

## **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:

$$\text{PEC (ng/L)} = (A \times 10^9 \times (1-R)) / (365 \times P \times V \times D)$$

- 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<sup>-1</sup> [6]
- D Dilution factor of wastewater by surface water flow (default value) = 10 [6]

$$\text{PEC} = 0.058 \text{ } \mu\text{g/L}$$

*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.

$$\text{PNEC} = 273 \text{ } \mu\text{g/L} / 10 = 27.3 \text{ } \mu\text{g/L}$$

## PEC/PNEC ratio

$$\text{PEC} = 0.058 \text{ } \mu\text{g/L}$$

$$\text{PNEC} = 27.3 \text{ } \mu\text{g/L}$$

$$\text{PEC/PNEC} = 0.002$$

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 <i>Desmodesmus subspicatus</i>         | OECD 201   | 72 h EC50 (growth rate) 3.11 mg/L TWM<br>72 h EC50 (biomass) 0.607 mg/L TWM<br>72 h NOEC (growth rate) 0.10 mg/L TWM | [1]  |
| Algal growth inhibition test with the blue-green alga <i>Synechococcus leopoliensis</i> | OECD 201   | 72 h EC50 (growth rate) >11.9 mg/L TWM<br>72 h EC50 (biomass) 3.50 mg/L TWA<br>72 h NOEC (growth rate) 3.30 mg/L TWA | [2]  |
| Acute Immobilisation Test with <i>Daphnia magna</i>                                     | OECD 202   | 24 h EC50 4.3 mg/L NC  | [11] |
| Acute Immobilisation Test with <i>Daphnia pulex</i>                                     | OECD 202   | 24 h EC50 13.9 mg/L NC   | [5]  |
| <i>Daphnia magna</i> , reproduction test  | OECD 202   | 24 h EC50 12.0 mg/L NC   | [11] |
| Acute toxicity to rainbow trout ( <i>Oncorhynchus mykiss</i> )                          | OECD 211   | 21 d NOEC (overall) 0.91 mg/L NC   | [15] |
| Fish, early-life stage toxicity test with zebrafish ( <i>Danio rerio</i> )              | OECD 203   | 96 h LC50 84 mg/L NC<br>96 h NOEC 50 mg/L NC   | [8]  |
| Toxicity to microorganisms (toxicity control)   | OECD 210   | 35 d NOEC (overall) 0.273 mg/L MMC   | [16] |
|   | 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><br><5% after 28 d<br><5% after 84 d<br><u>DOC elimination</u><br>11% after 84 d<br><u>Primary degradation (LC-UV)</u><br>~75% after 84 d   | [3]  |
|                            | NA         | <u>BOD ÷ ThOD (mineralisation)</u><br>0% after 21 d   | [8]  |
| Anaerobic biodegradability | ISO 11734  | Inorganic carbon (biogas formation)<br>-7% after 62 d (concentration: 20 mg TOC/L)<br>-28% after 62 d (concentration: 30 mg TOC/L)<br><u>Primary degradation (LC-UV)</u><br>91% after 62 d (concentration: 20 mg TOC/L)<br>84% after 62 d (concentration: 30 mg TOC/L)<br>(unknown transformation product formed) | [4]  |

| Study  | Guideline | Results                                | Ref. |
|--|-----------|--|------|
| Aerobic transformation in aquatic sediment systems | OECD 308  | Mineralisation <2% after 100 d         | [12] |
|  |           | Half-life (water phase) 34 d           |      |
|  |           | Half-life (total system) 311 d         |      |
|  | OECD 308  | Mineralisation 0.1% after 30 d         | [13] |
|  |           | Half-life (water phase) ~10 d          |      |
|  |           | Half-life (total system) >>60 d        |      |
|  |           | <u>Diazepam in sediment</u>            |      |
|  |           | 80.6–82.4% after 30 d                  |      |
|  |           | 74.5–75.5% after 30 d (extractables)   |      |
|  |           | 5.2–8.0% after 30 d (non-extractables) |      |
| Adsorption to sediment                             | OECD 308  | Koc 192 L/kg                           | [12] |
| Photodegradation in deionised H <sub>2</sub> O     | NA        | 25% after 5 d                          | [3]  |
| Hydrolysis in deionised H <sub>2</sub> O           | 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 <sub>i</sub> 3.4               | [9]  |
| n-Octanol/Water Partition Coefficient | NA        | log D <sub>OW</sub> 2.58 (pH 7.2) | [9]  |

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