOEL = 2 ppm NOEL (males)= 0.32 mg/kg bw/day NOEL (females) = 0.43 mg/kg bw/day		90-d	Bigot (1996) cited in (EC, 2004) Vol.3 B6, p 230

	Effect concentrtaion	Organism	Duration	Reference
	NOEL = 9.5 ppm NOEL (males)= 0.27 mg/kg bw/day NOEL (females) = 0.29 mg/kg bw/day	*Canis familiaris*	90-d	Dange (1996) cited in (EC, 2004) Vol.3 B6, p 231
	NOEL = 0.5 ppm NOEL (males)= 0.028 mg/kg bw/day over 53 weeks NOEL (females) = 0.039 mg/kg bw/day over 53 weeks NOEL (males)= 0.025 mg/kg bw/day over 104 weeks NOEL (females) = 0.032 mg/kg bw/day over 104 weeks	*Rattus norvegicus*	chronic	Bigot (1998) cited in (EC, 2004) Vol.3 B6, p 237
	NOEL (maternal toxicity) = 0.2 mg/kg bw/day NOEL (developmental toxicity) =1.0 mg/kg bw /day	*Rattus norvegicus*	chronic	Foulon (1997) cited in (EC, 2004) Vol.3 B6, p 239
	NOEL = 2 mg/kg bw	*Rattus norvegicus*	chronic	Hughes (1996) cited in (EC, 2004) Vol.3 B6, p 241
	Avian toxicity (oral)			
	Effect concentrtaion	Organism	Duration	Reference
	5.41 mg/kg bw	*Colinus virginianus*	acute	Redersen and Solatycki (1993) cited in (EC, 2004) Vol.3 B9, p 511
	437 mg/kg bw	*Anas platyrhynchos*	acute	Helsten and Solatycki (1994) cited in (EC, 2004) Vol.3 B9, p 512
	110-120 mg/kg diet (2 studies)	*Colinus virginianus*	short term (5d)	Gallagher et al., (1999) and (2000) cited in (EC, 2004) Vol.3 B9, p 516 and p 517