Environmental Risk Assessment Summary
Saquinavir

Introduction

The publication of environmental risk assessment (ERA) summaries is part of Roche's engagement on developing a better understanding of issues regarding pharmaceuticals in the environment (PiE).

New pharmaceutical substances are investigated for biodegradability and initial ecotoxicity during their development. For registration, a full state-of-the-art environmental risk assessment is developed based on chronic environmental effects and advanced environmental fate data, as required by the pertinent regulations. While not a regulatory requirement, Roche also investigates older pharmaceutical substances, normally at a simpler scale, in order to assess their environmental risks.

For active pharmaceutical ingredients, the potential environmental risk is calculated from the ratio between the predicted environmental concentration (PEC) of the substance in the aquatic environment based on a conservative emission scenario and the predicted no effect concentration (PNEC), a concentration below which no adverse effects on the environment have to be expected.

Summary

Saquinavir mesylate is the active pharmaceutical ingredient in the Roche product Invirase [16]. Invirase is indicated for the treatment of HIV-1 infected adult patients. Invirase should only be given in combination with Ritonavir and other antiretroviral medicinal products [14].

Saquinavir free base was the active pharmaceutical ingredient in the Roche product Fortovase. Fortovase was withdrawn from the market in 2006 [12].

Saquinavir is essentially completely metabolised by cytochrome P450, with the specific isoenzyme CYP3A4. Ritonavir inhibits the metabolism of Saquinavir, thereby increasing ("boosting") the plasma levels of Saquinavir. Renal clearance is only a minor elimination pathway, the principal route of metabolism and excretion for Saquinavir being via the liver [14].

Results from standard tests suggest that both, Saquinavir mesylate and Saquinavir free base, are not readily biodegradable [2] [28]. In a sediment/water fate test ^{14}C-Saquinavir free base could not be mineralised to $^{14}CO_2$ within 84 days of incubation [10]. However, Saquinavir free base dissipated from the water phase with a half live ($DT_{50,\ water}$) of 1.45 days with respect to the total radioactive residues (TRR) measured at day 0. At the end of the test equilibrium was reached, resulting in approximately 50% of activity in sediment extracts and in bound residues [10]. In a study with suspended solids in estuarine waters the calculated DT_{50} for the dissipation from water in systems with untreated wastewater and high suspended solids concentration were 4.1 and 3.5 days, respectively. The DT_{50} in $HgCl_2$ sterilised systems was 5 days indicating that the observed dissipation was mainly due to adsorption processes [1].

The PEC/PNEC ratio is 0.0012. With reference to the Guideline on the Environmental Risk Assessment on Medicinal Products for Human Use of the European Medicines Agency [13], a PEC/PNEC ratio of <1 means that Saquinavir and/or its metabolites are unlikely to represent a risk to the aquatic environment.

Predicted Environmental Concentration (PEC)

The PEC is based on the following data:

$PEC\ (\mu g/L) = (A \times 10^9 \times (1–R)) \div (365 \times P \times V \times D)$

A Total patient consumption in the European country with the highest yearly per capita use in the period 2013–2017 (data from IQVIA [19]
R Removal rate during sewage treatment (default value) = 0 [13]
P Number of inhabitants in the country with the highest per capita use in the respective year of the period 2013–2017 [15]; resulting in a consumption of 8.84 mg/y per inhabitant
V Volume of wastewater per inhabitant and day (default value) = 200 L day^{-1} [13]
D Dilution factor of wastewater by surface water flow (default value) = 10 [13]

PEC = 0.012 µg/L

Note: Saquinavir is at least partially metabolised in the body. Since little is known about the ecotoxicity of these metabolites, it is assumed as a worst case that they have the same ecotoxicological relevance as Saquinavir.

Predicted No Effect Concentration (PNEC)

Acute studies have been performed with Saquinavir mesylate and Saquinavir free base based on OECD Test Guidelines [21] for species from three trophic levels (green algae, invertebrates, fish). The lowest EC50 is >10.2 mg/L with respect to growth rate for Saquinavir mesylate with the green alga *Raphidocelis subcapitata* [7] [22]. Applying an assessment factor of 1000 according to the REACH Guidance [11], this results in a PNEC value of 10.2 µg/L.

PNEC = 10,200 µg/L ÷ 1000 = 10.2 µg/L

PEC/PNEC Ratio

PEC = 0.012 µg/L
PNEC = 10.2 µg/L

PEC/PNEC = 0.0012

With reference to the Guideline on the Environmental Risk Assessment on Medicinal Products for Human Use of the European Medicines Agency [13], a PEC/PNEC ratio of 0.0012 (i.e. <1) means that Saquinavir and/or its metabolites are unlikely to represent a risk to the aquatic environment.

Aquatic Toxicity Data for Saquinavir mesylate (SQV-M) and Saquinavir Free Base (SQV-B)

Study	Guideline	Results	Test item	Ref.
Growth inhibition test with green algae(*Raphidocelis subcapitata*)	FDA 4.01 (OECD 201)	72 h EC50 (growth rate) >10.2 mg/L GMC 72 h EC50 (yield) 5.82 mg/L GMC 72 h EC10 (growth rate) 2.11 mg/L GMC 72 h EC10 (yield) 0.925 mg/L GMC 72 h NOEC 1.12 mg/L GMC	SQV-M	[7], [22]
Growth inhibition test with green algae (*Desmodesmus subspicatus*)	OECD 201	72 h EC50 (growth rate) >100 mg/L NC 72 h EC50 (yield) >100 mg/L NC 72 h NOEC 14.7 mg/L TWM HTC	SQV-B	[3]
Acute immobilisation test with *Daphnia magna*	FDA 4.08	48 h EC50 >100 mg/L NC 48 h NOEC 36.0 mg/L NC	SQV-M	[23]
	OECD 202	48 h EC50 >100 mg/L NC 48 h NOEC 17.9 mg/L TWM HTC	SQV-B	[4]
Acute toxicity to rainbow trout (*Oncorhynchus mykiss*)	FDA 4.11	96 h LC50 >50 mg/L NC 96 h NOEC 38.8 mg/L MMC HTC	SQV-M	[24]
Acute toxicity to Guppy (*Poecilia reticulata*)	OECD 203	96 h LC50 >100 mg/L NC 96 h NOEC 5.49 mg/L TWM HTC	SQV-B	[5]
Activated sludge, respiration inhibition test	OECD 209	3 h EC50 >58.8 mg/L NC 3 h NOEC 58.8 mg/L NC HTC	SQV-M	[25]
Microbial growth inhibition	FDA 4.02	MIC 312 mg/L NC, *Anabaena flos-aquae* MIC >312 mg/L NC, *Aspergillus niger* MIC >312 mg/L NC, *Azotobacter vinelandii* MIC >312 mg/L NC, *Fusarium acuminatum* MIC >312 mg/L NC, *Pseudomonas putida*	SQV-M	[26]
Toxicity to nitrifying bacteria	ISO 9509	4 h EC50 >100 mg/L NC 4 h NOEC 37.5 mg/L IMC HTC	SQV-B	[6]
Toxicity to earthworm	FDA 4.02	LC50 >686 mg/kg soil MMC NOEC 686 mg/kg soil MMC HTC	SQV-M	[27]

EC10 Concentration of the test substance that results in 10% effect
EC50 Concentration of the test substance that results in 50% effect
NOEC No observed effect concentration
GMC Geometric mean measured concentration
HTC Highest tested concentration
IMC Initial measured concentration
NC Nominal concentration
TWM Time-weighted mean measured concentration

Environmental Fate Data for Saquinavir mesylate (SQV-M) and Saquinavir Free Base (SQV-B)

Study	Guideline	Results	Test item	Ref.
Ready biodegradability	FDA 3.11 (OECD 301 B)	CO2 production (mineralisation) <4% after 28 d	SQV-M	[28]
	OECD 301 F	BOD ÷ ThOD (mineralisation) 0% after 28 d DOC elimination 84% after 28 d Short-term and long-term adsorption 22% after 24 h, measured by LC-UV 52% after 28 days, measured by LC-UV	SQV-B	[2]
Sediment/water fate test	NA	$DT_{50, water}$ 1.45 days $DT_{75, water}$ 5.8 days $DT_{90, water}$ 16 days 100% of initially measured TRR in sediment after 84 days of incubation (approx.. 50% of in sediment extracts and in bound residues, respectively)	SQV-B	[10]
Sorption in estuarine waters	NA	$DT_{50, water}$ 4.1 days (untreated wastewater) $DT_{50, water}$ 3.5 days (high suspended solids concentration) $DT_{50, water}$ 5 days ($HgCl_2$ sterilised)	SQV-B	[1]
Hydrolysis	NA	0% after 5 days (deionised H_2O, 22 °C)	SQV-B	[2]
Photodegradation	NA	0% after 5 days (deionised H_2O, 22 °C)	SQV-B	[2]
Adsorption/Desorption to soil	OECD 106	K_{OC} 10692 L/kg (silt loam) K_{OC} 22919 l/kg (clay loam) K_{OC} 13711 L/kg (loam) immobile in soil	SQV-M	[29]

BOD	Biochemical oxygen demand
CO_2	Carbon dioxide produced
DOC	Dissolved organic carbon
DT_{50}	Half life
ThOD	Theoretical oxygen demand
TRR	Total Radioactive Residues

Physical Chemical Data for Saquinavir mesylate (SQV-M) and Saquinavir Free Base (SQV-B)

Study	Guideline	Results	Test item	Ref.
Water solubility	NA	2200 mg/L (25 °C)	SQV-M	[18]
	NA	~38 mg/L (various ecotoxicity tests)	SQV-M	[18]
	NA	2.2 mg/L	SQV-M	[20]
	NA	30 mg/L (25 °C)	SQV-B	[16]
	NA	~18 mg/L (various ecotoxicity tests)	SQV-B	[16]
	NA	31 mg/L (stirring time 24 h, 22 °C)	SQV-B	[2]
Dissociation constant pKa	NA	pKa_1 5.11, pKa_2 8.31	SQV-M	[20]
	NA	pKa 6.89	SQV-B	[16]
n-Octanol-water partition coefficient	NA	$logP_{OW}$ 2.12	SQV-M	[18]
	NA	$logP_{OW}$ 2.12	SQV-M	[20]
	NA	$logP_{OW}$ 3.34	SQB-B	[16]
	NA	$logP_{OW}$ 2.58	SQB-B	[1]
n-Octanol-water distribution coefficient	NA	$logD_{OW}$ 2.75 (*p*H 7.4)	SQB-B	[16]
	NA	$logD_{OW}$ 2.56 (*p*H 8)	SQB-B	[1]

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