Guidance Document #09
Feed Ingredients Environmental Safety (Phase 2)
May 2025
At Step 7: Steering Committee Endorsement

FEED INGREDIENTS ENVIRONMENTAL RISK ASSESSMENT (PHASE 2)

May 2025

Endorsed by the Steering Committee
April 2025

It is recommended for the companies planning to submit applications/dossiers for pre-market authorization, to contact the jurisdictions of the countries to confirm their acceptance of the current guidance document.

The International Cooperation for Convergence of Technical Requirements for the Assessment of Feed Ingredients (ICCF) was launched in 2017 and aims to develop and establish common guidance documents to provide technical recommendations for the assessment of feed ingredients, including new uses of existing feed ingredients.

This guidance document has been developed by the appropriate ICCF Experts Working Group and was subject to consultation by the Parties, in accordance with the ICCF Process.

The founding members of the ICCF include the Canadian Food Inspection Agency (CFIA), the European Commission (DG SANTE), the U.S. Food and Drug Administration (FDA), as well as the American Feed Industry Association (AFIA), the Animal Nutrition Association of Canada (ANAC), the EU Association of Specialty Feed Ingredients and their Mixtures (FEFANA) and the International Feed Industry Federation (IFIF).

TABLE OF CONTENT

LIST OF EQUATIONS

TABLE OF TABLES

FEED INGREDIENTS ENVIRONMENTAL RISK ASSESSMENT PHASE 2

1. INTRODUCTION

1.1. Objective of the guidance

This guidance document is a follow-up guidance to the ICCF Guidance Document on Environmental Risk Assessment Approach (Phase 1). The application of the recommendations in this guidance document (Phase 2) will depend on the outcome of the Phase 1 risk assessment. For feed ingredients exempted from further evaluation in Phase 1, the application of this guidance document is not necessary.

The purpose of this guidance document is to provide recommendations on the application of environmental fate and effects data to be used in a Phase 2 risk assessment. This guidance document identifies options for refining the Predicted Environmental Concentration (PEC) and deriving Predicted No Effect Concentrations (PNEC) of the feed ingredient or its constituent entity(ies) in the environmental compartment(s) of concern. While the guidance document provides these recommendations, applicants are advised to consult the appropriate regulatory authorities and their specific guidelines during the development phase of new feed ingredients or for a new use of an approved or authorized feed ingredient. This will ensure that the information provided is appropriate and acceptable for a specific pre-market approval or authorization.

1.2. Definitions

The definitions applicable in the context of this guidance document are provided in the ICCF Glossary of Terms.

1.3. Scope of the guidance

This guidance document is intended for feed ingredients containing constituent entity(ies), which do not meet the exclusion criteria established in the Phase 1 risk assessment and/or with a PEC greater than the threshold value for the environmental compartment(s) of concern. For these constituent entities, further evaluation to determine the potential for effects in non-target species is required.

The Phase 2 environmental risk assessment guidance does not apply to feed ingredients for use in marine aquaculture systems. Applicants intending to pursue authorization or approval of a feed ingredient for use in marine aquaculture systems should contact the appropriate regulatory

authority. This guidance document should be interpreted in conjunction with national legislation and specific regulatory requirements.

2. GENERAL PRINCIPLES

If, in Phase 1, the PEC in any environmental compartment exceeds the jurisdiction specific threshold value (Table 1), further risk assessment using this guidance document is necessary.

Table 1 – PEC threshold values adopted by different jurisdictions and used to determine the need for a Phase 2 assessment.

Jurisdiction	PEC Threshold values		References
	Terrestrial (µg/kg)	Aquatic (µg/L)	
United States	100	1	CVM Guidance for Industry #89
European Union	10	0.1	EC Regulation 429/2008

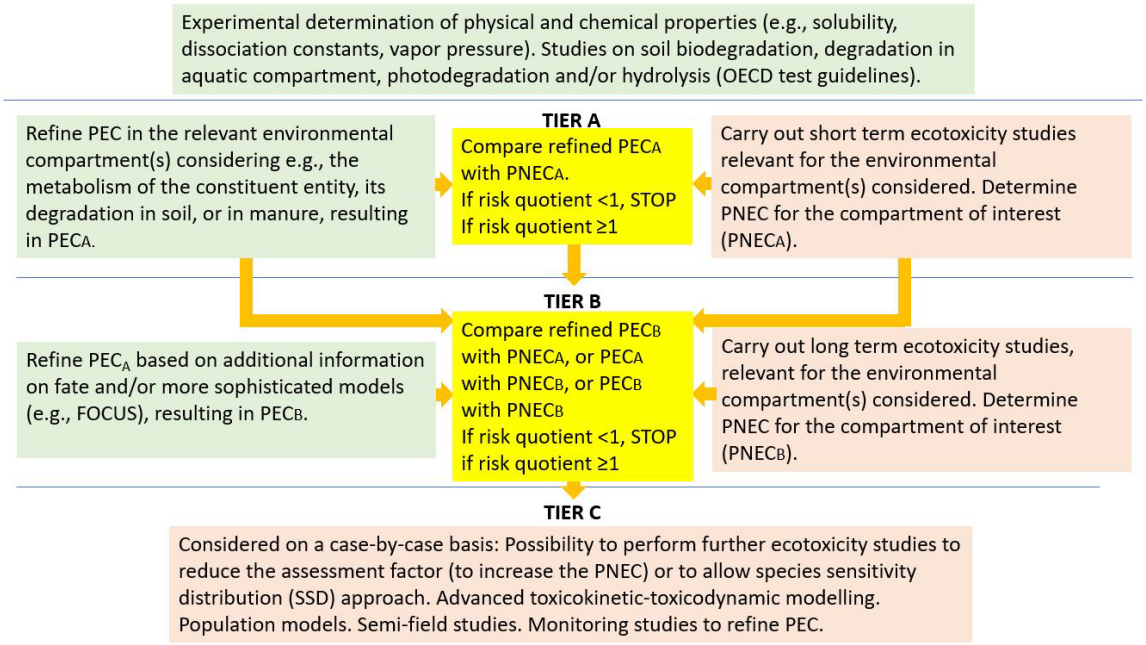
Source: ICCF EWG 'Environmental Risk Assessment Approach', 2023

If, in Phase 1, the feed ingredient or one of its constituent entities was screened as a potential Persistent, Bioaccumulative and Toxic or very Persistent and very Bioaccumulative substance, it is recommended, in Phase 2, to follow the criteria of Annex III of Regulation (EC) 1907/2006 for further assessment.

The Phase 2 risk assessment is based on three (3) main activities, performed in a three (3) tiered approach:

- The refinement of the PEC values calculated in Phase 1 (ICCF Guidance Document on Environmental Risk Assessment Approach (Phase 1), sections 3.1 and 3.2),
- The derivation of the PNEC for the relevant environmental compartment(s) of concern. The selection of ecotoxicological studies is also based on a tiered approach, as described in Figure 1,
- The calculation of the Risk Quotient (RQ) (RQ = PEC/PNEC): If the RQ is less than one (1), no further assessment is necessary. A RQ greater than or equal to one (1) indicates the potential for an environmental risk, because the concentration of the constituent entity(ies) expected in the environment from the proposed conditions of use may elicit an effect on non-target organisms. Therefore, further assessment is thus needed.

Figure 1 – Tiered approach for the Phase 2 environmental risk assessment.



The three (3) Tiers are evaluated sequentially:

- **Tier A:**
  - Refine PEC values calculated in Phase 1 by considering the physical and chemical characteristics and environmental fate of the constituent entity(ies) (PEC_A).
  - Determine the PNEC value(s) for non-target organisms in the relevant environmental compartment(s) from acute/short-term ecotoxicity studies [1] ($PNEC_A$).
  - Calculate the RQ ($RQ=PEC_A/PNEC_A$)
- **Tier B**[2]: if the RQ, calculated under Tier A, is greater than or equal to one (1).
  - Refine PEC_A values based on models, such as the Forum for Coordination of pesticide fate models and their Use (FOCUS) model (PEC_B),
  - Determine PNEC value(s) for non-target organisms in the relevant environmental compartment(s) from chronic/long-term ecotoxicity studies ($PNEC_B$).
  - Calculate the RQ ($RQ = PEC_B/PNEC_B$ or $PEC_A/PNEC_B$ or $PEC_B/PNEC_A$)
- **Tier C:** if the RQ, calculated under Tier B is greater than or equal to one (1).
  - Refine $PNEC_B$ by performing additional ecotoxicity studies to reduce the assessment factor and/or use a Species Sensitivity Distribution (SSD) approach ($PNEC_c$), or
  - Refine $PNEC_B$ by using an advanced modelling approach (e.g. toxicokinetic/toxicodynamic models or population models) ($PNEC_c$), or
  - Perform semi-field studies and/or pre-/post-market monitoring studies, depending on the regulatory authority to refine the PEC (PEC_c). This will support a better understanding of the environmental fate and effects of the constituent entity(ies) under environmental conditions.
  - Calculate the RQ ($RQ = PEC_c/PNEC_c$)

If at the end of the Tier C, the RQ is still greater than or equal to one (1), the constituent entity may elicit an environmental impact, when used under the proposed conditions.

3. PHYSICAL AND CHEMICAL PROPERTIES AND ENVIRONMENTAL FATE OF THE CONSTITUENT ENTITY(IES)

Knowledge of physical and chemical properties is needed to evaluate the fate and the toxicity of the constituent entity(ies). While information may be gathered using *in-silico* estimations (e.g. calculations, Quantitative Structure Activity Relationship (QSARs), read-across),

[1] In some cases, chronic/long-term ecotoxicity studies may be acceptable
[2] In certain cases and circumstances, it may be more appropriate to start with the Tier B

it is recommended to determine these parameters experimentally, preferably using existing Organisation for Economic Co-operation and Development (OECD) test guidelines. If adequately justified, other internationally recognised methods or experimental studies may be used (e.g. International Organization for Standardization [ISO]).

Physical and Chemical Properties

- Solubility in water,
- Dissociation constants in water,
- Vapour pressure,
- Distribution/partitioning between octanol and water,
- Adsorption on organic carbon in soil and water.

Environmental Fate

- Biodegradation in soil,
- Biodegradation in total manure,
- Degradation in the aquatic compartment,
- Photodegradation,
- Hydrolysis.

Depending on the constituent entity(ies) contained in the feed ingredient, it may not be necessary to perform all studies listed below. The need for performing certain studies should be related to the need for refining or calculating the PEC values for the soil and/or water compartment, in view of achieving a RQ (RQ = PEC/PNEC) less than 1. Various options are available for reducing the RQ. Hence, the applicant should explain and justify the choice made for the specific feed ingredient. For instance, biodegradation in total manure may not be needed if there is evidence that the constituent entity(ies) is biodegraded in soil.

3.1. Physical and chemical properties of the constituent entity(ies)

3.1.1. Solubility in water

It is recommended to determine the water solubility of the constituent entity(ies) following OECD guideline No 105.

3.1.2. Dissociation constants in water

It is recommended to determine the dissociation constant(s) of the constituent entity(ies) in water following OECD guideline No 112.

3.1.3. Vapour pressure

It is recommended to determine the vapour pressure of the constituent entity(ies) following OECD guideline No 104.

3.1.4. Octanol/Water partition coefficient

It is recommended to determine the octanol/water partition coefficient (K_{ow}) of the constituent entity(ies) following OECD guidelines No 107, 117 and/or 123. The K_{ow} value may trigger the need for additional information on the capacity of the constituent entity(ies) to bioaccumulate, potentially leading to secondary poisoning (See Section 4.3)

3.1.5. Soil adsorption coefficients

It is recommended to determine the adsorption coefficient (K_d) and the organic carbon normalized adsorption coefficient (K_{oc}) of the constituent entity(ies) on soil following OECD guidelines No 106 and/or 121.

3.2. Environmental fate of the constituent entity(ies)

3.2.1. Soil biodegradation

It is recommended to determine the soil biodegradation of the constituent entity(ies) following OECD guideline No 307. As kinetic results (such as degradation rates and degradation half-lives) are dependent on the soil temperature, it is recommended to use environmentally relevant temperatures. In general, the default temperature of 12°C should be considered and additional temperatures may be considered, depending on the environmental conditions in the various jurisdictions. Data obtained from the study (at 20 °C) can be extrapolated to the default temperature using the Arrhenius equation:

$$k = Ae^{\frac{-Ea}{RT}}$$

Where:

- k is the rate constant
- A is the pre-exponential factor
- e is Euler's number corresponding to 2.71828
- Ea is the molar activation energy for the reaction (J/mol)
- R is the universal gas constant
- T is the absolute temperature (°K)

The evaluation of the biodegradability of the constituent entity(ies) in the terrestrial environment is important when considering the concentration of the constituent entity(ies) in the soil, when manure is spread on the field. The soil biodegradability provides important

information on the behaviour of the constituent entity(ies) when released in the soil and may indicate the degradation or potential for soil accumulation.

3.2.2. Biodegradation in total manure

Biodegradation in solid, liquid, or total manure (liquid + solid) may be evaluated following an appropriate guideline, such as the European Medicine Agency (EMA) guidelines on environmental assessment[3], or the OECD Guideline No 320. The need to evaluate biodegradation in solid, liquid and/or total manure should be discussed with the regulatory authority of the jurisdiction where the feed ingredient is intended to be marketed.

3.2.3. Degradation in the aquatic environment

It is recommended to evaluate the degradation of the constituent entity(ies) in the aquatic environment following OECD guidelines No 302C, 308, 309 and/or 310.

The applicant may use the OECD Guidelines series No 301 for evaluating constituent entity(ies) that are readily biodegradable. However, the results of these studies would only serve as supporting information in the assessment of the biodegradability of the constituent entity(ies) in the aquatic environment.

The evaluation of the biodegradability of the constituent entity(ies) in the aquatic environment may be used as a refinement of the PEC_{water}, if models exist for the jurisdiction, where the feed ingredient is expected to be registered (e.g. FOCUS for the European Union). Aquatic biodegradability provides important information on the behaviour of the constituent entity(ies) when released in water.

3.2.4. Photodegradation

It is recommended to evaluate the photodegradation of the constituent entity(ies) following OECD guideline No 316.

3.2.5. Hydrolysis

It is recommended to evaluate the hydrolysis of the constituent entity(ies) following OECD guideline No 111.

[3] European Medicine Agency (2016). Guideline on environmental impact assessment for veterinary chemical products in support of the VICH guidelines GL6 and GL38, 77 pages.

4. TIER A

4.1. Evaluation of the exposure: Predicted Environmental Concentrations (PEC)

The PEC in soil (PEC_{soil}) of the feed ingredient, or any constituent entity(ies) of concern, calculated in Phase 1 can be refined, to better reflect the conditions of use, based on different options listed below:

- the potential *in vivo* metabolism of any constituent entity(ies) of concern, which may be calculated using the results of the absorption, distribution, metabolism and excretion (ADME) evaluation (ICCF Guidance Document on ADME Evaluation in the context of risk assessment of feed ingredients) (fraction of the constituent entity(ies) excreted) or by measuring the concentration of the constituent entity(ies) of concern in the total manure at the maximum recommended intake of the feed ingredient, and/or
- the potential degradation of the constituent entity(ies) in soil, which may be used to indicate the potential for accumulation, and/or
- the potential degradation of the constituent entity(ies) in total manure during storage.

For the aquatic compartment, the PEC in water (PEC_{water}) of the feed ingredient or any constituent entity(ies) of concern may be refined based on the following options:

- the potential degradation of the constituent entity(ies) in the aquatic compartment, and/or
- the potential photolysis of the constituent entity(ies) in water, and/or
- the potential hydrolysis of the constituent entity(ies) in water, and/or
- the potential adsorption and degradation of the constituent entity(ies) in sediment/soil, and/or
- the potential *in vivo* metabolism of the constituent entity(ies), in the case of land-based aquaculture.

4.1.1. Refinement of the Predicted Environmental Concentration in soil (PEC_{soil})

4.1.1.1. Refinement of PEC_{soil} based on metabolism

The PEC_{soil} may be refined, using the concentration of the constituent entity(ies) and/or its metabolites of concern in total manure.

Metabolites, representing 10% or more of the constituent entity(ies) administered, and/or not forming part of known biochemical pathways, should be considered parent compound(s), and

added to the constituent entity(ies) concentration when estimating the PEC_{soil}. Conversely, metabolites that

- represent less than 10% of the constituent entity(ies) administered, and/or
- are known to not pose a risk to the environment and/or
- enter known biochemical pathways,

can be subtracted from the concentration of the parent constituent entity(ies) predicted to be excreted in the environment.

The PEC_{soil} value can then be refined using Equation 1.

Equation 1 – Calculation of the refined of PEC_{soil} based on metabolism of the constituent entity.

$$PEC_{soilA} = PEC_{soil} \times Exc$$

Where:

- PEC_{soilA} = the refined Tier A Predicted Environmental Concentration in soil based on the metabolism of the constituent entity (mg/kg).
- PEC_{soil} = the Predicted Environmental Concentration in soil calculated in Phase 1 (mg/kg).
- Exc = the fraction of constituent entity and its metabolite(s) excreted by the animals (total manure) and entering the environment.

4.1.1.2. Refinement of PEC_{soil} based on degradation in total manure

When total manure is stored, the constituent entity(ies) and/or its metabolite(s) may degrade in the total manure. In this case, the PEC_{soil} may be further refined based on the potential degradation of the constituent entity(ies) and/or its metabolites (see Section 3.2.2).

Degradation products, representing 10% or more of the constituent entity(ies) administered, and/or not entering known biochemical pathways; should be considered as parent compound(s). Their concentration in manure should be added to the concentration of constituent entity(ies) when refining the PEC_{soil}. Conversely, degradation products representing

- less than 10%, and/or
- are known not to pose an environmental risk, and/or
- enter known biochemical pathways,

may be subtracted from the concentration of the parent constituent entity(ies).

The PEC_{soil} value can then be recalculated using Equations 5 to 9.

Equation 2 – Calculation of the concentration of the constituent entity in total manure

$$PC_{mA} = PC_m \times Exc$$

Where:

- PC_{mA} = the refined Tier A predicted concentration of the constituent entity in the total manure, based on the metabolism of the constituent entity (mg/kg N, mg/kg P, mg/kg total manure).
- PC_m = the predicted concentration of constituent entity in the total manure calculated in Phase 1 (mg/kg N, mg/kg P, mg/kg total manure).
- Exc = the fraction of constituent entity and its metabolite(s) of concern excreted by the animals (total manure) and entering the environment.

Equation 3 – Calculation of the concentration of the constituent entity in the total manure at the time of application in the field (one application per year), considering degradation.

$$PC_{msA} = PC_{mA} \times e^{-k \times T_{st}/2}$$

Where:

- PC_{msA} = the Tier A predicted concentration of constituent entity in total manure at the time of application (mg/kg N, mg/kg P, mg/kg total manure).
- PC_{mA} = the refined Tier A predicted concentration of constituent entity in the total manure (Equation 2).
- k = the rate constant calculated according to Equation 4 (/d).
- Tst/2 = the average length of time of total manure storage (days) (default value: 182.5 days).

Equation 4 – Rate constant of degradation

$$k = \frac{ln2}{DT_{50}}$$

Where:

- k = the constant rate of degradation.
- DT_{50} = the time needed to achieve 50% degradation of the constituent entity in total manure (d).
- ln = the Napierian logarithm or natural logarithm.

Equation 5 – Calculation of the refined PEC_{soil} based on degradation in total manure of the constituent entity.

$$PEC_{soilA} = \frac{PC_{msA} \times Maximum\ load}{Mass\ of\ dry\ soil}$$

Where:

- PEC_{soilA} = the refined Tier A Predicted Environmental Concentration of the constituent entity in soil (mg/kg) after consideration of degradation during total manure storage.
- PC_{msA} = the refined Tier A Predicted Concentration of the constituent entity in the total manure at the time of application (Equation 3) (mg/kg N or mg/kg P or mg/kg total manure).
- Maximum load = the maximum amount of total manure to be spread/injected on a hectare of soil during the defined period, based on its nitrogen (kg N/ha), phosphorus (kg P/ha) or its total mass (kg/ha), as used in Phase 1.
- Mass of soil = the quantity of soil in which the total manure is mixed and is calculated as described in Equation 6.

Equation 6 – Mass of soil

$$Mass\ of\ soil = 1500 \times 10000 \times depth\ of\ soil$$

Where:

- Mass of soil = the quantity of soil in which the total manure is mixed (kg).
- 1500 = the bulk density of dried soil (kg/m^3).
- 10000 = the number of m^2 in one (1) ha.
- Depth of soil in m = the depth of soil to which the total manure is mixed (0.05 m for total manure spreading system with no till or 0.15 m for conventional tilling or total manure injection systems).

4.1.1.3. Refinement of PEC_{soil} based on degradation in soil (potential accumulation in soil)

When excreted, the constituent entity(ies) and/or its metabolite(s) may degrade in the soil. To account for degradation, the PEC_{soil} may be refined based on the DT of the constituent entity(ies) and/or its metabolites (see Section 3.2.1) using Equation 7. For readily biodegradable constituent entities, the calculation of $PEC_{soilplateauA}$ is not necessary.

The potential for the constituent entity(ies) to accumulate in soil should be considered, if the DT_{50} value is greater than or equal to 60 days or the DT_{90} is greater than or equal to one (1) year. The PEC_{soil} is recalculated at steady state ($PEC_{soilplateauA}$) using Equations 7 to 9.

Equation 7 – Calculation of the refined of PEC_{soil} based on the potential of the constituent entity to degrade in soil.

$$\mathrm{PEC}_{soilDTA} = \mathrm{PEC}_{soilA} \times e^{((-0.693\times 365)/\mathrm{DT}_{50})}$$

Where:

- $PEC_{soilDTA}$ = the refined Tier A Predicted Environmental Concentration of the constituent entity in soil after one (1) year (mg/kg), considering its degradation in soil.
- PEC_{soilA} = the Tier A Predicted Environmental Concentration of the feed ingredient in soil (mg/kg), either calculated in Phase 1 or refined based on metabolism (Section 4.1.1.1) and/or refined based on the degradation in total manure (Section 4.1.1.2).
- DT_{50} = the time needed to degrade 50% of the constituent entity in soil at 12 °C (days).
- 365 = the number of days in a year.

Equation 8 – Calculation of the fraction of the constituent entity degraded in one year.

$$Fd = \frac{PEC_{soilA} - PEC_{soilDTA}}{PEC_{soilA}}$$

Where:

- Fd = the fraction of the constituent entity degraded in one (1) year.
- PEC_{soilA} = the Tier A Predicted Environmental Concentration of the feed ingredient in soil (mg/kg), either calculated in Phase 1 or refined based on metabolism (Section 4.1.1.1) and/or refined based on the degradation in total manure (Section 4.1.1.2).
- $PEC_{soilDTA}$ = the refined Tier A Predicted Environmental Concentration of the constituent entity in soil (mg/kg), after considering the degradation of the constituent entity in the soil (Equation 7).

Equation 9 – Calculation of the refined PEC_{soil} at steady state (plateau)

$$PEC_{soilplateauA} = \frac{PEC_{soilA}}{Fd}$$

Where:

- $PEC_{soilplateauA}$ = the refined Tier A Predicted Environmental Concentration of the constituent entity in soil (mg/kg), at steady state.
- PEC_{soilA} = the Tier A Predicted Environmental Concentration of the constituent entity in soil (mg/kg), either calculated in Phase 1 or refined based on metabolism (Section 4.1.1.1) and/or refined based on the degradation in total manure (Section 4.1.1.2).
- Fd = the fraction of the constituent entity degraded in one year (Equation 8).

Kinetic results (such as degradation rates and degradation half-lives) should correspond to an environmentally relevant temperature, i.e. by default 12 °C.

4.1.2. Calculation of the Predicted Environmental Concentration in freshwater ($PEC_{freshwater}$)

Constituent entities of feed ingredients fed to terrestrial species may be transported to surface waters due to run-off from excretion to land (e.g. pasture animals) or from total manure amended cropland.

The PEC for run-off water ($PEC_{porewater}$) is calculated based on a worst-case assumption, where mitigating factors such as:

- movement, desorption, and transformation of the constituent entity(ies), whether at the site of total manure excretion and/or application, or
- interactions of the constituent entity(ies) with crops,

are not considered.

The concentration of the constituent entity(ies) in surface water is estimated assuming that one (1) part run-off/drainage water will be diluted by two (2) parts receiving water. The soil depth is set equal to 0.2 m and is used to calculate the PEC in wet weight soil ($PEC_{soilwwA}$), which is then used to calculate $PEC_{porewaterA}$.

As a result, the PEC in surface water ($PEC_{freshwaterA}$) should be calculated as part of the Phase 2 risk assessment. $PEC_{freshwaterA}$ is derived from $PEC_{porewaterA}$.

The $PEC_{porewater}$ is calculated using Equations 10 to 15.

Equation 10 – Calculation of $PEC_{soilwwA}$

$$PEC_{soilwwA} = \frac{PC_{msA} \times Q}{1700 \times 10000 \times DEPTH_{field}}$$

Where:

- $PEC_{soilwwA}$ = the Tier A Predicted Environmental Concentration of the constituent entity in soil (wet weight) (mg/kg).
- PC_{msA} = the Tier A predicted concentration of the constituent entity in the total manure at the time of application (mg/kg N or mg/kg P or mg/kg total manure), either calculated in Phase 1, or after refinement based on degradation in total manure (Section 4.1.1.2).
- Q = the annual application rate to soil (kg N/ha, kg P/ha, or kg total manure applied/ha).
- 1700 = the bulk density of (wet) soil (kg/m^3).
- 10000 = the number of m^2 in one (1) ha (m^2/ha).
- $DEPTH_{field}$ = the mixing depth in soil (m) (default value: 0.2 m).

Equation 11 – Calculation of the air-water partition coefficient

$$K_{airwater} = \frac{VP \times MOLW}{SOL \times 8.314 \times 285}$$

Where:

- $K_{airwater}$ = the air-water partition coefficient of the constituent entity in soil (m^3/m^3).
- VP = the vapour pressure of the constituent entity (see Section 3.1.3) (Pa).
- MOLW = the molecular mass of the constituent entity (g/mol).
- SOL = the solubility of the constituent entity in water (see Section 3.1.1) (mg/L).
- 8.314 = the gas constant (Pa m^3/mol/°K).
- 285 = the temperature at air-water interface (°K).

Equation 12 – Calculation of the solids-water partition coefficient in soil (v/w)

$$k_{psoil} = 0.02 \times K_{oc}$$

Where:

- K_{psoil} = the solids-water partition coefficient in soil (L/kg).
- 0.02 = the weight fraction of organic carbon in dry weight soil (kg/kg).
- K_{oc} = the organic carbon normalised soil water partition coefficient (L/kg) (soil adsorption/desorption – Section 3.1.5).

Equation 13 – Calculation of the soil-water partition coefficient (v/v)

$$K_{soilwater} = (0.2 \times K_{airwater}) + 0.2 + (0.6 \times \frac{K_{psoil}}{1000} \times 2500)$$

Where:

- $K_{soilwater}$ = the soil-water partition coefficient (m^3/m^3).
- 0.2 = the fraction of air in fresh field soil (m^3/m^3).
- $K_{airwater}$ = the air-water partition coefficient, calculated using Equation 11 (m^3/m^3).
- 0.2 = the fraction of water in fresh field soil (m^3/m^3).
- 0.6 = the fraction of solids in fresh field soil (m^3/m^3).
- K_{psoil} = the solids-water partition coefficient in soil calculated using Equation 12 (L/kg).
- 2500 = the bulk density of soil solids (kg/m^3).

Equation 14 – Calculation of $PEC_{porewaterA}$

$$PEC_{porewaterA} = \frac{PEC_{soilwwA} \times 1700}{K_{soilwater} \times 1000}$$

Where:

- $PEC_{porewaterA}$ = the Tier A Predicted Environmental Concentration of the constituent entity in porewater (mg/L).
- $PEC_{soilwwA}$ = the Tier A Predicted Environmental Concentration of the constituent entity in soil calculated using Equation 10 (wet weight) (mg/kg).
- 1700 = the bulk density of (wet) soil (kg/m^3).
- $K_{soilwater}$ = the solids-water partition coefficient in soil calculated using Equation 13 (m^3/m^3).
- 1000 = the conversion of m^3 to L

The estimated concentration of the constituent entity(ies) after run-off from soil or drainage of the soil to water bodies ($PEC_{freshwaterA}$) is calculated according to Equation 15.

Equation 15 – Calculation of the Predicted Environmental Concentration in freshwater ($PEC_{freshwater}$)

$$PEC_{freshwaterA} = \frac{PEC_{porewaterA}}{3}$$

Where:

- $PEC_{freshwaterA}$ = the Tier A Predicted Environmental Concentration of the constituent entity in freshwater (mg/L).
- $PEC_{porewaterA}$ = the Tier A Predicted Environmental Concentration of the constituent entity in porewater calculated using Equation 14 (mg/L).

4.1.3. Calculation of the Predicted Environmental Concentration in freshwater sediments ($PEC_{sedimentA}$)

For any constituent entity(ies) with a log K_{oc} or log K_{ow} greater than three (3), the potential impact of the constituent entity(ies) in freshwater sediment may need to be assessed[4].

The $PEC_{sedimentA}$ is calculated from the $PEC_{freshwaterA}$ (Equation 15) using the equilibrium partitioning approach in Equation 16.

Equation 16 – Calculation of $PEC_{sedimentA}$

$$PECs_{edimentA} = \frac{K_{suspwater}}{1150} \times PEC_{freshwaterA} \times 1000 \times CONV_{susp}$$

Where:

- $PEC_{sedimentA}$[5] = the Tier A Predicted Environmental Concentration of the constituent entity in the sediment of freshwater (mg/kg dry weight).
- $K_{suspwater}$ = the suspended matter-water partition coefficient (m^3/m^3).
- 1150 = the bulk density of wet suspended matter (kg/m^3).
- $PEC_{freshwaterA}$ = the Tier A Predicted Environmental Concentration of the constituent entity for fresh water (mg/L) calculated using Equation 15.
- 1000 = the conversion from liter (L) to m^3.
- CONVsusp = the conversion from wet to dry weight suspended matter (kg wet weight/kg dry weight), using Equations 17 to 19.

[4] In this guidance document, we recommend a value of three (3). However, this value may depend on the jurisdiction [e.g. in United States (USA), the threshold value is 4].

[5] If the $PNEC_{sedimentA}$ (see Section 4.2.3.3.3) is expressed in mg/kg ww, then it is not necessary to use the factor $CONV_{susp}$ in the calculation (Equation 16)

Equation 17 – Calculation of the suspended matter-water partition coefficient

$$K_{suspwater} = 0.9 \times (F_{solidsusp} \times \frac{K_{psusp}}{1000} \times 2500)$$

Where:

- $K_{suspwater}$ = the suspended matter-water partition coefficient (m^3/m^3).
- 0.9 =the volume fraction of water in the suspended matter (m^3/m^3).
- $F_{solidsusp}$ = the fraction of solids in suspended matter (m^3/m^3 of water-solid – default value 0.1).
- K_{psusp} = the solids-water partition coefficient in suspended matter (v/w) using Equation 19 (L/kg).
- 1000 = the conversion from liter (L) to m^3.
- 2500 = the bulk density of solid in suspended matter (kg dry weight/m^3).

Equation 18 – Calculation of the conversion factor for suspended matter concentration

$$CONV_{susp} = \frac{1150}{F_{solidsusp} \times 2500}$$

Where:

- $CONV_{susp}$ = the conversion factor from wet weight to dry weight suspended matter concentrations (kg wet weight/kg dry weight).
- 1150 = the bulk density of wet suspended matter (kg/m^3).
- $F_{solidsusp}$ = the fraction of solids in suspended matter (m^3/m^3 of water-solid – default value 0.1).
- 2500 = the bulk density of solids in suspended matter(kg/m^3).

Equation 19 - The solids-water partition coefficient in suspended matter (v/w)

$$K_{psusp} = 0.1 \times K_{oc}$$

Where:

- K_{psusp} = the solids-water partition coefficient in suspended matter (L/kg).
- 0.1 = the fraction of organic carbon in suspended matter (kg/kg).
- K_{oc} = the organic carbon normalized partition coefficient (see Section 3.1.5) (L/kg).

4.1.4. Refinement of PEC_{water} based on metabolism (land-based aquaculture)

In land-based aquaculture systems, the constituent entity(ies) may be discharged to surface water. The PEC_{water} calculated in Phase 1 can be further refined based on the metabolism of the constituent entity(ies) in aquatic target species using Equation 20.

Equation 20 – Calculation of the refined PEC_{waterA} based on metabolism.

$$PEC_{waterA} = PEC_{water} \times Exc$$

Where:

- PEC_{waterA} = the refined Tier A Predicted Environmental Concentration of the constituent entity in water used in land-based aquaculture (mg/L).
- PEC_{water} = the Predicted Environmental Concentration of the constituent entity in water used in land-based aquaculture calculated in Phase 1 (mg/L).
- Exc = the fraction of constituent entity and its metabolite(s) excreted by the animals (urine + faeces) and entering the environment.

Retention of the constituent entity(ies) in the system could also be considered for PEC_{waterA} refinement.

Further refinement options for the PEC_{water} depend on the type of freshwater aquaculture system (e.g. pond, open flow/raceway) and it is recommended to consult the regulatory authority of the jurisdiction, where the feed ingredient is intended to be marketed, and their specific guidance document(s)/guideline(s) for further information.

4.2. Evaluation of the effect: Derivation of Predicted No Effect Concentrations from acute/short-term ecotoxicity studies

The following ecotoxicity studies are relevant to feed ingredients and/or any constituent entity(ies) of concern that are intended for terrestrial and/or aquatic species. As noted above, the terrestrial compartment is directly exposed to constituent entities, by direct excretion from the target animal (e.g. pasture animals) or from application of total manure to croplands. In addition, it is expected that the aquatic compartment may also be indirectly exposed to the constituent entities due to run-off from the site of total manure excretion or amended croplands. Therefore, it is recommended that acute/short term ecotoxicity studies be conducted for both the terrestrial and aquatic non-target organisms. It is preferred that the relevant ecotoxicity studies are conducted according to existing OECD guidelines. When properly justified, other internationally recognised methods or experimental study designs may be used (e.g. ISO standards).

4.2.1. Terrestrial compartment

The bioavailability of the constituent entity(ies), and the toxicity observed, may be influenced by soil properties such as organic carbon, clay content, soil pH and soil moisture content. To avoid variability from these properties, and allow an adequate RQ calculation, it may be necessary to normalize the results for a standard soil (organic matter 3.4% or organic carbon content of 2.0±0.5%).

4.2.1.1. Soil microorganisms

It is recommended to evaluate the effect of the constituent entity(ies) on soil micro-organisms following the OECD guideline No 216.

4.2.1.2. Earthworms

It is recommended to evaluate the acute/short-term effect of the constituent entity(ies) on earthworms following the OECD guideline No 207.

In some jurisdictions, chronic/long- term effects in earthworms is required in the Tier A assessment. If this is the case, it is recommended that the chronic/long-term effects in earthworms be evaluated following OECD guidelines No 220 or 222, depending on the expected route of exposure.

4.2.1.3. Terrestrial plants

It is recommended to evaluate the effect of the constituent entity(ies) on terrestrial plants following the OECD guideline No 208, considering a minimum of four (4) plant species.

Alternatively, the study could be conducted following the OECD guideline No 208 with six (6) plant species (at least two (2) monocotyledonous and two (2) dicotyledonous species), which permit the calculation of the respective EC_{50}'s for the constituent entity(ies). This would allow for the use of the same study in Tier B, when needed, provided that an EC_{10} or NOEC can later be derived.

The current approaches for the EU and the US are provided in Table 1.~~When envisaging the use of this alternative, it is recommended to contact the regulatory authority of the jurisdiction, where the feed ingredient is intended to be marketed.~~

Table 2 – Evaluation of potential toxicity on terrestrial plants

Alternatives	European Union (EU)	United States of America (USA)
Four (4) plant species ~~(OECD guideline 208)~~		X
Six (6) plants species ~~(OECD guideline 208)~~	X	

4.2.2. Freshwater compartment

4.2.2.1. Algae

It is recommended to evaluate the effect of the constituent entity(ies) on algae, following the OECD guideline No 201.

4.2.2.2. Daphnia magna

It is recommended to evaluate the effect of the constituent entity(ies) on *Daphnia magna* following the OECD guideline No 202.

4.2.2.3. Fish

It is recommended to evaluate the effect of the constituent entity(ies) on fish following the OECD guideline No 203.

4.2.3. How to derive the Tier A Predicted No Effect Concentrations

The Tier A PNEC is determined by dividing the toxicity endpoints derived from each of the terrestrial and aquatic taxonomic levels identified in Section 4.2.2 by the relevant assessment factor (AF) (Equation 21). Assessment factors account for uncertainties related to intra- and inter-laboratory variation in toxicity data, intra- and interspecies variation, and extrapolation from laboratory study results to the field.

Equation 21 – Application of assessment factors to derive the PNEC.

$$PNEC = \frac{Tox_{endpoint}}{AF}$$

Where:

- PNEC = the Predicted No Effect Concentration of the constituent entity for the relevant non-target organism (mg/kg or mg/L).
- $Tox_{endpoint}$ = the toxicity endpoint (mg/kg or mg/L).

- AF = the Assessment Factor.

4.2.3.1. Toxicity endpoint selection for the terrestrial compartment

The lowest effect concentration of the constituent entity(ies) is selected for each of the taxonomic levels (i.e. microorganisms, earthworms, terrestrial plants) in the terrestrial compartment based on the ecotoxicity studies recommended in Section 4.2.1.

Ecotoxicity study scope	Toxicity endpoints considered		Remark	
Soil microorganisms *Section 4.2.1.1*	Lower than or equal to 25 % of the control		Nitrogen transformation	
Earthworms *Section 4.2.1.2*	Acute/short term EC_{50}	Chronic/long term NOEC	Acute/short term mortality	Chronic/long term reproductive output
Terrestrial plants *Section 4.2.1.3*	EC_{50}		The most sensitive effects value for all endpoints examined for each plant species tested.	

4.2.3.2. Toxicity endpoint selection for the freshwater compartment

The lowest effect concentration of the constituent entity(ies) is selected for each of the taxonomic levels (i.e. algae, *Daphnia magna*, fish) in the aquatic compartment based on the ecotoxicity studies recommended in Section 4.2.2.

Ecotoxicity study scope	Toxicity endpoints considered	Remark
Algae *Section 4.2.2.1*	EC_{50}	Growth rate after 72 h exposure
Daphnia magna *Section 4.2.2.2*	EC_{50}	Immobilisation after 48h exposure
Fish *Section 4.2.2.3*	LC_{50}	Mortality after 96h exposure

4.2.3.3. Assessment factors

Assessment factors vary depending on each jurisdiction. It is therefore advised to consult the regulatory authority of the jurisdiction, where the feed ingredient is intended to be marketed (see Tables 2 and 3, for examples). No assessment factor is applied for studies on soil microorganisms.

Table 3 – Assessment factors adopted by different jurisdictions and used to determine the $PNEC_{soilA}$ values.

Jurisdiction	$PNEC_{soilA}$		
	Earthworms EC_{50}	Earthworms NOEC	Terrestrial plants EC_{50}
United States	NA(1)	10	100
European Union	1000	10	100

(1)NA = not applicable

Table 4 – Assessment factors adopted by different jurisdictions and used to determine the $PNEC_{freshwaterA}$ values.

Jurisdiction	$PNEC_{freshwaterA}$		
	Algae EC_{50}	*Daphna magna* EC_{50}	Fish LC_{50}
United States	100	1000	1000
European Union	1000	1000	1000

4.2.3.3.1. Predicted No Effect Concentration for the terrestrial compartment

The Tier A PNEC in the terrestrial compartment ($PNEC_{soilA}$) is determined by dividing the appropriate toxicity endpoint defined in Section 4.2.3.1/4.2.3.3.1 by the relevant assessment factor in Section 4.2.3.3 (or as discussed with the regulatory authority of the jurisdiction, where the feed ingredient is intended to be marketed)

Equation 22 – Evaluation of $PNEC_{soilA}$

$$PNEC_{soilA} = \frac{Tox_{endpointsoil}}{AF}$$

Where:

- $PNEC_{soilA}$ = the Tier A Predicted No Effect Concentration of the constituent entity in soil (mg/kg).
- $Tox_{endpointsoil}$ = the toxicity endpoint (mg/kg), defined in Section 4.2.3.
- AF = Assessment Factor.

4.2.3.3.2. Predicted No Effect Concentration for the freshwater compartment

The Tier A PNEC in the freshwater compartment ($PNEC_{freshwaterA}$) is determined by dividing the appropriate toxicity endpoint defined in Section 4.2.3.2 by the relevant assessment factor in Section 4.2.3.3 (or as discussed with the regulatory authority of the jurisdiction, where the feed ingredient is intended to be marketed)

Equation 23 – Evaluation of $PNEC_{freshwaterA}$

$$PNEC_{freshwaterA} = \frac{Tox_{endpointfreshwater}}{AF}$$

Where:

- $PNEC_{freshwaterA}$ = the Tier A Predicted No Effect Concentration of the constituent entity in freshwater (mg/L).
- $Tox_{endpointfreshwater}$ = the toxicity endpoint (mg/L), defined in in Section 4.2.3.
- AF = the Assessment Factor (See Table 3).

4.2.3.3.3. Predicted No Effect Concentration for the freshwater sediment compartment

Depending on the physical and chemical characteristics of the constituent entity, its potential effect on freshwater sediment-dwelling organisms should be considered. For example, a constituent entity with a strong tendency to partition to sediment should be further evaluated. In addition, depending on the jurisdiction, specific requirements for when to evaluate the sediment compartment may exist. For example, the EU requires that the sediment compartment be considered if the log K_{ow} is greater than 3, while the US considers the results of the Tier A *Daphnia magna* immobilization test (see Section 4.2.2.2).

The $PNEC_{sedimentA}$ is then derived from the $PNEC_{freshwaterA}$ using the equilibrium partition coefficient approach using Equation 24. Furthermore, if the log K_{ow} is greater than five (5), an additional assessment factor of ten (10) is applied to the results obtained in Equation 24, to account for risk due to sediment ingestion.

Equation 24 – Calculation of the Predicted No-Effect Concentration in sediments of fresh water ($PNEC_{sedfimentrA}$)

$$PNEC_{sedimentA} = \frac{K_{suspwater}}{1150} \times PNEC_{freshwaterA} \times 1000 \times \mathrm{CONV}_{susp}$$

Where:

- $PNEC_{sedimentA}$ = the Tier A Predicted No Effect Concentration for sediment dwelling organisms (mg/kg_{dw}).
- $K_{suspwater}$ = the suspended matter-water partition coefficient, calculated using Equation 17 (m^3/m^3).
- 1150 = the bulk density of wet suspended matter (kg/m^3).
- $PNEC_{freshwaterA}$ = the Tier A Predicted No Effect Concentration for fresh water assessed in accordance with Section 4.2.3.2 (mg/L).
- 1000 = the conversion from liter to m^3.
- $CONV_{susp}$ = the conversion factor for suspended matter concentration (kg_{ww}/kg_{dw}) using Equation 19.

4.2.4. Risk Quotient and conclusion

The environmental risk of the feed ingredient and/or its constituent entity is characterized using the RQ method (RQ = PEC/PNEC) to compare the refined or calculated Tier A PEC values (See Section 4.1) with the determined Tier A PNEC value (Section 4.2.3) for the relevant environmental compartment.

If the RQ is lower than one (1), no further information is necessary and the constituent entity(ies) of the feed ingredient is not expected to result in significant environmental risk, under the proposed conditions of use. If no constituent entities of the feed ingredient are expected to result in significant environmental risk, the feed ingredient is also not expected to result in significant environmental risk, under the proposed conditions of use.

If the RQ is greater than or equal to one (1), the applicant should provide further information on the environmental fate and/or the ecotoxicity of the constituent entity(ies) of concern, by following the recommendations described in Tier B (Section 5).

4.3. Bioaccumulation and secondary poisoning

The log K_{ow} value should be used to determine the potential for a constituent entity to bioaccumulate in the environment. If the log K_{ow} value is above a certain threshold, the applicant should provide further information on the potential of the constituent entity(ies) to bioaccumulate and the risk for secondary poisoning in non-target species should be assessed. This information should consider evidence from ADME studies (ICCF Guidance Document on ADME

Evaluation in the context of risk assessment of feed ingredients) and/or biodegradation studies (see Section 3.2).

The log K_{ow} threshold varies depending on the jurisdiction and their specific objectives with regards to the potential for bioaccumulation of the constituent entity(ies) in the environment. It is advised to consult the regulatory authority of the jurisdiction, where the feed ingredient is intended to be marketed (see Table 4 for examples).

Table 5 – Threshold value of log K_{ow} adopted by different jurisdictions and used to determine the need for bioaccumulation and secondary poisoning.

Jurisdiction	Threshold value (log K_{ow})	Reference
United States	4	CVM Guidance for Industry #166
European Union	3	European Chemicals Agency (ECHA), 2018

When it is necessary, the Bioconcentration Factor (BCF) of the constituent entity(ies) should be determined, based on:

- a Quantitative Structure Activity Relationship (QSAR)[6], or
- an evaluation of the persistence of the constituent entity(ies) and, if persistent based on the half-life, the potential to bioaccumulate based on measured data in aquatic species[7] should be determined, or
- the measurement of the BCF in fish following the OECD guideline No 305.

If the BCF is greater than 1000, it is expected that the constituent entity(ies) may bioaccumulate in the environment and the evaluation of secondary poisoning should be assessed[8, 9].

[6] Further details on the application of QSAR can be found in the ECHA guidance Chapter R6
[7] Further details may be obtained using the REACH part C.
[8] Note that secondary poisoning assessment may not be required by the jurisdiction where the feed ingredient is planned to be marketed. Advice should be sought from the relevant jurisdiction, before evaluating further the secondary poisoning potential of the feed ingredient.
[9] For further information: feed additives applications tool (www.efsa.europa.eu) FERA calculation tool, according to the section 3.3.2 of the EMA Guideline on environmental impact assessment for veterinary medicinal products in support of the VICH guidelines GL6 and GL38) (www.ema.europa.eu)

5. TIER B

In Tier B, the exposure from freshwater and/or freshwater sediment can be further refined by using advanced models to reduce the PEC values from Tier A. In addition, the PNEC can be further refined based on chronic/long-term ecotoxicity studies that will allow the application of a lower AF.

5.1. Refinement of the Predicted Environmental Concentration in the surface water and sediment for feed ingredients intended for use in terrestrial and/or aquatic species

The $PEC_{freshwaterA}$ may be further refined using advanced models, such as FOCUS to obtain the $PEC_{freshwaterB}$ and/or $PEC_{sedimentB}$.

5.2. Evaluation of the effect: Derivation of Predicted No Effect Concentrations from chronic ecotoxicity studies

The following ecotoxicity studies are recommended for feed ingredients, or any constituent entity(ies) of concern they contain that are intended to be used in terrestrial and/or aquatic species. It is recommended to conduct the chronic/long-term ecotoxicity studies according to existing OECD guidelines. When properly justified, other internationally recognised methods or experimental studies may be used (e.g. ISO).

5.2.1. Terrestrial compartment

The bioavailability of the constituent entity(ies), and the toxicity observed, may be influenced by soil properties such as organic carbon, clay content, soil pH, and soil moisture content. To avoid variability from these properties, and allow an adequate calculation of the RQ, it may be necessary to normalize the results for a standard soil (organic matter 3.4% or organic carbon content of $2.0 \pm 0.5\%$).

It is recommended that the following chronic/long-term ecotoxicity studies be conducted for feed ingredients intended for terrestrial species. In some instances, it may be appropriate to use these ecotoxicity studies for feed ingredients fed to aquatic species (e.g. if faeces collected from land-based aquaculture systems are applied to cropland).

5.2.1.1. Soil microorganisms

It is recommended to evaluate chronic/long-term exposure effects of the constituent entity(ies) on soil microorganisms following the OECD guideline No 216. The duration of the study may vary from a minimum of 28 days to a maximum of 100 days, depending on the constituent entity(ies) tested. The study shall be run until the differences in nitrate formation between treated and untreated group is greater than or equal to 25%.

5.2.1.2. Earthworms

If chronic/long-term exposure effects on earthworms have already been evaluated under Tier A (See Section 4.2.1.2), refinement of the toxicity endpoint is not necessary; and no additional studies are needed. Otherwise, it is recommended to evaluate the chronic/long-term exposure effects of the constituent entity(ies) on earthworms following the OECD guideline No 220/222. Testing another soil invertebrate (either springtail *Folsomia candida* following OECD Guideline No 232 or on the predatory mite *Hypoaspis acleifer* following OECD guideline No 226) may be needed in some jurisdictions, depending on the relative sensitivity of the most sensitive terrestrial plant in relation to earthworms (see Section 5.2.1.3).

5.2.1.3. Terrestrial plants

It is recommended to evaluate the chronic/long-term exposure effects of the constituent entity(ies) on terrestrial plants by conducting a second toxicity study following the OECD guideline No 208, using two additional species from the most sensitive species category from the Tier A study (see Section 4.2.1.3), in addition to using the same sensitive species. If six (6) plant species were evaluated in the Tier A terrestrial plant study, then the EC_{10} or NOEC from the same study may be used in the Tier B. However, the applicant should contact the regulatory authority of the jurisdiction, where the feed ingredient is intended to be marketed, to confirm if this option is appropriate. Out of the six (6) terrestrial plants tested, at least two (2) should be monocotyledonous and two (2) dicotyledonous.

5.2.2. Freshwater compartment

The following chronic/long-term ecotoxicity studies are recommended for feed ingredients intended for terrestrial and/or aquatic species.

5.2.2.1. Algae

It is recommended to evaluate the chronic/long-term exposure effects of the constituent entity(ies) on algae, following OECD guideline No 201. In Tier B, the EC_{10} or a NOEC shall be determined.

Alternatively, the test conducted under Tier A may be used to derive the EC_{10} or NOEC. The use of this alternative would require contacting the regulatory authority of the jurisdiction, where the feed ingredient is intended to be marketed.

5.2.2.2. Daphnia magna

It is recommended to evaluate the chronic/long-term exposure effects of the constituent entity(ies) on *Daphnia magna* following the OECD guideline No 211. However, if the acute toxicity level/concentration determined in Tier A for *Daphnia magna* (see Section 4.2.2.2), and fish (see Section 4.2.2.3) is one (1) order of magnitude lower than the acute effect concentration in algae (E_rC_{50}) (see Section 4.2.2.1), this chronic/long-term study may be necessary, depending on the jurisdiction.

5.2.2.3. Fish

It is recommended to evaluate the chronic/longer-term effect of the constituent entity(ies) on fish following the OECD guideline No 210. However, if the acute toxicity level/concentration determined in Tier A for *Daphnia magna* (see Section 4.2.2.2) and fish (see Section 4.2.2.3) is one (1) order of magnitude lower than the acute effect concentration in algae (EC_{50}) (see Section 4.2.2.1), this chronic/long-term study may be necessary, depending on the jurisdiction.

5.2.3. Freshwater Sediment Compartment

In addition to the ecotoxicity studies described above, it is recommended to evaluate the toxicity of the constituent entity(ies) on benthic non-target organisms in accordance with the relevant guidelines provided by ISO, the US Environmental Protection Agency (EPA) or OECD, if appropriate.

If the RQ for the freshwater sediment compartment calculated in Tier A is greater than or equal to one (1), the risk of sediment exposure to benthic organisms shall be evaluated. For this purpose, it is recommended to evaluate the chronic/long-term exposure effects of the constituent entity(ies) on benthic organisms using one of the OECD guidelines No 218, 219, 225, 239, or 315. The applicant should justify the choice of the guidance documents applied, based on potential advice from the regulatory authority of the jurisdiction, where the feed ingredient is intended to be marketed.

The organic carbon content of sediment may influence bioavailability and consequently the toxicity of the constituent entity. Therefore, for comparison of sediment tests, the organic carbon content of the test sediment should be within a certain range (about 2.0±0.5%). For the risk characterization, the toxicity estimates underlying the $PNEC_{freshwater}$ should be normalized to the

same organic carbon content that is used in the PEC calculation, i.e. 10 % of organic carbon in the dry sediment.

5.2.4. How to derive the Tier B Predicted No Effect Concentrations

The Tier B PNEC is determined by dividing the toxicity endpoints derived from each of the terrestrial and aquatic taxonomic levels identified in Sections 5.2.1, 5.2.2 and/or 5.2.3 by the relevant assessment factor (AF) (Equation 21). Assessment factors account for the uncertainties related to intra- and inter-laboratory variation in toxicity data, intra- and interspecies variation, and the extrapolation from laboratory studies results to the field.

5.2.4.1. Toxicity endpoint selection for the terrestrial compartment

The lowest effect or no observed effect concentration of the constituent entity(ies) is selected for each of the taxonomic levels (i.e. microorganisms, earthworms, terrestrial plants) in the terrestrial compartment, based on ecotoxicity studies recommended in Section 5.2.1.

Ecotoxicity study scope	Toxicity endpoints considered	Remark
Soil microorganisms *Section 5.2.1.1*	Lower than or equal to 25 % of the control	
Earthworms *Section 5.2.1.2*	EC_{10} or NOEC	A second soil invertebrate may be necessary.
Terrestrial plants *Section 5.2.1.3*	EC_{10} or NOEC	The most sensitive endpoint of all plants tested and retesting of two additional species of the most sensitive category (USA). In EU the same study as in Tier A may be adequate provided that EC_{10} or NOEC can be derived.

5.2.4.2. Toxicity endpoint selection for the freshwater compartment

The lowest effect or no observed effect concentration of the constituent entity(ies) is selected based on the ecotoxicity studies conducted from those recommended in Section 5.2.2.

Ecotoxicity study scope	Toxicity endpoints considered	Remark
Algae *Section 5.2.2.1*	E_rC_{10} or NOEC	72h to 96h If the EC_{10} or NOEC are not reported on the growth, it may be replaced by the EC_{10}, or NOEC measured on the yield.
Daphnia magna *Section 5.2.2.2*	EC_{10} or NOEC	Reproductive output after 21 days of exposure
Fish *Section 5.2.2.3*	EC_{10} or NOEC	Duration dependent on the species tested

5.2.4.3. Toxicity endpoint selection for the freshwater sediment compartment

The lowest effect or no observed effect concentration of the constituent entity(ies) is selected for each of the taxonomic levels (i.e. algae, *Daphnia magna*, fish*)* based on the ecotoxicity studies recommended in Section 5.2.3.

5.2.4.4. Assessment factors

Assessment factors vary depending on the jurisdiction and their specific objectives with regards to the Tier B PNEC concentration of constituent entity(ies) in the environment. It is therefore advised to consult the regulatory authority of the jurisdiction, where the feed ingredient is intended to be marketed (see Tables 5 to 7, for examples)

Table 6 – Assessment factors adopted by different jurisdictions and used to determine the $PNEC_{soilB}$ values.

Jurisdiction	$PNEC_{soilB}$		
	Soil microorganisms	Earthworms EC_{10} or NOEC	Terrestrial plants EC_{10} or NOEC
United States	NA	NA	10
European Union	1	10	10

Table 7 – Assessment factors adopted by different jurisdictions and used to determine the $PNEC_{freshwaterB}$ values.

Jurisdiction	$PNEC_{freshwaterB}$		
	Algae E_rC_{10} or NOEC	*Daphnia magna* EC_{10} or NOEC	Fish EC_{10} or NOEC
United States	10	10	10
European Union	100, 50, or 10*	100, 50, or 10*	100, 50, or 10*

* AF depending on the number of studies: 100 for one (1) chronic/long-term study in algae, 50 for two (2) chronic/long-term studies (algae and *Daphnia magna* or fish), 10 for three (3) chronic/long-term studies (algae, *Daphnia magna* and fish)

Table 8 – Assessment factors adopted by different jurisdictions and used to determine the $PNEC_{sedfreshwaterB}$ values.

Jurisdiction	$PNEC_{sedfreshwaterB}$		
	One study on *Chironomid*	Two studies (*Chironomid* and *Lumbriculus*)	Three studies (*Chironomid* and *Lumbriculus* and another benthic species)
United States	10	NA	NA
European Union	100	50	10

5.2.4.5. Predicted No Effect Concentration for the terrestrial compartment

The Tier B PNEC in the terrestrial compartment ($PNEC_{soilB}$) is determined by dividing the appropriate toxicity endpoint defined in Section 5.2.4.1 by the relevant assessment factor in Section 5.2.4.4 (or as discussed with the regulatory authority of the jurisdiction, where the feed ingredient is intended to be marketed).

Equation 25 – Evaluation of $PNEC_{soilB}$

$$PNEC_{soilB} = \frac{Tox_{endpointsoilB}}{AF}$$

Where:

- $PNEC_{soilB}$ = the Tier B Predicted No Effect Concentration of the constituent entity in soil (mg/kg).
- $Tox_{endpointsoilB}$ = the toxicity endpoint (mg/kg), based on the evaluation of the studies in Section 5.2.1.
- AF = the Assessment Factor (See Table 5).

5.2.4.6. Predicted No Effect Concentration for the freshwater compartment

The Tier B PNEC in the freshwater compartment ($PNEC_{freshwaterB}$) is determined by dividing the appropriate toxicity endpoint defined in Section 5.2.2 by the relevant assessment factor in Section 5.2.4.4 (or as discussed with the regulatory authority of the jurisdiction, where the feed ingredient is intended to be marketed).

Equation 26 – Evaluation of $PNEC_{freshwaterA}$

$$PNEC_{freshwaterB} = \frac{Tox_{endpointfreshwaterB}}{AF}$$

Where:

- $PNEC_{freshwaterB}$ = the Tier B Predicted No Effect Concentration of the constituent entity in fresh water (mg/L).
- $Toxendpoint_{freshwaterB}$ = the toxicity endpoint (mg/L), based on the evaluation of the studies in Section 5.2.2.
- AF = the Assessment Factor (See Table 6).

5.2.4.7. Predicted No Effect Concentration for the freshwater sediment compartment

The Tier B PNEC in the freshwater sediment compartment ($PNEC_{sedimentB}$) is determined by dividing the appropriate toxicity endpoint defined in Section 5.2.3 by the relevant assessment factor in Section 5.2.4.4 (or as discussed with the regulatory authority of the jurisdiction, where the feed ingredient is intended to be marketed).

Equation 27 – Evaluation of $PNEC_{sediment}$

$$PNEC_{sedimentB} = \frac{Tox_{endpointsediment}}{AF}$$

Where:

- $PNEC_{sedimentB}$ = the Tier B Predicted No Effect Concentration of the constituent entity in freshwater sediment (mg/kg or mg/L).
- $Tox_{endpointsediment}$ = the toxicity endpoint (mg/kg or mg/L), based on the evaluation of the studies in Section 5.2.3.
- AF = the Assessment Factor (See Table 7).

5.2.5. Risk quotient and conclusion

The environmental risk of the constituent entity(ies) is characterized using the RQ method (RQ = PEC/PNEC) to compare the refined Tier B PEC values (see Section 5.1) or, if appropriate, with the Tier A PEC value (see Section 4.1) with the determined Tier B PNEC value (Section 5.2.4) for the relevant environmental compartment.

If the RQ is lower than one (1), no further information is necessary and the constituent entity(ies) of the feed ingredient is not expected to result in significant environmental risk, under the proposed conditions of use. If no constituent entities of the feed ingredient are expected to result in a significant environmental risk, the feed ingredient is also not expected to result in significant environmental risk, under the proposed conditions of use.

If the RQ is greater than or equal to one (1), the applicant should provide further information on the environmental fate and/or the ecotoxicity of the constituent entity(ies), by following the recommendations described in Tier C (Section 6)

6. TIER C

The further evaluation of the environmental safety of the constituent entity(ies) may involve:

- Conducting chronic/long-term laboratory toxicity tests with additional species, allowing the Species Sensitivity Distribution (SSD) approach, and/or
- Using advanced modelling approaches (e.g. toxicokinetic/toxicodynamic, or population models), and/or
- Conducting semi-field experiments, and/or

- Conducting pre-/post-market monitoring studies to better understand the behaviour and toxicity of the feed ingredient under existing conditions, depending on the regulatory authority.

As this situation is highly dependent on the constituent entity(ies), it is recommended to seek advice from the regulatory authority of the jurisdiction, where the feed ingredient is planned to be used.

7. ABBREVIATIONS

ADME	Absorption, Distribution, Metabolism, and Excretion
AF	Assessment Factor
BCF	Bioconcentration Factor
DT	Degradation Time
dw	Dry weight
EC_{10}	Effect Concentration on 10 % of the population
EC_{50}	Median Effective Concentration
EMA	European medicine Agency
ErC_{10}	Effect concentration based on the growth rate on 10 % of the population
FOCUS	Forum for Coordination of pesticide fate models and their Use
LC_{50}	Median Lethal Concentration
K_d	Adsorption coefficient
K_{oc}	Organic-carbon normalized adsorption coefficient
K_{ow}	Octanol/Water partition coefficient
NOEC	No-Observed Effect Concentration
PEC	Predicted Environmental Concentration
PNEC	Predicted No Effect Concentration
QSAR	Quantitative Structure Activity Relationship
RQ	Risk Quotient
SSD	Species Sensitivity Distribution

ww	Wet weight

8. BIBLIOGRAPHY

8.1. ICCF

ICCF EWG 'Feed Ingredients Environmental Safety Assessment Approach' (2023). Tarrés-Call J., Cluster, R., Johnson-Couch, K., Patterson, S., Maier, M., Heintz, M. Nasir, Z., Teeter, J. Guidance on feed ingredient environmental risk assessment approach. 22 pages.

ICCF EWG 'ADME Evaluation' (2024). Betancourt A., Chen C. Q., Manini P., Ladics G., Smith M., Hahn U., Fraatz K. Guidance on ADME evaluation in the context of risk assessment of feed ingredients. 29 pages.

8.2. OECD

Organisation for Economic Co-operation and Development (OECD) (1995). OECD Guidelines No 105 on water solubility - OECD Guidelines for the Testing of Chemicals, 7 pages.

Organisation for Economic Co-operation and Development (OECD) (1981). OECD Guidelines No 112 on dissociation constants in water (titration method – spectrophotometric method – conductometric method) – OECD Guidelines for the Testing of Chemicals,7 pages.

Organisation for Economic Co-operation and Development (OECD) (2022). OECD Guidelines No 117 on estimation of the partition coefficient (n-octanol/water), High Performance Liquid Chromatography (HPLC) method - OECD Guidelines for the Testing of Chemicals, 12 pages.

Organisation for Economic Co-operation and Development (OECD) (2001). OECD Guidelines No 121 on estimation of the adsorption coefficient (K_{oc}) on soil and on sewage sludge using High Performance Liquid Chromatography (HPLC) – OECD Guidelines for the Testing of Chemicals, 11 pages.

Organisation for Economic Co-operation and Development (OECD) (2006). OECD Guidelines No 201 on freshwater algae and cyanobacteria, growth inhibiting test - OECD Guidelines for the Testing of Chemicals, 25 pages.

Organisation for Economic Co-operation and Development (OECD) (2004) OECD Guidelines No 202 on Daphnia sp., acute immobilisation Test - OECD Guidelines for the Testing of Chemicals, 12 pages.

Organisation for Economic Co-operation and Development (OECD) (2019). OECD Guidelines No 203 on fish acute toxicity test - OECD Guidelines for the Testing of Chemicals, 24 pages.

Organisation for Economic Co-operation and Development (OECD) (1984). OECD Guidelines No 207 on earthworm acute toxicity test - OECD Guidelines for the Testing of Chemicals,9 pages.

Organisation for Economic Co-operation and Development (OECD) (2006). OECD Guidelines No 208 on terrestrial plant test: seedling emergence and seedling growth test - OECD Guidelines for the Testing of Chemicals, 21 pages.

Organisation for Economic Co-operation and Development (OECD) (2013). OECD Guidelines No 210 on fish, early-life stage toxicity test - OECD Guidelines for the Testing of Chemicals, 24 pages.

Organisation for Economic Co-operation and Development (OECD) (2012). OECD Guidelines No 211 on daphnia magna reproduction test - OECD Guidelines for the Testing of Chemicals, 26 pages.

Organisation for Economic Co-operation and Development (OECD) (2000). OECD Guidelines No 216 on soil microorganisms: Nitrogen transformation test - OECD Guidelines for the Testing of Chemicals, 10 pages.

Organisation for Economic Co-operation and Development (OECD) (2023). OECD Guidelines No 218 on sediment-water chironomid toxicity using spiked sediment - OECD Guidelines for the Testing of Chemicals, 29 pages.

Organisation for Economic Co-operation and Development (OECD) (2023). OECD Guidelines No 219 on sediment-water chironomid toxicity using spiked water - OECD Guidelines for the Testing of Chemicals, 28 pages.

Organisation for Economic Co-operation and Development (OECD) (2016). OECD Guidelines No 220 on enchytraeid Reproduction Test - OECD Guidelines for the Testing of Chemicals, 24 pages.

Organisation for Economic Co-operation and Development (OECD) (2016). OECD Guidelines No 222 on earthworm reproduction test (*Eisenia fetida*/*Eisenia Andrei*) - OECD Guidelines for the Testing of Chemicals, 21 pages.

Organisation for Economic Co-operation and Development (OECD) (2007) OECD Guidelines No 225 on sediment-water Lumbriculus toxicity test using spike sediment - OECD Guidelines for the Testing of Chemicals, 31 pages.

Organisation for Economic Co-operation and Development (OECD) (2016). OECD Guidelines No 226 on predatory mite (*Hypoaspis Geolaelaps*) reproduction test in soil - OECD Guidelines for the Testing of Chemicals, 26 pages.

Organisation for Economic Co-operation and Development (OECD) (2016). OECD Guidelines No 232 on collembolan reproduction test in soil - OECD Guidelines for the Testing of Chemicals, 22 pages.

Organisation for Economic Co-operation and Development (OECD) (2014). OECD Guidelines No 239 on water-sediment *myriophyllum spicatum* toxicity test - OECD Guidelines for the Testing of Chemicals, 18 pages.

Organisation for Economic Co-operation and Development (OECD) (2009). OECD Guidelines No 302 C on inherent biodegradability: modified MITI test – OECD Guidelines for the Testing of Chemicals, 22 pages.

Organisation for Economic Co-operation and Development (OECD) (2012). OECD Guidelines No 305 on the bioaccumulation in fish: aqueous and dietary exposure - OECD Guidelines for the Testing of Chemicals, 72 pages.

Organisation for Economic Co-operation and Development (OECD) (2002). OECD Guidelines No 307 on aerobic and anaerobic transformation in soil - OECD Guidelines for the Testing of Chemicals, 17 pages.

Organisation for Economic Co-operation and Development (OECD) (2002). OECD Guidelines No 308 on aerobic and anaerobic transformation in aquatic sediment systems - OECD Guidelines for the Testing of Chemicals, 19 pages.

Organisation for Economic Co-operation and Development (OECD) (2004). OECD Guidelines No 309 on aerobic mineralisation in surface water – simulation biodegradation test - OECD Guidelines for the Testing of Chemicals, 21 pages.

Organisation for Economic Co-operation and Development (OECD) (2014). OECD Guidelines No 310 on readily biodegradability – CO2 in sealed vessels (headspace test) - OECD Guidelines for the Testing of Chemicals, 18 pages.

Organisation for Economic Co-operation and Development (OECD) (2008). OECD Guidelines No 315 on bioaccumulation in sediment-dwelling benthic oligochaetes - OECD Guidelines for the Testing of Chemicals, 33 pages.

Organisation for Economic Co-operation and Development (OECD) (2023). OECD Guidelines No 316 on phototransformation of chemicals in water – direct photolysis - OECD Guidelines for the Testing of Chemicals, 56 pages.

Organisation for Economic Co-operation and Development (OECD) (2010). OECD Guidelines No 317 on the bioaccumulation in terrestrial oligochaetes - OECD Guidelines for the Testing of Chemicals, 30 pages.

Organisation for Economic Co-operation and Development (OECD) (2022). OECD Guideline No 320 determining anaerobic transformation of chemicals in liquid total manure, 30 June 2022 – OECD Guidelines for the Testing of Chemicals, 30 pages.

8.3. VICH

VICH (2004). Environmental Impact Assessment for Veterinary Medicinal products – Phase II guidance, 38 pages.

8.4. European Union

Regulation (EC) No 1907/2006 of the European Parliament and of the Council of 18 December 2006 concerning the Registration, Evaluation, Authorisation and Registration of Chemicals (REACH) and establishing a European Chemicals Agency, amending Directive 1999/45/European Commission (EC) and repealing Council Regulation (EEC) No 793/93 and Commission Regulation (European Commission (EC)° NO 1488/94 as well as Council Directive 76/769/EEC and Commission Directives 91/155/EEC, 93/67/EEC, 93/105/European Commission (EC) and 2000/21/European Commission (EC). Official Journal of the European Union L396, 30.12.2006, p.1, http://data.europa.eu/eli/reg/2006/1907/2024-06-06 (consolidated version).

Commission Regulation (EC) No 429/2008 of 25 April 2008 on detailed rules for the implementation of regulation (EC) No 1831/2003 of the European Parliament and of the Council as regards the preparation and the presentation of applications and the assessment and the authorisation od feed additives – Official Journal of the European Union L133/1, http://data.europa.eu/eli/reg/2008/429/2021-03-27 (consolidated version).

European Medicine Agency (2016). Guideline on environmental impact assessment for veterinary chemical products in support of the VICH guidelines GL6 and GL38, 77 pages.

European Chemical Authority (2008). Guidance on information requirements and chemical safety assessment. Chapter R.6: QSARSs and grouping of chemicals, 134 pages.

European Chemical Authority (2008). Guidance on information requirements and chemical safety assessment. Chapter R.10: Characterisation of dose [concentration]-response for environment, 65 pages.

European Chemical Authority (2016). Guidance on information requirements and chemical safety assessment: Chapter R.16: Environmental exposure assessment, 178 pages.

European Chemical Authority (2017. Guidance on information requirements and chemical safety assessment: Patc C: PBT/vPvB assessment. 22 pages.

8.5. United States

CVM Guidance for Industry 89 (2001) – Environmental impact assessment 'EIA"s) for veterinary medicinal products (VMP's) – Pase I (VICH GL 6) – Final guidance. 10 pages

CVM Guidance for Industry 166 (2006) – Environmental impact assessment (EIA's) for veterinary medicinal products (VMP's) – Phase II (VICH GL 38) – Final guidance. 37 pages.