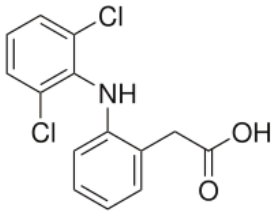
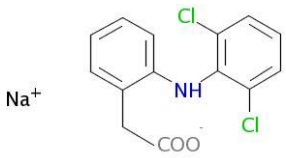
DICLOFENAC

This EQS dossier was prepared by the Sub-Group on Review of the Priority Substances List (under Working Group E of the Common Implementation Strategy for the Water Framework Directive).

The dossier was reviewed by the Scientific Committee on Health and Environmental Risks (SCHER), which made several comments. The SCHER raised questions about the influence of the form of diclofenac in the studies on its solubility and thus on the results of the studies. Information has been added to the dossier. In the course of reviewing the studies, other information was found which has been included in the toxicity tables and discussed in the text. Additional explanation has been given for the use of an additional assessment factor of 10 for the marine EQS. The dossier no longer attempts to propose a MAC. Given the uncertainty regarding the BCF, emphasis is placed for the time being on the $QS_{water,eco}$ *rather than the* $QS_{secpois}$ *The situation will need to be reviewed as more data become available.*

1 CHEMICAL IDENTITY

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

	
Molecular weight (g.mol^{-1})	296.15 318.13 (Diclofenac sodium salt)

Relation Molecular weight Diclofenac / Diclofenac sodium salt = 0.9309. No difference between Diclofenac / Diclofenac sodium salt was made with the effect data. A standardisation of the test results is not made because of the small difference in molecular weight of both compounds.

2 EXISTING EVALUATIONS AND REGULATORY INFORMATION

Annex III EQS Dir. (2008/105/EC)	Not Included
Existing Substances Reg. (793/93/EC)	Liste No -
Pesticides(91/414/EEC)	Not included in Annex I
Biocides (98/8/EC)	Not included in Annex I
PBT substances	No, based on low octanol/water partition coefficient and low plasma levels found in fish exposed to sewage treatment plant effluent containing diclofenac
Substances of Very High Concern (1907/2006/EC)	No
POPs (Stockholm convention)	No
Other relevant chemical regulation (veterinary products, medicament, ...)	Regulated by directive 2001/83/EC, EMEA/CHMP/SWP/4447/00
Endocrine disrupter	Available information / Not investigated

3 PROPOSED QUALITY STANDARDS (QS)

ENVIRONMENTAL QUALITY STANDARD (EQS)

$QS_{freshwater,\ eco}$ is considered the “critical QS” for derivation of an Environmental Quality Standard in view of the uncertainty regarding the calculation from the biota EQS of the corresponding water EQS.

However, there is a residual uncertainty regarding whether the proposed AA-EQS aquatic biocoenosis (freshwater,eco) value is protective for avian predators (secondary poisoning) assuming a biota $QS_{secondary\ poisoning}$ of 1 [µg.kg$^{-1}_{biota\ ww}$]. An indicative QS of 0.007 µg/l was calculated with an AF of 1000 and an

extrapolated whole body BCF of 147 for fish. An assessment factor of 1000 was used, because chronic avian toxicity data are lacking.

	Value	Comments
Proposed AA-EQS for [freshwaters] [µg.L^{-1}] **Proposed AA-EQS for [saltwaters] [µg.L^{-1}]**	0.1 0.01	**See section 7.1**
Proposed MAC-EQS for [freshwater] [µg.L^{-1}] **Proposed MAC-EQS for [marine waters] [µg.L^{-1}]**	insufficient data insufficient data	**See section 7.1**

3.1 SPECIFIC QUALITY STANDARD (QS)

Protection objective*	**Unit**	**Value**	**Comments**
Pelagic community (freshwater)	[µg.l^{-1}]	AA-QS = 0.1	See section 7.1
Pelagic community (marine waters)	[µg.l^{-1}]	AA-QS = 0.01	
Benthic community (freshwater)	[µg.kg^{-1} $_{dw}$]		e.g. EqP, see section 7.1
	[µg.l^{-1}]		
Benthic community (marine)	[µg.kg^{-1} $_{dw}$]		
		-	
Predators (secondary poisoning)	[µg.kg^{-1} $_{biota\ ww}$]	1	See section 7.2
	[µg.l^{-1}]	tentative 0.007 (freshwaters) (marine waters)	
Human health via consumption of fishery products	[µg.kg^{-1} $_{biota\ ww}$]		See section 7.3
	[µg.l^{-1}]	(freshwaters) (marine waters)	
Human health via consumption of water	[µg.l^{-1}]		

4 MAJOR USES AND ENVIRONMENTAL EMISSIONS

4.1 USES AND QUANTITIES

Diclofenac is an active pharmaceutical ingredient (non-steroidal anti-inflammatory drug (NSAID), antiphlogistic) used by patients for the treatment of inflammation and pain predominantly via oral and dermal application.

Sold amounts in Sweden in 2002: 3960 kg (Carlsson 2005).

Use as human pharmaceutical in Germany in 2001: 85800.7 kg (BLAC 2003).

Diclofenac use as human pharmaceutical in Germany in 2001-2009 (Data source: IMS Health MIDAS, 2010) in kg/year

2001	2002	2003	2004	2005	2006	2007	2008	2009

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

67.175,1	88.312,1	93.347,9	83.822,0	92.354,2	88.881,6	90.390,8	91.731,8	91.583,0

4.2 ESTIMATED ENVIRONMENTAL EMISSIONS

5 ENVIRONMENTAL BEHAVIOUR

5.1 ENVIRONMENTAL DISTRIBUTION

		Master reference
Water solubility (mg.l^{-1})	2.37 at 20°C (Diclofenac)	Fini et al., 1993
	1500 at 20°C (Diclofenac sodium)	Caelo 2010
	53100 at 25°C (Diclofenac sodium)	Novartis internal data
Volatilisation		
Vapour pressure (Pa)	6,14 10^{-8} mm Hg 1,59 x 10^{-7} Torr	Neely & Blau, 1993 ACS Daten Bank, 2004
Henry's Law constant (Pa.m^3.mol^{-1})		
Adsorption	**The range - is used for derivation of quality standards.**	
Organic carbon – water partition coefficient (K_{OC})	1450 mL/g (pH=1, calculated) 874 mL/g (pH=4, calculated) 2,30 mL/g (pH=7, calculated) 1 mL/g (pH=8-10, calculated)	ACS Daten Bank, 2004 ACS Daten Bank, 2004 ACS Daten Bank, 2004 ACS Daten Bank, 2004
	Sludge K_{oc} = 47 - 1310 L/Kg Sludge K_{oc} = 31 – 701 cm^3/g Sludge logK_{oc} = 0.78 (from measurements in a STP in Hamburg) Soil K_{oc} = 121.0 - 2310.0 cm^3/g Soil K_{oc} = 200 – 631 L/kg Soil K_{oc} = 107.3 – 167.3 cm^3/g (0-5 cm soil layer Soil K_{oc} = 61.7 – 83.2 cm^3/g (15-25 cm soil layer) Sediment logK_{oc} = 2.45 - 3.74	Ternes, 2004 Urase, 2005 BLAC, 2003 Drillia, 2005 Xu, 2009 Chefetz, 2008 Scheytt, 2005
Suspended matter – water partition coefficient($K_{susp-water}$)	-	
Bioaccumulation	**The BCF value - on fish is used for derivation of quality standards.**	

Octanol-water partition coefficient (Log Kow)	logKow = 4.02 logP = 3.28 ± 0.36 (calculated)	SYRACUSE SCIENCE CENTER, 2002
	logKow = 4.51 (pH ~ 3) logD = 1.31 (pH = 7.4)	Avdeef et al, 1998
BCF (measured)	Fish, 2 d, exposure concentration (520 µg/L) (Diclofenac) Plasma: 5 – 11	Brown, 2007
	Fish, 12 d Plasma: 2.5 – 29 (Diclofenac)	Fick, 2010
	Fish, *Oncorhynchus mykiss,* exposure concentration (1.06 µg/L) Muscle: 69 Gills: 763 Kidney: 971 Liver: 2732	Schwaiger et al, 2004
	Fish, *Oncorhynchus mykiss,* 21 d exposure concentration (0.5 - 25 µg/L) Bile: 440 - 660	Mehinto et al, 2010
	Fish, *Oncorhynchus mykiss,* 10 d Bile: 320 - 950	Kallio et al, 2010
	Estimated whole body BCF at concentration corresponding to the QS biota 147	see section 7.2
	Mussel, *Mytilus edulis trossulus*, exposure (1 µg/l) whole body BCF: 180	Ericson et al, 2010

5.2 ABIOTIC AND BIOTIC DEGRADATIONS

		Master reference
Hydrolysis	DT_{50}= d at °C (distilled water) DT_{50}= d at °C (salt water)	
Photolysis	Rapid degradation of DCF to a level <1% of the initial concentration after 4 days exposure to sunlight (DT50 < 4d)	Buser, 1998
	DT_{50}= 2.4 days (in salt and organic-free water, 50° N in winter)	Andreozzi, 2003
	DT_{50}= 39 min (in natural water and Milli-Q water, 45° N in summer)	Packer, 2003
Biodegradation	$DT_{50\ (type\ of\ water)}$= 5.5 – 18.6 d Significant depletion by sediment microbial activity (93 %	Gröning, 2007

	depletion of diclofenac after 5 days $t_{½}$ = 5.5 – 18.6 days in sediment systems (bench-scale annular flume; flat sediment surface vs moving sediment)	Kunkel, 2008

6 AQUATIC ENVIRONMENTAL CONCENTRATIONS

6.1 ESTIMATED CONCENTRATIONS

Compartment	Predicted environmental concentration (PEC)	Master reference
Freshwater		
Marine waters (coastal and/or transitional)		
Sediment		
Biota (freshwater)		
Biota (marine)		
Biota (marine predators)		

6.2 MEASURED CONCENTRATIONS

Compartment	Measured environmental concentration (MEC)	Master reference
Freshwater	EU database PEC 1 = 0.205 µg/L PEC 2 = 0.237 µg/L	DGEnv, 2010.
EU database, Maximum of the average by station (n=49), year 2003 - 2005	0.71 µg/L	DGEnv, 2010.
Germany, Maximum of the average by station (n=72), year 2008	0.71 µg/L	Umweltbundesamt, 2010
Germany, Maximum of analyses, year 2008	1.7 µg/L	Umweltbundesamt, 2010
Maximum environmental concentration	1,200 µg/L	Boxall, 2008 Knappe, 2007
Marine waters (coastal and/or transitional)	6.2 ng/L in the estuary of the river Elbe, Not detected in samples from different North Sea areas	Weigel, 2002

	Max. 195 ng/l and median of < 8 ng/l in UK estuaries Max. 6 ng/l; median: 3 ng/l (Canada Atlantic coast, receiving untreated sewage)	Thomas, 2004 Comeau, 2008
WWTP effluent	4.7 µg/L 0.25 – 5.45 µg/L	Heberer, 2002 Andreozzi, 2003
Sediment		
Biota	In plasma of rainbow trout exposed to sewage effluents: 12 ng/ml at one site; below limit of detection (<3 ng/ml) at two other sites.	Brown, 2007
Biota (marine predators)		

Further monitoring data for water can be found in Santos et al. (2010). A Limit of Quantification (SPE-HPLC-MS/MS) is 10 ng/l for surface water and sewage is reported with 10 ng/l (Hollender et al. 2009). Modelled and measured loads of wastewater treatment plants (WWTPs) for Diclofenac to river catchments in Switzerland showed, that a value of 0.1 µg/l is exceeded in many river sections (Ort et al. 2009).

7 EFFECTS AND QUALITY STANDARDS

7.1 ACUTE AND CHRONIC AQUATIC ECOTOXICITY

ACUTE EFFECTS			**Master reference**
Algae & aquatic plants ($mg.l^{-1}$)	**Freshwater**	*Desmodesmus subspicatus* / 3 d EC_{50} : 71.9 mg/l (Diclofenac Sodium) Reliability : 2	Cleuvers, 2003
		Desmodesmus subspicatus / 3 d / growth ErC_{50} : 375.6 mg/l EbC_{50} : 135.4 mg/l (Diclofenac Sodium) Reliability : 2	Maletzki, 2010
		Lemna minor / 7 d / growth rate EC_{50} : 7.5 mg/l (Diclofenac Sodium) Reliability : 2	Cleuvers, 2003
	Marine	*Gender species* / d or h EC_{50} :	
Invertebrates ($mg.l^{-1}$)	**Freshwater**	*Daphnia magna* / 2d EC_{50} : 22.43 mg/L (Diclofenac Sodium)	Ferrari, 2003
		Daphnia magna / 48 h EC_{50} : 68 mg/l (Diclofenac Sodium)	Cleuvers, 2003
		Cerodaphnia dubia / 48 h EC_{50} = 22.7 mg/l (Diclofenac Sodium)	Ferrari, 2003 & 2004
	Marine	*Gender species* / d or h EC_{50} :	
	Sediment	*Gender species* / d or h EC_{50} :	
Fish ($mg.l^{-1}$)	**Freshwater**	*Danio rerio* / 4d EC_{50} : 0.09 mg/L Teratogenicity Reliability : 4	Dietrich &,Prietz, 1999
		Danio rerio / 4d LC_{50} : 0.480 mg/L Reliability : 4	Dietrich &,Prietz, 1999

		Danio rerio / 96 h LC_{50} : 82 mg/l Reliability : 4	Novartis internal data
	Marine	*Gender species* / d or h EC_{50} :	
	Sediment	*Gender species* / d or h EC_{50} :	
Other taxonomic groups		*Vibrio fisheri* **/** 0.5 h EC_{50} : 11.454 mg/L (Diclofenac Sodium)	Ferrari, 2003

CHRONIC EFFECTS			**Master reference**
Algae & aquatic plants (mg.l^{-1})	**Freshwater**	*Pseudokirchneriella subcapitata* / 4d NOEC：10 mg/l	Ferrari, 2003
		Desmodesmus subspicatus / 3 d / NOEC : 25 mg/l (Diclofenac Sodium) Reliability : 2	Maletzki, 2010
		Lemna minor / 7 d / growth rate EC_{50} : 7.5 mg/l (Diclofenac sodium) Reliability : 2	Cleuvers, 2003
	Marine	*Gender species* / d NOEC：	
Invertebrates (mg.l^{-1})	**Freshwater**	*Ceriodaphnia dubia* NOEC：1mg/L (Diclofenac Sodium)	Ferrari, 2003 & 2004
	Marine	*Gender species* / d NOEC：	
	Sediment	*Gender species* / d NOEC：	
Fish (mg.l^{-1})	**Freshwater**	***Oncorhynchus mykiss* 28 d**, histopathological lesions, gills and kidney (Diclofenac) **NOEC：0.001 mg/L** Reliability : 2	Schwaiger et al, 2004
		Oncorhynchus mykiss 28 d, Cytological effects in gills, liver and kidney **LOEC**：0.001 mg/L calculated NOEC 0.0005 mg/L (Diclofenac) Reliability : 2	Triebskorn et al, 2004,
		Oncorhynchus mykiss 28 d, ultrastructural lesions in gills, liver and kidney **LOEC**：0.001 mg/L calculated NOEC 0.0005 mg/L (Diclofenac) Reliability : 2	Triebskorn et al. 2007
		Oncorhynchus mykiss 21 d, histopathological lesions, kidney NOEC：0.001 mg/L Reliability : 2	Mehinto, 2010

		Oncorhynchus mykiss 21 d, histopathological lesions, kidney NOEC：0.0005 mg/L Prostaglandinsynthesismodulation in cox 1 and cox 2 for liver, gills and kidneys which are also involved in the inflammation response Reliability : 2 Diclofenac	Mehinto, 2010
		Salmo trutta / 21 days NOEC : liver effects (monocyte infiltration/accumulation which are also involved in the inflammation response) 0.0005 mg/l (Diclofenac Sodium) Reliability : 2	Hoeger, 2005
		Danio rerio / 10 d (ELS) / mortality NOEC: 4 mg/l (Diclofenac Sodium) Reliability : 3	Ferrari, 2003 & 2004
	Marine	*Gender species* / d NOEC：	
	Sediment	*Gender species* / d NOEC：	
Other taxonomic groups		Brachionus calyciflorus (Diclofenac Sodium) NOEC : 12. 5 mg/L	Ferrari, 2003

Tentative QS_{water}	Relevant study for derivation of QS	Assessment factor	Tentative QS
$MAC_{freshwater, eco}$			
$MAC_{marine\ water, eco}$			
$AA\text{-}QS_{freshwater, eco}$	*Oncorhynchus mykiss* / 28 days NOEC : 1.0 µg/l	10	0.1 $\mu g.l^{-1}$
$AA\text{-}QS_{marine\ water, eco}$		100	0.01 $\mu g.l^{-1}$
$AA\text{-}QS_{freshwater, sed.}$	-	EqP	- $\mu g.kg^{-1}_{ww}$ - $\mu g.kg^{-1}_{dw}$
$AA\text{-}QS_{marine\ water, sed.}$	-	EqP	- $\mu g.kg^{-1}_{ww}$ - $\mu g.kg^{-1}_{dw}$

There are NOEC-values for groups of aquatic organisms like algae, crustaceans, fish and rotifers (see table above). The lowest measured for brown trout *Salmo trutta f. fario* with a NOEC of 0.5 µg/L was determined in the Hoeger et al. 2005 study. This NOEC is based on effects in liver (monocyte infiltration/accumulation) that did not exhibit a statistical concentration-response relationship and were not present at the high concentrations tested. Therefore it is not used for EQS derivation.

Triebskorn et al (2004) found cytological effects at 1 µg/L, the lowest concentration tested, in liver, kidney, gills and intestine in rainbow trout (*Oncorhynchus mykiss)* after 28 day exposure and confirmed these results by an ultrastructural analysis in a second publication (Triebskorn et al. 2007). The lowest concentration caused effects of between 10 and 20% compared to the control values. According to the TGD-EQS (EC 2011) a NOEC can be calculated by dividing the LOEC value by a factor of 2, deriving a NOEC of 0.5 µg/L.

Mehinto et al.(2010) found in *Oncorhynchus mykiss* a NOEC of 1 µg/L for histopathology and several immunorelevant genes were modulated at this concentration level in liver, kidneys and gills, leading to lower NOECs of 0.5 µg/L.

For the same species a NOEC (adverse histological effects, necrosis in kidney and gills) of 1 µg/L was determined in a prolonged 28 d fish study by Schwaiger et al, 2004). The three studies (Schwaiger et al 2004; Triebskorn et al. 2004, and Triebskorn et al. 2007) used 0.12 ‰DMSO to dilute Diclofenac in the test system. This is slightly above the upper limit of what is allowable according to the OECD guidelines, including the OECD 305 guideline. However, DMSO is a frequently used solvent in these kinds of tests. Further, both a control and a solvent control were applied and there was no difference between them. Such low amounts of solvents do not interfere with the bioaccumulation process and will not cause any harmful effects to fish. In a similar study a NOEC of 0,5 µg/l was calculated on the basis of a LOEC of 1.0 µg/l for histopathological lesions in the kidney observed in a 21 d study with juvenile female rainbow trout by Mehinto et al. 2010, without using DMSO.

Although histological effects are not a standard endpoint, the effects are regarded as relevant, because they may induce effects on reproduction or growth in a full life cycle fish test. This is discussed further below.

On the basis of the Triebskorn et al (2004, 2007) study, a calculated NOEC of 0.5 µg/l could be used to derive the $EQS_{freshwater,eco}$. Indeed, in addition to the measured NOEC of 0.5 µg/l from Hoeger et al (2006), there are other measured LOECs and NOECs at or below 1 µg/l, at least relating to ultrastructural or immunotoxicological effects (e.g. Triebskorn et al. and Mehinto et al. 2010) that could be population relevant.

Although it may not be protective enough, because higher than the NOECs for some other endpoints, this dossier selects for the time being the measured NOEC of 1.0 µg/l for necrosis in rainbow trout (*Oncorhynchus mykiss*) from the Schwaiger et al (2004) study. For the calculation of the EQS in accordance with the TGD-EQS and Appendix 5 WRRL (2000/60/EG) a safety factor of 10 is applied..

Calculated **$AA\text{-}QS_{freshwater, eco}$**: 1.0 µg/L / 10 = 0.1 µg/L

However, noting that this could be underprotective, it is suggested that the EQS be reviewed at the time of the next priority substance review taking into account the LOECs and any other reliable studies produced in the meantime,

The use of histopathological (including ultrastructural) and immunotoxic effects as critical data for EQS setting is sometimes disputed. However, in the absence of other (sub)chronic studies, neither the population relevant effects of histopathological alterations nor a potential for bioaccumulation can be excluded. Although a direct correlation cannot always be established for effects in the field, e.g. due to multiple stressors, population relevance is very likely because:

a) histopathological damage of kidneys at environmental concentrations makes infections more likely.

b) immune functions are modulated at the same and lower concentration levels, which means an inadequate immune response to pathogens. That could mean a higher infection rate, oxidative stress, loss of immune functions and also a higher cancer risk. So a reduction in survival rate and fitness is likely.

Evidence for population relevance of histopathological effects comes from the following:

a) In Switzerland, a 60 % decline in the trout population has been observed, and 41% of the rest of the surviving trout are infected with a proliferative kidney disease caused by parasites, meaning a bad health status, which indicates also a reduction in fitness (Burkhardt-Holm et al. 2005).

b) Modelled immune suppression in the Chinook salmon produced the greatest changes in the population growth rate (Spromberg and Meador 2005),

Other chemical stressors (e.g.estrogenic substances),and additional environmental and endogenic factors cannot be neglected in the field, and may make it difficult to establish a clear correlation to only one stressor. However, the line of evidence for diclofenac (at least 4 studies showing histopathological effects, relevant exposure concentrations, population decline in Swiss rivers linked with kidney disease) supports a link between the histopathological and population effects.

To derive an AA-QS$_{marine\ water,\ eco}$ an AF of 100 is applied to the same NOEC, as follows, there being no chronic studies with marine fish species or other salt water species available.

Calculated **AA-QS$_{marine\ water,\ eco}$**: 1.0 µg/L / 100 = 0.01 µg/L

Scientifically there is normally no obvious reason, not to use endpoints of marine species for the environmental risk assessment or vice versa. [See the US EPA Guideline OPPTS 850.1075 (1996), the final US EPA EA for Diazinon (US-EPA, 1995),] Hutchinson et al. (1998) and Wheeler et al. (2002). The only exceptions are metals which are ionized in salt water. The differences found in the literature between freshwater and marine species are mainly due to different taxonomic levels and the respective developmental and physiological differences. Therefore it can be assumed, that marine fish species will not be more sensitive than freshwater fish species.

But with no data at all for species like echinoderms, or molluscs, an extra Assessment Factor of 10 is proposed, as it is not clear that fish are indeed the most sensitive group. Tissues of mussels (which are filter-feeders and filter large amounts of water like fish gills) might be very sensitive to diclofenac as well.

In section 2.9.1 the TGD-EQS (EC 2011) says. " It follows that uncertainty may be increased if data for sensitive taxa are missing when dealing with substances with a specific mode of action like insecticides, herbicides or antibiotics… " This is consistent with the provisions of REACH for marine effects assessment where a larger AF is recommended to cover the increased uncertainty resulting from the larger diversity of marine ecosystems and the limited availability of effects data for marine life forms.

Because the acute fish data are of low (or uncontrolled) reliability, and fish are the most sensitive species in the chronic dataset, the data set on acute effects is regarded as insufficient for the derivation of MAC-QS values.

7.2 SECONDARY POISONING

Secondary poisoning of top predators		**Master reference**
Mammalian oral toxicity	Mouse / Oral /	Caleo 2010

LD50: 140-390 mg.kg^{-1}(Diclofenac sodium)

	Reliability : 4 NOEC : mg.kg$^{-1}_{biota\ ww}$ (CF=)	
	Baboon / Oral / 12 months / Endponit not specified) Reliability : 4 LOAEL : 5 mg.kg$^{-1}_{bw}$.d^{-1}	Novartis internal data
Avian oral toxicity	*Gyps bengalensis* / Oral / Single dose / Mortality LD 50 : 0.225 mg.kg$^{-1}_{bw}$.d^{-1} LC 50: 1 mg.kg$^{-1}_{feed\ ww}$	Green et al, 2007 Swan et al, 2006
	Columba livia / Oral / 7 d Clinical signs (depression, somnolence, sitting on hocks with closed eyes, reduced feed and water intake) LOAEL: <= 0.25 mg.kg$^{-1}_{bw}$.d^{-1}	Hussain et al, 2008
	Columba livia / Oral / 7 d Mortality post treatment (7d) and body weight LOAEL (20%) : <= 0.25 mg.kg$^{-1}_{bw}$.d^{-1} LOEC : <=2.5 mg.kg$^{-1}_{feed\ ww}$ (CF= 10)	Hussain et al, 2008
	Gallus gallus / Oral / 7 d body weight post treatment (Day 21d) LOAEL : <= 0.25 mg.kg$^{-1}_{bw}$.d^{-1} LOEC : <=2.5 mg.kg$^{-1}_{feed\ ww}$ (CF= 10)	Hussain et al, 2008

Tentative QS$_{biota}$	**Relevant study for derivation of QS**	**Assessment factor**	**Tentative QS**
Biota	LC50 : 1 mg.kg$^{-1}_{biota\ ww}$	1000	1 µg.kg$^{-1}_{biota\ ww}$ corresponding to 0.007 µg.L^{-1} (freshwater) - µg.L^{-1} (marine waters)

Relevant BCF trigger is met. Secondary poisoning should be evaluated, because a high avian toxicity has been observed. But only a tentative QS can be derived, because chronic or subchronic test results are lacking.

Residual uncertainty:

- Whole body BCF at low concentrations might be underestimated.
- No information on chronic toxicity is available..

Five studies are available describing the uptake of diclofenac from the aquatic environment in fish. Two of the studies looked at the concentration in plasma after rainbow trout were exposed to effluents of sewage treatment plants for periods lasting at least 12 days (Fick et al 2010, Brown et al 2007). The exposure concentrations in these experiments fluctuated with the changes in effluent composition. In the study by Brown et al (2007) fish were also exposed to a high concentration of diclofenac (520 µg/L) together with other pharmaceuticals with short exposure duration of 48 hours. In the studies the BCF in the effluent studies ranges from 2.5 to 29 L/kg. The BCF from the short-term laboratory study was 7. The most important limitation on the use of these values for the assessment of secondary poisoning, is that these BCF values are not representative of whole body BCF values. This is also reflected by the regression equation that the authors present for blood-water partitioning, which is less than 20% of the value that is estimated for whole body BCFs. The study performed by Zhang et al. (2010) using space-resolved solid-phase microextraction in the adipose fin and muscle shows, that Diclofenac did not bioconcentrate, but the study is unacceptable to derive a whole body BCF for fish

Another study investigated the accumulation of diclofenac in the bile of rainbow trout after 21 days exposure at different exposure concentrations (Mehinto et al 2010). The BCF values for bile ranged from about 440 to 660 L/kg. However, these BCF values are again not representative of whole body concentrations.

The only accumulation study in which fish muscle and other organs were measured is the study by Schwaiger et al (2004). In this study, rainbow trout were exposed to diclofenac at concentrations ranging from 1 to 500 µg/L in a flow-through system. DMSO was used at a concentration that was at maximum 0.12 promille. This is not significantly higher than the value recommended by the OECD (0.1 promille). DMSO is not in the list of possible solvents to be used from the OECD 205. However, DMSO is quite routinely used in toxicity testing. Yakata (2006) found that DMSO and other studied dispersants at a concentration of 0.1 promille neither hinders the intake of the test substance into fish nor results in underestimation of the bioconcentration potential. Although the study used a high fish loading of 40 g fish per liter, the exposure concentrations were closely maintained. The concentrations were measured in gills, muscle, liver and kidney. The accumulation in all organs was strongly dependent on the aquatic exposure concentration, reaching plateau levels at high concentrations (BCF is approximately inversely correlated with exposure concentration). The reason for this is unknown. In the study it is shown that at all concentrations except the lowest the gills are affected by diclofenac. This might have caused a lower uptake of diclofenac by the gills. Another explanation might be that at higher concentrations metabolism of diclofenac is induced, resulting in more effective elimination from the body. BCFs are mentioned for the lowest and highest concentrations and are presented in a figure for the concentrations in between. An overview of these BCF values is given in the table below.

BCF values for muscle, gills, kidney, and liver determined at different exposure concentrations

Exposure concentration [µg/L]	muscle	gills	kidney	liver
1.06	69	763	971	2732
4.95	26.6667	213.333	293.333	680
20.13		66.6667	93.3333	213.333
100.9			26.6667	53.3333
501.2	0.3	3	5	12

To estimate a whole body BCF from these values, the contribution of these organs to the whole body weight must be known. Hoeger et al (2008) report a value of 60% for the weight of muscle tissue relative to the total body weight. In the study by Garnier-Laplace et al (2000) regressions are presented to estimate these percentages from the body weight of the fish. With a body weight of 167.6 g as used by Schwaiger et al (2004), the resulting percentage muscle relative to the whole body weight is 61.9%. The two values are in good accordance and so the latter value for the specific weight of the used fish is selected. For gills, only the study by Garnier-Laplace et al (2000) presents information to estimate the percentages of the total body weight of the fish. The estimated value is 3.2%. For kidney, the regression presented in the same study results in a relative weight of only 0.06%, possibly due to the uncertainty of the extrapolation to larger fish. From the study by Hoeger et al it (2008) it appears that kidney and gills contain almost the same amount of diclofenac 36 hours after an intraperitoneal injection with diclofenac. Given the BCF values for gills and kidney this would imply a similar relative weight of kidney and gills. For liver, there are several estimates of the relative weight compared to the total body weight. Denton & Yousef (1976) report a regression based on fish weight, that results in 1.44%. A similar equation by Schmelzing and Claus (1990) leads to 1.34-1.37%. Literature data reviewed by Schmelzing and Claus (1990) vary from 1.33-165%. Only the data presented by Garnier-Laplace et al (2000) deviate from this and end up at 3.00% for fish of 167.6 g. The data might be extrapolated to far to give a reasonable estimate. The value of 1.44% has been selected for further calculations.

The whole body BCF is then calculated from the sum of the relative weights times the BCF values of these four tissues. Because the sum of the relative weights is only 67%, the estimate of the whole body BCF must be considered as an underestimate of the real whole body BCF. From the study by Hoeger et al (2008) it appears that by far the largest proportion of diclofenac is recovered from the intestines. However, this was after an intraperitoneal injection and it is not clear how this affect the overall distribution. The BCF values estimated at 1, 5, and 500 µg/L are 107, 33, and 0.45 L/kg.

Several toxicity studies with diclofenac are available, most notably the studies with vultures as a result of the massive intoxication on the Indian subcontinent. A single oral dose of diclofenac resulted in an LD50 of 225 µg/kg body weight for the Oriental white-backed vulture (*Gyps bengalensis*) (Green et al 2007, Swan et al 2006 based on the data from Oaks et al 2004). In this study, 20 vultures were either administered diclofenac orally (at doses of 2.5 and 0.25 mg/kg body weight) or fed tissues from goats or buffaloes treated with diclofenac, a few hours before slaughter (resulting doses ranged from 0.007 to 0.940 mg/kg body weight). All control birds (two for the oral dose and six for the dosing via meat) survived. The LD50 was calculated by removing one outlier that died despite of a very low dose of diclofenac. Histopathological examination showed that the bird had low uric acid concentration in the plasma, comparable with the other birds that received low doses. Otherwise the LD50 would have been 98 µg/kg body weight.

Swan et al (2006) examined if the European Griffon vulture (*Gyps fulvus*) and the African white-backed vulture (*Gyps africanus*) were equally sensitive. Two African white-backed vultures and three Griffon vultures received a single dose of 800 µg/kg body weight and died within two of dosing, while all controls survived. A similar experiment was repeated by Naidoo et al (2009) with Cape Griffon Vulture (*Gyps coprotheres*). Both birds died after receiving a dose of 800 µg/kg body weight. These experiments confirmed the general susceptibility of all *Gyps* species to diclofenac.

To examine if American vultures would be equally sensitive as Eurasian vultures, Rattner et al (2008) exposed Turkey vultures (*Cathartes aura*) to increasing concentrations of Diclofenac. Two control vultures were included and eight vultures were exposed to concentrations ranging from 0.08 mg/kg to 2.5 mg/kg body weight. All vultures survived the observation period of seven days. After three weeks, five previously exposed vultures were given a single oral dose of 2.5 to 25 mg/kg body weight, with inclusion of one extra control vulture. No mortality occurred and there were no signs of overt toxicity. Apparently, this species is much less sensitive to diclofenac than the species from the *Gyps* genus. This lower sensitivity goes hand in hand with lower uric acid levels in the plasma of Turkey vultures dosed with diclofenac in comparison with species from the *Gyps* genus.

Four other types of birds were tested in a study by Hussain et al (2008). Broiler chicks (*Gallus gallus*, 15 days old), pigeons (*Columba livia*, 3 months old), Japanese quail (*Coturnix japonica*, 4 weeks old) and mynah (*Acridotheres tristis*, independent young) were orally exposed to diclofenac at dose rates of 0 (control), 0.25, 2.5, 10 and 20 mg/kg body weight, for seven consecutive days. Mortality was observed until two weeks after exposure ended. The LD50 calculated with a log-logistic model from the presented results was 4.1 mg/kg body weight/day for broiler chicks. For pigeons this value was 15.6 mg/kg body weight/day. For Japanese quail and mynah there was an onset of toxicity at the two higher dosages, but the LD50s were higher than 20 mg/kg body weight for these species. For broiler chicks and pigeons, the LD50 was accompanied by a significant reduction in body weight at all doses.

Other studies with chicken resulted in similar or slightly higher LD50s. Naidoo et al (2007) applied single intramuscular doses to hens of 18 weeks of age at five dosages of 0.6 to 10 mg/kg body weight. The LD50 was 9.8 mg/kg body weight. Assuming 50% oral bioavailability, this would be equivalent to an oral dose of 19.6 mg/kg body weight. Reddy et al (2006) applied a single intramuscular dose of 5 mg/kg body weight in poultry of both sexes of 6 weeks of age. 40% mortality occurred. At the same dose in the study by Naidoo et (2007) 33% mortality occurred. In a recent study with 6-week-old White Leghorns, diclofenac was administered at oral doses of 2 and 20 mg/kg body weight (Jain et al 2009). In the control group and 2 mg/kg body weight dose all six birds survived. At 20 mg/kg body weight, 3 out of six birds died within twelve hours. Apparently, the repeated dose for 7 consecutive days causes the LD50 to be about a factor of 5 lower than the LD50s from single dose studies.

The experiments with vultures are all performed as single dose studies. The Oriental white-backed vulture has a body weight of 4.75 kg and consumes 1.023 kg meat per meal. This meal is sufficient for the vultures for a period of three days (Swan et al, 2006; Green et al 2006, 2007). The assessment factor of 3000 from the TGD applies to the LC50 from an OECD 205 test, which is for an exposure of five days via food. The single oral doses correspond better to the draft OECD guideline 223. The TGD states that acute lethal doses are not acceptable for extrapolation to chronic toxicity, as these are not dietary tests. However, at the same time, the TGD states that a dose from an avian study can be expressed as a concentration in food for the purpose of secondary poisoning. The experiment with Oriental white-backed vultures is mainly a diet study, because the vultures were fed with contaminated meat. Expressed as dose, there was no obvious difference between the vultures that were exposed orally or via the diet. The difference between the vultures and birds normally used in toxicity testing is that the latter group more or less eats continuously over time, while the vultures consume only one meal over three days.

An overview of the derived LD50s with equivalent concentrations in food is presented in the table below. The LD50 for vultures is clearly the most critical endpoint, although it is useful to realize that at similar doses (0.25 mg/kg_{bw}/d), reduced body weight was observed for broiler chicks and juvenile pigeons. It appears that chicken, although taxonomically not closely related to the vultures, are rather sensitive to diclofenac as well. In contrast, another genus of vultures appears to be rather insensitive. It is important to note that it has been suggested that not only vultures, but also raptors, storks, cranes and owls may be very sensitive to non-steroidal anti-inflammatory drugs (NSAID), including diclofenac (Cuthbert et al 2007).

Species name	Scientific name	LD50 [mg/kg_{bw}/d]	LC50 [mg/kg feed]
Orientak white-backed vulture	*Gyps bengalensis*	0.225	1.0
Gyps fulvus	*Griffon vulture*	<0.80	
African white-backed vulture	*Gyps africanus*	<0.80	
Cape Griffon Vulture	*Gyps coprotheres*	<0.80	
Turkey vultures	*Cathartes aura*	>25	
Chicken	*Gallus gallus*	4.1	32.8
Pigeon	*Columba livia domestica*	15.6	
Japanese quail	*Coturnix japonica*	>20 (55)	
Mynah	*Achridotheres tristis*	>20 (55)	

With a meat consumption of 1.023 kg per meal and an average weight of 4.75 kg for Oriental white-backed vulture, the concentration in food is 1043 µg/kg food. Because the tested species can be considered as key indicator wildlife species and the type of food and food consumption are already specific for the species, an assessment factor of 1000 may be applied instead of 3000. The $QS_{biota,\ sec\ pois}$ thus becomes 1 µg/kg.

With a bioconcentration factor of 147 the corresponding $QS_{water,\ sec\ pois}$ is 0.007 µg/L.

A similar $QS_{biota,\ sec\ pois}$ 3,3 µg/kg can be calculated based on the sublethal effect data for chicken body weight LOAEL : <= 0.25 $mg.kg^{-1}_{bw}.d^{-1}$ (Day 21, post treatment of short-term study performed by Hussain et al (2008); a short-term NOAEL of <= 0.125 $mg.kg^{-1}_{bw}.d^{-1}$ can be extrapolated). With a CF = 10 and AF = 300 $QS_{biota,\ sec\ pois}$ 3.3 µg/kg can be calculated. The corresponding $QS_{water,\ sec\ pois}$ would be 0.023 µg/L.

As noted earlier, no chronic or sub chronic study for trout covering population relevant endpoints (e.g. growth, reproduction) is available. Furthermore, no fully accepted test on bioaccumulation for rainbow trout (OECD 305) is available.

The study by Schwaiger used 0.12 ‰ DMSO to dilute diclofenac in the test system. This is slightly above the upper limit of what is recommended in OECD guidelines, including the OECD guideline 305 („Bioconcentration: Flow-through Fish Test"). However, DMSO is a frequently used solvent in these kinds of tests and hence the use of DMSO is generally permissible. Furthermore, it has to be pointed out that both a negative control and a solvent control were applied, with no significant difference between the negative and the solvent control. This is backed by Mehinto et al. 2010, who found similar histopathological effects in the same concentration range without DMSO.

In a very recent publication Richards et al (2011) were able to detect diclofenac in the fur of wild Eurasian otter (*Lutra lutra*), indicating an uptake of diclofenac by fish-eating mammals. Although these results are qualitative and need further confirmation, they are an indication that secondary poisoning should not be ignored.

Comparison of the AA-$QS_{,\ sec\ pois}$ of 0.007 µg/L with the AA-$QS_{freshwater,eco}$ of 0.1 µg/L leads to the conclusion that the critical QS is the secondary poisoning EQS. However, this value could be subject to refinement on the basis of a whole-body fish BCF assessment and a chronic avian test. The TGD-EQS notes that long-term studies establishing long-term NOECs are preferred, and that if the QS for water lies within the range of possible extrapolated values of the QS for biota, when considering the uncertainties of the extrapolation, it is not possible to determine with high confidence which is the 'critical' QS. For this reason, the freshwater,eco QS is proposed for the time being as the critical EQS.

7.3 HUMAN HEALTH

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

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

Human health via consumption of drinking water		**Master reference**
Existing drinking water standard(s)	no preferred regulatory standard	
Any guideline		

Not evaluated

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