Environmental Risk Assessment Summary
Diazepam

Introduction

The publication of environmental risk assessment 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

Diazepam is one of the first benzodiazepines used in medicine: an active pharmaceutical ingredient (API) with anxiolytic, sedative, muscle-relaxant, anti-convulsive and anti-epileptic properties. Diazepam was originally developed by F. Hoffmann-La Roche Ltd. in the 1960s [17].

Diazepam is the active pharmaceutical ingredient used in the Roche product Valium [9].

Subsequent to oral application, diazepam shows rapid uptake, high bioavailability and plasma protein binding. Hepatic metabolism is the main pathway for elimination of diazepam in man. Human excretion of diazepam is mainly by urinary pathway in the form of conjugates. Quantitative estimates of the relative amounts of diazepam and its metabolites as a fraction of total excretion vary strongly. An older reference reports up to 50% of orally ingested diazepam to be excreted as the parent by urinary pathway. In contrast, based on several literature data, a mean estimate of approximately 11% of ingested diazepam being excreted as the parent or its glucuronide conjugate is reported [17].

Diazepam is not inherently biodegradable. In a test according to OECD 302 C no significant biodegradation (<5%) was observed after 28 and 84 days, respectively. However, primary degradation of 84% was observed by LC-UV analysis after 84 days of exposure [1].

The PEC/PNEC ratio is 0.002. With reference to the Guideline on the Environmental Risk Assessment on Medicinal Products for Human Use of the European Medicines Agency [6], a PEC/PNEC ratio of ≤1 means that Diazepam 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 (mg/L) = (A x 10^9 x (1-R)) / (365 x P x V x D)

A Total patient consumption of Diazepam in the European country with the highest yearly per capita use in the period 2013–2017 (data from IQVIA [10])
R Removal rate during sewage treatment (default value) = 0 [6]
P Number of inhabitants in the country with the highest per capita use in the respective year of the period 2013–2017 [7]; resulting in a consumption of 42.5 mg/inhabitant
V Volume of wastewater per inhabitant and day (default value) = 200 L day^{-1} [6]
D Dilution factor of wastewater by surface water flow (default value) = 10 [6]

PEC = 0.058 µg/L

Note: Diazepam 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 Diazepam.

Predicted No Effect Concentration (PNEC)

Chronic studies have been performed for species from three trophic levels, algae, *Daphnia* and fish based on OECD Test Guidelines [14]. The lowest No Observed Effect Concentration (NOEC) is 273 µg/L of the 35 d early-life stage toxicity test with zebra fish (*Danio rerio*) [16]. Applying an assessment factor of 10 according to the EMA Guideline [6] results in a PNEC value of 27.3 µg/L.

PNEC = 273 µg/L / 150 = 27.3 µg/L

PEC/PNEC ratio

PEC = 0.058 µg/L
PNEC = 27.3 µg/L

PEC/PNEC = 0.002

With reference to the Guideline on the Environmental Risk Assessment on Medicinal Products for Human Use of the European Medicines Agency [6], a PEC/PNEC ratio of 0.002 (i.e. ≤1) means that Diazepam and/or its metabolites are unlikely to represent a risk to the aquatic environment.

Aquatic Toxicity Data for Diazepam

Study	Guideline	Results	Ref.
Algal growth inhibition test with the green alga *Desmodesmus subspicatus*	OECD 201	72 h EC50 (growth rate) 3.11 mg/L TWM 72 h EC50 (biomass) 0.607 mg/L TWM 72 h NOEC (growth rate) 0.10 mg/L TWM	[1]
Algal growth inhibition test with the blue-green alga *Synechococcus leopoliensis*	OECD 201	72 h EC50 (growth rate) >11.9 mg/L TWM 72 h EC50 (biomass) 3.50 mg/L TWA 72 h NOEC (growth rate) 3.30 mg/L TWA	[2]
Acute Immobilisation Test with *Daphnia magna*	OECD 202	24 h EC50 4.3 mg/L NC 24 h EC50 13.9 mg/L NC	[11] [5]
Acute Immobilisation Test with *Daphnia pulex*	OECD 202	24 h EC50 12.0 mg/L NC	[11]
Daphnia magna, reproduction test	OECD 211	21 d NOEC (overall) 0.91 mg/L NC	[15]
Acute toxicity to rainbow trout (*Oncorhynchus mykiss*)	OECD 203	96 h LC50 84 mg/L NC 96 h NOEC 50 mg/L NC	[8]
Fish, early-life stage toxicity test with zebrafish (*Danio rerio*)	OECD 210	35 d NOEC (overall) 0.273 mg/L MMC	[16]
Toxicity to microorganisms (toxicity control)	OECD 301 F	21 d NOEC 100 mg/L NC	[8]

EC50 Concentration of the test substance that results in 50% effect
LC50 Concentration of the test substance that results in 50% mortality
MMC Mean measured concentrations
NC Nominal concentration
NOEC No Observed Effect Concentration
TWM Time weighted mean concentration

Environmental Fate Data for Diazepam

Study	Guideline	Results	Ref.
Inherent biodegradability	OECD 302 C	<u>BOD ÷ ThOD (mineralisation)</u> <5% after 28 d <5% after 84 d <u>DOC elimination</u> 11% after 84 d <u>Primary degradation (LC-UV)</u> ~75% after 84 d	[3]
	NA	<u>BOD ÷ ThOD (mineralisation)</u> 0% after 21 d	[8]
Anaerobic biodegradability	ISO 11734	Inorganic carbon (biogas formation) –7% after 62 d (concentration: 20 mg TOC/L) –28% after 62 d (concentration: 30 mg TOC/L) <u>Primary degradation (LC-UV)</u> 91% after 62 d (concentration: 20 mg TOC/L) 84% after 62 d (concentration: 30 mg TOC/L) (unknown transformation product formed)	[4]

Study	Guideline	Results	Ref.
Aerobic transformation in aquatic sediment systems	OECD 308	Mineralisation <2% after 100 d Half-life (water phase) 34 d Half-life (total system) 311 d	[12]
	OECD 308	Mineralisation 0.1% after 30 d Half-life (water phase) ~10 d Half-life (total system) >>60 d Diazepam in sediment 80.6–82.4% after 30 d 74.5–75.5% after 30 d (extractables) 5.2–8.0% after 30 d (non-extractables)	[13]
Adsorption to sediment	OECD 308	Koc 192 L/kg	[12]
Photodegradation in deionised H_2O	NA	25% after 5 d	[3]
Hydrolysis in deionised H_2O	NA	2% after 5 d	[3]

BOD Biochemical oxygen demand
DOC Dissolved organic carbon
ThOD Theoretical oxygen demand
TOC Total organic carbon

Physical Chemical Data for Diazepam

Study	Guideline	Results	Ref.
Water solubility	NA	50 mg/L (20 °C)	[9]
Dissociation constant	NA	pK_1 3.4	[9]
n-Octanol/Water Partition Coefficient	NA	log D_{OW} 2.58 (pH 7.2)	[9]

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