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Guideline on assessing the environmental and human health risks of veterinary medicinal products in groundwater

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Guideline on assessing the toxicological risk to human health and groundwater communities from veterinary pharmaceuticals in groundwater

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Executive summary

Groundwater is a source of drinking water and also provides a unique ecosystem with vulnerable aquatic communities. This guideline provides a methodology for performing a risk assessment of residues of veterinary medicinal products (VMPs) in groundwater, serving both as a source of drinking water and as an ecosystem. A risk assessment needs to be performed when the concentration of a VMP in groundwater is equal to or above 0.1 µg/l. In addition, this guideline highlights case studies where a risk assessment may be needed for highly toxic or persistent substances, when the predicted concentration in groundwater is lower than 0.1 µg/l. The guideline complements existing guidelines such as the CVMP guideline on environmental impact assessment for veterinary medicinal products in support of the VICH guidelines GL6 and GL38, which provide a methodology for groundwater for exposure assessment.

1. Introduction and legal background

This present guideline complements the VICH guidelines GL6 (VICH 2000) and GL38 (VICH 2005) on the environmental risk assessment (ERA) of VMPs. The need for an assessment of VMPs in groundwater is included in the VICH GL 38 “Environmental Impact Assessment for Veterinary Medicinal Products Phase II” (VICH, 2005) and the CVMP guideline “*Environmental Impact Assessment for Veterinary Medicinal Products in support of the VICH guidelines GL6 and GL38*” (EMA, 2016), referred to as the CVMP Technical Guidance Document (CVMP TGD). According to the CVMP TGD, the predicted environmental concentration (PEC) in groundwater of a veterinary medicine needs to be compared with the value of 0.1 µg/l. The value of 0.1 µg/l is the groundwater quality standard (GQS) for pesticides and biocides (including their relevant metabolites, degradation and reaction products) according to the Groundwater Directive 2014/80/EU Annex I, amending Directive 2006/118/EC (GWD) and according to the Drinking Water Directive (Council Directive 98/83/EC). Up to now, and based on the recommendations in the CVMP TGD, the CVMP has followed the approach below:

- Concentrations of active ingredients (a.i.) in groundwater equal to or above 0.1 µg/l have been considered unacceptable as VMPs, regardless of their hazardous properties. In this situation, applicants could refine the PEC groundwater with additional data (e.g., modelling, more studies e.g. on degradation in manure, mitigation measures). In situations where the refined PEC_{groundwater} is equal to or higher than 0.1 µg/l, restrictions to the proposed uses were required to avoid predicted values equal to or above 0.1 µg/l, and/or the human health risks and environmental risks were further assessed using the risk quotient approach.
- Concentrations of a.i. below 0.1 µg/l, have been considered as acceptable, as no risk is anticipated and therefore no further regulatory action is required.

When the value of 0.1 µg/l was introduced into the European groundwater legislation as a GQS, it was established as a precautionary value as this was the usual limit of detection for pesticides in waters (with concentrations below this limit being difficult to quantify due to analytical limitations). Consequently, predicted concentrations below 0.1 µg/l were assumed as equivalent to ‘no emission into groundwater’, thus concluding that any obligation to prevent entry of hazardous substances into groundwater was fulfilled. However, analytical methods have continuously improved with the result that it is now often possible to quantify substances below the current limit value of 0.1 µg/l. Thus, the limit value of 0.1 µg/l is no longer accepted as equivalent to ‘no emission into groundwater’. In addition, experience has shown that for certain substances concentrations in groundwater below 0.1

µg/l have the potential to impact on vulnerable aquatic communities. For certain VMPs, the predicted no effect concentrations (PNEC) are below 0.1 µg/l and a review of environmental quality standards (EQS) for surface waters has revealed that annual average environmental quality standard (AA-EQS) could be even three or more orders of magnitude lower than the GQS of 0.1 µg/l (UK TAG, 2012). For example, the AA-EQS for cypermethrin (a listed priority substance) in surface waters is 8×10^{-5} µg/l (Directive 2013/39/EU). Taxonomic groups present in groundwater (such as arthropods) are especially sensitive to such substances. Thus, this example shows that a universal groundwater quality standard of 0.1 µg/l may not be viewed as an acceptable value for protecting surface water and groundwater species.

It is also important to consider that the current Article 6(1) of the GWD requires Member States to take measures to prevent the input into the groundwater compartment of substances listed in points 1 to 6 of Annex VIII of the Water Framework Directive (2000/60/EC) (so called, 'zero-tolerance substances'). Annex I of the GWD also lays down GQS for entry into groundwater of pesticides and their metabolites. However, while certain VMPs contain a.i. that may also be used as pesticides or biocides or a.i. that meet the definitions of hazardous substances according to Annex VIII of Directive 2000/60/EC (e.g. organohalogenes), the GWD does not contain any specific provisions for VMPs nor for those a.i. in VMPs that are also used as pesticides or biocides.

The Phase II ERA guideline for VMPs (VICH, 2005) notes that 'groundwater is a natural resource and should not only be assessed with regards to public health but also to possible harmful effects to the biota of groundwater'. The guideline, however, does not provide any further information on how to assess possible harmful effects to human health or ecosystems, i.e., when the predicted concentration in groundwater is estimated to be ≥ 0.1 µg/l and for situations where the PNEC for the aquatic compartment is below the value of 0.1 µg/l.

2. Scope

This guideline outlines the methodology for performing a human health and environmental risk assessment for groundwater, addressing:

1. The environmental and human health risk assessment approach for the groundwater compartment for VMPs with a predicted environmental concentration (PEC) equal to or above the GQS of 0.1 µg/l.
2. An environmental risk assessment for VMPs with a PEC in groundwater below 0.1 µg/l when experimental evidence indicates that concentrations below 0.1 µg/l might pose a risk to groundwater ecosystems.

When determining the potential impact of a VMP on groundwater, consideration has also to be given by the applicant to relevant European Union legislation on groundwater (Council Directive 2006/118/EC) and drinking water (Council Directive 98/83/EC). In view of this, the CVMP considers that substances used as VMPs that are also used as pesticides and/or biocides should be subject to the same limits, as laid down in Annex II of the GWD, i.e. they should not result in concentrations in groundwater equal to or above 0.1 µg/l. For those a.i. that come within the scope of points 1 to 6 of Annex VIII to the WFD input into groundwater should be prevented, in line with the provisions set in this Directive for these categories of substances. If, for those a.i. used as VMPs, entry cannot be prevented, then the risks to human health and the environmental compartment would need to be addressed and considered within the context of the overall benefit/risk balance of the product.

3. Quantification of risk to humans from residues in groundwater when used as drinking water

The risk to humans of VMPs in groundwater is associated with the use of groundwater as a source of drinking water. The risk assessment in this guideline is therefore based on scenarios following human consumption of drinking water derived from groundwater.

In order to assess the human health risk of groundwater contamination by VMPs, it should be assumed that groundwater may be used as drinking water without further purification. Thus, a maximum tolerable concentration in drinking water (MTC_{dw}) ($\mu\text{g}/\text{l}$) needs to be calculated following the methodology used by the World Health Organization (WHO, 2017).

$$MTC_{dw} = \frac{ADI \times BW \times P}{C_w} \quad (\text{Equation 1})$$

The recommended default values to be used in the calculation of the MTC_{dw} and their units are listed in Table 1.

Table 1. Recommended default values to be used in the calculation of the MTC_{dw} in Equation 1 and their units (based on World Health Organisation (WHO), 2017. Guidelines for Drinking-water Quality. Fourth Edition).

	Parameter description	Unit	Default value
BW	Body weight	[kg]	60
MTC_{dw}	Maximum tolerable concentration in drinking water	$[\mu\text{g} \cdot \text{l}^{-1}]$	–
ADI*	Acceptable daily intake	$[\mu\text{g} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}]$	–
P	Fraction of the ADI to be used by the intake of drinking water	[–]	0.1**
C_w	Daily consumption of drinking water	$[\text{l} \cdot \text{day}^{-1}]$	2

* Assessment of the risk of groundwater contamination by VMPs for human health is only expected to be applicable for substances for which an ADI has been determined. Substances for which an ADI was not set because they were considered not to pose a risk for consumers of meat, milk and eggs will not pose any risk via consumption of drinking water either. Substances for which no ADI could be established due to unacceptable toxicity, e.g. genotoxic carcinogenic effects, are not accepted for use in food producing animals and will therefore not be subject to Phase II environmental assessment and assessment of risk for humans via exposure from drinking water.

** It is noted that the WHO uses up to 0.2 for P. However, for the evaluation of residues of veterinary medicines in groundwater in the EU an indicative value of 0.1 is considered adequate. See text for further details.

The default values of 60 kg for the weight of the human body and 2 litres for the daily consumption of drinking water are based on the recommendations made by WHO and used within the WHO methodology. Any deviation from the default value of P in Table 1 needs to be accompanied by a solid scientific justification.

The CVMP establishes acceptable daily intake (ADI) values for most active ingredients for use in VMPs that are administered to food producing animals. The ADI is a toxicological risk limit and is identical to the Tolerable Daily Intake (TDI), which is used within, for example, the WHO terminology. It represents a toxicologically safe value even if exposure is prolonged. CVMP recommends to use a P value of 10% as an indicative percentage of the ADI to be used by drinking water. However, on a case

by case basis, deviation from the 10% default value may be justified as the acceptable daily intake via drinking water depends on the anticipated daily intake via other sources. This means that the actual P value may in practice be higher or lower than 0.1. A fundamental principle is that the total intake of the active ingredient by consumers (via water and food products) does not exceed the ADI.

Based on the information on the predicted concentration in groundwater ($PEC_{\text{groundwater}}$) calculated according to CVMP TGD (2016) and the maximum tolerable concentration in drinking water (MTC_{dw}), the potential human risk of the active ingredient via drinking water can be assessed by the risk quotient in Equation 2 below.

$$RQ_{\text{gw}} = \frac{PEC_{\text{gw}}}{MTC_{\text{dw}}} \text{ (Equation 2)}$$

In cases where the $RQ_{\text{groundwater}}$ is equal to or exceeds 1.0, a human health risk has been identified. However, it may be possible to refine the calculations and lower the risk quotient. This may include a refinement of the $PEC_{\text{groundwater}}$, using more elaborate modelling approaches described in the CVMP TGD (2016). Refinement of the risk may also be achieved by deviating from the default P value of 10%, as the fraction of the ADI to be used by the intake of drinking water as mentioned above, resulting in a modification of the MTC_{dw} .

If the refined $RQ_{\text{groundwater}}$ is below 1.0, the risk to human health is considered acceptable. If the refined $RQ_{\text{groundwater}}$ is equal to or exceeds 1.0, risk mitigation measures have to be applied or the risk should be considered as part of the benefit/risk assessment.

4. Quantification of risk to groundwater ecosystems

In addition to the human health risk assessment, an environmental risk assessment for groundwater ecosystems is also needed. Contamination of groundwater may permanently eradicate unique groundwater communities due to their inability to recolonise any affected habitats (Di Lorenzo et al., 2015a, b). In addition, if key species and ecological functions are affected, then purification processes may be disturbed and the original state may not be restored.

4.1. Definition of groundwater ecosystems of concern

The environmental risk assessment according to the VICH guidelines GL6 (VICH, 2000) and GL38 (VICH, 2005) makes use of a standard aquatic ecosystem and data from model aquatic test species and these are not truly representative of groundwater ecosystems/biota.

Freshwater aquatic ecosystems are defined by a few representative habitats e.g. a stream or a pond, whereas the most representative groundwater habitats are hypogean karsts (fractures, channels, caves), and alluvial gravel interstitial systems. For the protection of the whole groundwater compartment, it is also necessary to protect spring water. Springs are an ecotone, a transition between groundwater and surface water bodies, and in this context they are also considered groundwater, as they may fill gravel pits and ponds or lakes without other influx from surface water.

4.2. Environmental risk assessment considerations

The assessment of the environmental risks of VMPs is framed by internationally agreed guidelines (i.e., VICH guidelines GL6 and GL38), which prescribe the use of data from a limited number of tests on fate, behaviour and ecotoxicity of the VMP. The current guidelines only include ecotoxicity testing for

surface water species (i.e., algae, daphnids and fish species). Experimental data obtained for organisms from these three trophic levels is used to derive a PNEC for the groundwater ecosystem and its groundwater biota. Groundwater biota are specifically adapted to live in the challenging conditions found in groundwater and are therefore potentially vulnerable to additional stress. This may be addressed by use of an additional assessment factor. In the longer term more reliable information may be provided from relevant ecotoxicological studies performed with groundwater species under realistic (i.e. groundwater-like) conditions (see also paragraph 4.2.4).

The groundwater ecosystem is considered more vulnerable than many other aquatic ecosystems. The concept of ecosystem vulnerability, is defined as the “exposure to contingencies and stress, and the difficulty in coping with them” (Millennium Ecosystem Assessment report, 2005), and comprises three major dimensions:

- i) *Exposure to stress, perturbations and shocks (e.g. toxicants in groundwater);*
- ii) *Sensitivity of exposed species to the stress or perturbation, including their capacity to anticipate and cope with the stress (e.g. groundwater species sensitivity to chemical stressors);*
- iii) *Resilience of the exposed ecosystems, and species in terms of their capacity to absorb shocks and perturbations while maintaining function.*

The 3 bullets mentioned above have been considered when determining the most appropriate assessment factors when extrapolating from data on surface water species.

4.2.1. Exposure to toxicants in groundwater

An important element to be considered for the ERA of groundwater ecosystems is the duration of exposure of chemicals to any resident communities. There are indications that groundwater ecosystems have a more prolonged period of exposure time, given that the groundwater maintains a constant temperature, whereas the temperature of surface water alters according to the surroundings. This aspect combined with a complete absence of sunlight (at least in the vast majority of cases), oligotrophy and low redox potential, could result in decreased biotic and photolytic degradation processes (Yagi et al., 2010; Bulog and Mali Bizjak, 2014) and lead to longer persistence of chemical substances. In most surface water systems (e.g. rivers), the residence time of water (the average amount of time that a molecule of water spends in a particular system) ranges from a few days to a few weeks (Worrall et al., 2014). However, in groundwater ecosystems such as porous aquifers, the residence time may be decades or even centuries (Freeze and Cherry, 1979). Consequently, whereas the baseline quality of the riverine surface water may recover after a relatively short period of time, the recovery in aquifers (if any) may require decades, or disturbances may even be irreversible.

4.2.2. Sensitivity of groundwater species to chemical stressors

There is a limited knowledge of the sensitivity of groundwater species to acute exposure to toxicants (Kolar and Finizio, 2017). The chronic effects, however, of chemical stressors to groundwater species¹ are not known (Kolar and Finizio, 2017; van Beelen, 2007; Avramov et al., 2013).

Groundwater species have developed a number of metabolic adaptations for extreme energy saving to deal with starvation. As a result of this, their metabolic rates (or depuration rates) are significantly lower than those of most other aquatic species. These adaptations can affect the species response to a

¹ In the groundwater compartment, the term species also comprise microorganisms and/or microbial communities

chemical stressor, and make them more sensitive to the long term effects when compared to surface water species (Di Lorenzo et al., 2014). Thus, the ability to respond to chemical stressors may be slower in groundwater species. However, it is also important to note that the metabolic potential of groundwater species under sudden favourable conditions is higher for faster energy recovery and restoration of body reserves (Simčič et al., 2005; Hervant et al., 1997). Faster restoration of body reserves may lead to higher uptake rates of lipophilic substances and storage in fatty tissues. The energetic cost of this response may have long term consequences on the survival and reproduction in exposed groundwater species.

4.2.3. Resilience and complexity of groundwater ecosystems

Groundwater ecosystems are characterised by lower levels of complexity (in terms of a lower number of trophic levels) due to the allotrophy² and heterotrophy³ of these systems. This lower level of complexity also implies that re-colonisation after perturbation may be extremely slow, and that the restoration of an affected biotic community is unlikely (Culver and Pipan, 2009). Thus, groundwater ecosystems have low resilience to perturbations. Groundwater communities are characterised by a high level of endemism, longevity, slow growth and low reproduction rates (Gibert et al., 1994). The biological adaptations of these species contribute to the low plasticity of their community when reacting to repeated or chronic pressure due to chemical stressors.

Due to the low resilience to perturbations by groundwater ecosystems, recovery from the toxic effects of VMPs may not be possible within a realistic timeframe of a few seasons or even decades (Kolar and Finizio, 2017). Consequently, the permanent loss of endemic groundwater species is a realistic threat (Bulog and Mali Bizjak, 2014).

4.2.4. Conclusion on quantification of risk to groundwater ecosystems

The arguments presented above show that groundwater ecosystem are fundamentally different and therefore may be more vulnerable than surface water ecosystems as they lack the ability to recover from perturbations. As a result of this vulnerability, an additional assessment factor of 10 should be applied to extrapolate the $PNEC_{\text{groundwater}}$ from the $PNEC_{\text{Surfacewater}}$ (Equation 3 below). A similar approach has already been adopted in relation to EQS derivation in marine ecosystems (EC, 2011).

$$PNEC_{\text{groundwater}} = PNEC_{\text{Surfacewater}}/10 \text{ (Equation 3)}$$

5. Risk assessment of groundwater ecosystems

5.1 Exposure assessment

The initial concentration of a VMP in groundwater ($PEC_{\text{groundwater}}$) is determined using the methods described in the CVMP TGD (2016). If the initial concentration is equal to or exceeds the GQS of 0.1 µg/l, the exposure assessment (PEC) can be refined following CVMP TGD (2016). First, the PEARL CVMP-metamodel for leaching to groundwater is run. Based on the outcome of the metamodel, it is established whether a FOCUS PEARL model (FOCUS, 2000) simulation is necessary. The FOCUS PEARL model (FOCUS, 2000) should be run using the relevant scenarios and the parameters outlined in Appendix I of the CVMP TGD. The exposure assessment can also potentially be refined by determining

² Referring to bioproduction being largely dependent on transport of resources from the surface.

³ Referring to the need to use organic carbon for growth.

a.i. degradation in manure in accordance with the respective CVMP guideline (EMA, 2011) and/or by using data on metabolism in the target animal.

5.2 Risk characterisation

In Phase II of the ERA, PNECs for surface water organisms (i.e. algae, Daphnia, fish) are derived based on experimental ecotoxicological data. $PNEC_{groundwater}$ can be extrapolated by considering an additional assessment factor of 10 to be applied to the PNEC of the most sensitive species (see section 4, equation 3).

The applicant can propose refinement of the $PNEC_{groundwater}$, using the standard Tier B surface water tests (but still including the additional assessment factor of 10). No refinement of $PNEC_{groundwater}$ with experimental studies on groundwater species is possible, until standardised ecotoxicological long term tests for groundwater species are available.

5.3 Risk assessment

The RQ for the groundwater compartment is determined using the following equation:

$$RQ_{groundwater} = PEC_{groundwater} / PNEC_{groundwater} \quad (\text{Equation 4})$$

- When $PEC_{groundwater}$ is below 0.1 µg/l and the $PNEC_{surfacewater}$ is above 1.0 µg/l, no risk is anticipated and the risk assessment stops.
- When the refined $PEC_{groundwater}$ is equal to or higher than 0.1 µg/l, human health risks (see section 3) and ecological risks are further assessed according to scenario 1.
- When $PEC_{groundwater}$ is below 0.1 µg/l and the $PNEC_{surfacewater}$ is below 1.0 µg/l, ecological risks are assessed according to scenario 2.

5.3.1 Scenario 1: $PEC_{groundwater} \geq 0.1 \mu\text{g/l}$

When the refined $PEC_{groundwater}$ is equal to or higher than 0.1 µg/l, ecological risks are further assessed by determining the $RQ_{groundwater}$.

If the $RQ_{groundwater}$ is ≥ 1 , indicating a risk to the groundwater compartment, the applicant should propose adequate risk mitigation measures to protect groundwater ecosystems. If no suitable risk mitigation measure/s can be applied, the risk for groundwater has to be addressed in the benefit/risk assessment evaluation.

5.3.2 Scenario 2: $PEC_{groundwater} < 0.1 \mu\text{g/l}$ and $PNEC_{surfacewater} \leq 1 \mu\text{g/l}$ derived from Tier B surface water toxicity tests

In cases where the $PEC_{groundwater}$ of VMPs will be $< 0.1 \mu\text{g/l}$, a risk assessment for the groundwater compartment will not be needed, unless Tier B ecotoxicity data has shown that the $PNEC_{surfacewater}$ is $\leq 1.0 \mu\text{g/l}$. In this circumstance, the calculated $PNEC_{groundwater}$ would be $\leq 0.1 \mu\text{g/l}$ (i.e., $PNEC_{groundwater} = PNEC_{surfacewater} / 10$), and the value of 0.1 µg/l might not be protective for groundwater species. Consequently, the $RQ_{groundwater}$ should be calculated in order to determine if there is a possible risk to the groundwater compartment.

When a risk to the groundwater ecosystem is indicated, the applicant should propose adequate risk mitigation measures to protect groundwater ecosystems. If no suitable risk mitigation measure/s can be applied, the risk for groundwater has to be addressed in the benefit risk assessment.

List of abbreviations

a.i	Active ingredient
AA-EQS	Annual Average Environmental Quality Standard
ADI	Acceptable Daily Intake
CVMP TGD	Committee for Medicinal Products for Veterinary Use (CVMP) Technical Guidance Document (TGD): Environmental impact assessment for veterinary medicinal products in support of the VICH guidelines GL6 and GL38
CVMP	Committee for Medicinal Products for Veterinary use
DT ₅₀	Degradation half-lives
ERA	Environmental Risk Assessment
FOCUS	Forum for the Coordination of Pesticide Fate Models and Their Use
GQS	Groundwater Quality Standard
GWD	Groundwater Directive
K _{oc}	Organic carbon partition coefficient
MRL	Maximum Residue Level
MTC _{dw}	Maximum Tolerable Concentration in drinking water
PEC	Predicted Environmental Concentration
PEC _{groundwater}	Predicted Environmental Concentration in groundwater
PNEC	Predicted No Effect Concentration
PNEC _{groundwater}	Predicted No Effect Concentration in groundwater
PNEC _{surfacewater}	Predicted No Effect Concentration in surface water
RQ _{groundwater}	Risk Quotient for groundwater
TDI	Tolerable Daily Intake
TGD	Technical Guidance Document
VICH	Veterinary International Conference on Harmonization
VMPs	Veterinary Medicinal Products
WFD	Water Framework Directive
WHO	World Health Organization

References

- Avramov, M., Rock, T. M., Pfister, G., Schramm, K-W., Schmidt S I., Griebler, C. 2013. Catecholamine levels in groundwater and stream amphipods and their response to temperature stress. *General and Comparative Endocrinology* 194, 110-117 e
- Bulog, B. and Mali Bizjak, L. 2014. Olm – cave salamander. In natural heritage of Bela krajina, Slovenia. Bela krajina Museum. 2014. pp 177-186. Retrived from http://web.bf.uni-lj.si/bi/NATURA-SLOVENIAE/pdf/NatSlo_18_1_8.pdf
- Culver, D. C. and Pipan, T. *The Biology of Caves and Other Subterranean Habitats*. Publisher, Oxford University Press. 273 pp 2009. ISBN 0191551449.
- Di Lorenzo, T., Di Marzio, W.D., Sáenz, M.E., Baratti, M., Dedonno, A.A., Iannucci, A., Cannicci., S., Messina, G., Galassi, D.M.P., 2014. Sensitivity of hypogean and epigean freshwater copepods to agricultural pollutants. *Environ Sci Pollut Res* 21: 4643-4655.
- Di Lorenzo, T., Borgoni, R, Ambrosini, R., Cifoni, M., Galassi, D.M.P., Petitta, M., 2015a. Occurrence of volatile organic compounds in shallow alluvial aquifers of a Mediterranean region: Baseline scenario and ecological implications. *Science of the Total Environment*, 538: 712-723.
- Di Lorenzo, T., Cifoni, M., Lombardo, P., Fiasca, B., Galassi, D. M. P. 2015b. Ammonium threshold values for groundwater quality in EU may not protect groundwater fauna: evidence from an alluvial aquifer in Italy. *Hydrobiologia*, 743: 139-150.
- European Commission (EU) 2011. Guidance Document No. 27 Technical Guidance For Deriving Environmental Quality Standards. Available online <https://circabc.europa.eu/sd/a/0cc3581b-5f65-4b6f-91c6-433a1e947838/TGD-EQS%20CIS-WFD%2027%20EC%202011.pdf>
- European Medicines Agency (EMA). 2016. Environmental Impact Assessment for Veterinary Medicinal Products in support of the VICH guidelines GL6 and GL38. EMEA/CVMP/ 418282/2005-Rev.1. http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2009/10/WC500004389.pdf
- European Medicines Agency (EMA). 2011. Guideline on determining the date of veterinary medicinal products in manure. EMEA/CVMP/ERA/430327/2009. http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2011/03/WC500104495.pdf
- European Medicines Agency (EMA). 2005 Environmental impact assessment for veterinary medicinal products in support of the VICH guidelines GL6 and GL38. Available online http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2009/10/WC500004386.pdf
- Freeze, R.A. and Cherry, J.A. 1979. *Groundwater*. Prentice-Hall, Englewood Cliffs, NJ, 604pp
- FOCUS 2000. FOCUS groundwater scenarios in the EU review of active substances" v.2.2 - Report of the FOCUS Groundwater Scenarios Workgroup, EC Document Reference Sanco/321/2000 rev.2.

http://esdac.jrc.ec.europa.eu/public_path/projects_data/focus/gw/docs/FOCUS_GW_Report_Main.pdf

Gibert, J., Danielopol, D.L., Stanford, J.A., 1994. Basic attributes of groundwater ecosystems and prospects for research. In J. Gibert, D.L. Danielopol, and J.A. Stanford, eds *Groundwater Ecology*. Academic Press, Inc. 7-40 pp

Hervant, F., Mathieu, J., Barré, H., Simon, K. and Pinon, C. 1997. Comparative study on the behavioral, ventilatory, and respiratory responses of hypogean and epigean crustaceans to long-term starvation and subsequent feeding. *Comparative Biochemistry and Physiology, Part A: Physiology*, 118:4, 1277–1283.

Kolar, B. and Finizio A., 2017. Assessment of environmental risks to groundwater ecosystems related to use of veterinary medicinal products. *Regulatory Toxicology and Pharmacology* 88: 303-309

Millennium Ecosystem Assessment. 2005. *Ecosystems and Human Well-being: Synthesis*. Island Press, Washington DC. ISBN 1-59726-040-1 Available online
<https://www.millenniumassessment.org/documents/document.356.aspx.pdf>

Official Journal of the European Communities. Commission Directive 2014/80/EU of 20 June 2014 amending Annex II to Directive 2006/118/EC of the European Parliament and of the Council on the protection of groundwater against pollution and deterioration Text with EEA relevance [2014] OJ L182/53

Official Journal of the European Communities. Directive 98/83/EC of 3 November 1998 on the quality of water intended for human consumption [1998] OJ L330/32

Official Journal of the European Union. Directive 2013/39/EU of the European Parliament and of the Council of 12 August 2013 amending Directives 2000/60/EC and 2008/105/EC as regards priority substances in the field of water policy [2013] OJ L226/1.

Official Journal of the European Union. Council Directive 2000/60/EC establishing a framework for Community action in the field of water policy (Water Framework Directive) [2000] OJ L327/1

Official Journal of the European Union. Directive 2006/118/EC of the European Parliament and of the Council of 12 December 2006 on the protection of groundwater against pollution and deterioration [2006] OJ L327/19

Simič, T., Lukančič, S. and Brancelj, A. 2005. Comparative study of electron transport system activity and oxygen consumption of amphipods from caves and surface habitats. *Freshwater Biology*, 50:3, 494–501.

UK Technical advisory group (UK TAG) - Proposals for further environmental quality standards for specific pollutants. Final report. 2012. Available online
https://www.wfduk.org/sites/default/files/Media/UKTAG%20Specific%20Pollutants_final_011412.pdf

van Beelen, P. 2007. Ecologische risicobeoordeling van grondwater. Rijksinstituut voor Volksgezondheid en Milieu (RIVM) no.711701055 , 44pp. Retrieved from

<http://www.rivm.nl/bibliotheek/rapporten/711701055.html>

Veterinary International Conference on Harmonization (VICH). 2000. GL 6 - Guideline on environmental impact assessment (EIAS) for veterinary medicinal products – phase I. CVMP/VICH/592/98-FINAL.

http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2009/10/WC500004394.pdf

Veterinary International Conference on Harmonization (VICH). 2005. GL 38 - Guideline on environmental impact assessment for veterinary medicinal products phase II. CVMP/VICH/790/03-FINAL.

http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2009/10/WC500004393.pdf

World Health Organisation (WHO), 2017. Guidelines for Drinking-water Quality. Fourth Edition.

Available online http://www.who.int/water_sanitation_health/publications/drinking-water-quality-guidelines-4-including-1st-addendum/en/

Worrall F., Howden, N.J.K., Burt, T.P. 2014. A method of estimating in-stream residence time of water in rivers *Journal of Hydrology*, 512, 274-284 <https://doi.org/10.1016/j.jhydrol.2014.02.050>

Yagi, J. M., Neuhauser, E. F., Ripp, J. A., Mauro, D. M. & Madsen, E. L. 2010. Subsurface ecosystem resilience: long-term attenuation of subsurface contaminants supports a dynamic microbial community. *The ISME Journal*, 4, 131–143.