Deliverable 13 – Proposal of pesticide environmental risk assessment for amphibians

CA18221 – PERIAMAR

PEsticide RIsk AssessMent for Amphibians and Reptiles

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Funded by the European Union

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1. Introduction

Amphibians are the most threatened group of vertebrates (IUCN 2024). Almost half of the amphibian species in Europe occur in arable lands (IUCN 2024). Populations inhabiting these agricultural landscapes seem to be declining (Arntzen et al. 2017) because of multiple factors, with environmental pollution listed as one of the most important threats. Pesticides could be playing a relevant role in these declines (Hayes et al. 2006). Some amphibian populations are frequently found within areas of pesticide application (e.g. great crested newt, Cooke 1986), and many other species can be exposed while crossing fields during their breeding migrations at the time of pesticide applications (Berger et al. 2013, 2018, Lenhardt et al. 2015). In addition, pesticides can be transported via drift or runoff to nearby small-sized water bodies where many amphibians breed, and although pesticide concentrations in these water bodies are expected to be relatively high, there is a scarcity of pesticide monitoring in these environments. The number of current-use pesticides whose toxicity has been tested on amphibians is low. Toxicological data show that some pesticides can have harmful effects after exposure to low, environmentally relevant concentrations in aquatic life-stages of amphibians (reviewed in Mann et al. 2009). Some data point to significant risks for terrestrial stages of amphibians exposed to recommended application rates of pesticides (Brühl et al. 2013).

Environmental risk assessment (ERA) should ensure that the requested or registered use of these substances poses no unacceptable risks to the environment. ERA is implemented through the characterisation of risks to representative non-target organisms, using both exposure and toxicity data. The absence of amphibians from the species assessed in the pesticide ERA implies that risks to these animals are extrapolated from assessments conducted on other vertebrates that act as surrogates, which has raised some concern. For instance, pond scenarios currently used in ERA to estimate pesticide concentrations are much larger than those used by the majority of amphibian populations for breeding (Adriaanse et al. 2001), which compromises their reliability in reflecting the real exposure of amphibian aquatic stages to pesticides. In terrestrial environments, dermal exposure of vertebrates to pesticides is currently not considered in ERA conducted on birds and mammals, but this route may be relevant for amphibians because of their high skin permeability (Quaranta et al. 2009, Kaufmann and Dohmen 2016). Available information suggests that acute toxicity (measured as mortality after short-term exposure) of pollutants to amphibian aquatic stages can be predicted from fish sensitivity data (Weltje et al. 2013, Ortiz-Santaliestra et al. 2018). However, much uncertainty remains on the potential coverage of chronic toxicity for amphibians from available fish toxicity data due to a lack of comparable information. In particular, for reproductive toxicity, the options for extrapolation are limited because of the important differences between amphibians and fish in reproductive physiology (Kvarnryd et al. 2011). Results about predictability of pesticide toxicity to terrestrial amphibian stages from avian or mammalian data are contradictory (Crane et al. 2016, Ortiz-Santaliestra et al. 2018), but there are some chemical groups, like pyrethroid or organochlorine insecticides, which generally seem to be more toxic to amphibians than to birds or mammals (Ortiz-Santaliestra et al. 2018).

EU Regulations 283/2013 and 284/2013, setting up the requirements for approval of pesticide active substances and PPP, states the need to consider risks to amphibians, using available and relevant data. Following this legal requirement, the panel on plant protection products and their residues of the European Food Safety Authority (EFSA) elaborated the scientific opinion

reviewing the situation of amphibians and reptiles with regards to the current pesticide ERA framework in the EU (EFSA PPR Panel et al. 2018). This scientific opinion drew attention towards the scarcity of knowledge and identified those aspects that need to be addressed to provide a protective ERA for amphibians while keeping vertebrate testing to a minimum, as required by the EU Directive 63/2010 on the use of animals for experimental purposes.

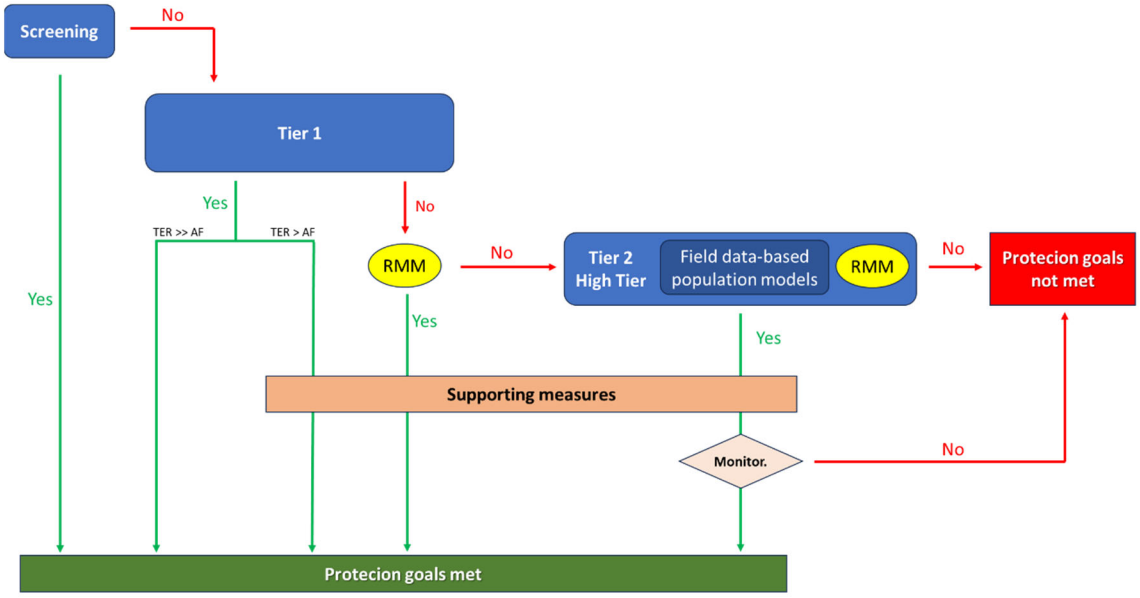
The COST Action PERIAMAR has been working, following the publication of the EFSA Scientific Opinion and from its kick-off in November 2019, on the elaboration of a risk assessment scheme for amphibians that addresses the regulatory gap in this context. This document summarizes the main aspects of that proposal, arising from the combination of the Action Working Groups that have addressed the different sections of the risk assessment.

Developing a completely new risk assessment scheme involves some challenges, especially when it refers to a group of vertebrates. These animals are affected by the need of minimize additional testing, and generating new ecotoxicity data to assess the risk PPPs pose to amphibians raises several ethical concerns due to the necessity of performing animal experimentation. Currently, many international regulatory frameworks emphasize the need to implement the 3 R's policy (reduce, refine, replace) and discourage animal testing. Specifically in the EU, (i) the Directive EC/63/2010 recommends implementing replacement protocols to minimize the number of animals used in experiments; (ii) the REACH policy emphasises the need to reduce animal testing, urging the industry and scientific community to develop alternative methodologies for such assays; (iii) the European Food Safety Authority (EFSA) released a scientific opinion on the state of pesticide risk assessment for amphibians and reptiles, strongly advocating for the development of non-animal alternative methodologies to be used at initial steps of the risk assessment for amphibians. Additionally, the SETAC Global Animal Alternatives Advisory Group also suggested that scientists should continue promoting the advancement of the 3Rs policy. Though for fish and mammals' standard alternative methods to animal experimentation already exist, a parallel has not yet been accomplished for amphibians.

2. Overview of the risk assessment scheme

A scheme is presented in which exposure and effect characterization are addressed from the data that are routinely included in the dossiers, which means that applicants do not need to conduct additional testing to the set of experiments already included in the applications. The use of laboratory tests is however, left at the applicant’s discretion as a way to improve the toxicity characterization, which otherwise is extrapolated from surrogate data. These tests, if conducted, will add to the amphibian toxicological knowledgebase, and shall be used to reduce the level of uncertainty and improve the extrapolation from surrogate toxicity data by getting a better adjustment of the relationship between taxa in toxicological sensitivity. Field studies are also considered if they are conducted with the purpose of supporting population models that must necessarily serve to estimate risks to the specific protection goal of population persistence.

The overall risk assessment scheme consists of an approach with a screening step and two tiers. Conversely, Considering the high uncertainty in the effect and exposure characterization, the scheme is based on the implementation of supporting measures that should contribute to population maintenance under scenarios of high, or uncertain pesticide risks. The following figure summarizes the proposed risk assessment scheme:



ERA is the implementation of the precautionary principle laid out in Regulation (EC) No 1107/2009. In this process information regarding effect and exposure are evaluated to assess the acceptability of risks. A PPP shall not be authorised unless the risks are considered acceptable. Therefore, in the absence of such information the PPP cannot be authorised nor used. With regards to amphibians and reptiles there is an apparent lack of information regarding the effect and exposure characterisation. In the past it was assumed that the risks to amphibians and reptiles are covered by surrogate species. With the knowledge gained after the EFSA scientific opinion that this is no longer the case, an ERA scheme needs to be implemented or PPP can no longer be authorised. The proposed scheme is therefore an important step to secure the use of PPP as important ways to protect plants and plant products as required by Regulation (EC) No 1107/2009.

3. Protection goals

The EFSA SO established different Specific Protection Goals (SPG) options defined from the role of amphibians as providers of ecosystem services (i.e. Service Providing Units, SPU). Thus, SPG are referred as 'limit of operation' when they allow for negligible effects in such a way that no consequences for the service provision are expected. These SPG are compared to another two options, 'below limit of operation' (a more conservative approach) and 'above limit of operation' (a scenario in which ecosystem services are affected and that is presented only for comparative purposes, not as an acceptable option).

For the present ERA proposal, the Specific Protection Goals proposed by the EFSA SO as 'limit of operation' are agreed. Those SPG are summarized in the following table:

	Ecological entity	Attribute	Magnitude/temporal scale
Adults and juveniles	Individual	Survival	Negligible effects
All life stages	Long-term persistence of populations	Abundance, distribution, population growth rate	Small effects up to months on species abundance, occupancy or PGR changes

Taking these SPG into account, the proposed risk assessment scheme will consider pre-metamorphic stages as long as their exposure to PPP is susceptible to cause an effect that can affect the long-term persistence of populations in the terms specified in the table.

Individual survival will be addressed through an assessment of risk associated with acute exposures to pesticides in the terrestrial and aquatic environments. Population persistence will be addressed through an assessment of risks associated with long-term exposure to pesticides in the terrestrial environment, using reproduction as the relevant individual-level endpoint. This SPG can also be addressed by means of spatially explicit population models supported by field data. In addition, long-term risks to pre-metamorphic (aquatic) stages, characterized from endpoints like reduced survival, growth or development, can be considered as an input for population dynamics estimation to match this SPG. This approach can be achieved through the implementation of mechanistic effects models predicting damage at the individual level (e.g. survival, growth, development) during the aquatic stages and until the end of the metamorphosis. The outputs of these models can be scaled-up to determine how that reduced survival, growth or development until the end of the metamorphosis can reduce individual survivorship to the moment at which sexual maturity is reached, hence reducing the breeding population size.

4. Definition of relevant scenarios for problem formulation

Following the information contained in the EFSA Scientific Opinion, a series of priority scenarios on which a risk assessment for amphibians is triggered has been identified. The first stage is to identify the relevant exposure routes:

Habitat	Aquatic					Terrestrial					
Matrix	Water/sediment					Overspray	Water puddle	Soil	Plants	Food	Air
Source	**Spray drift (DR)**	**Run-off (RO)**	**Drainage (DN)**	Atmospheric deposition	Food, plants, water, sediment	Spraying	Formed by RO	Spraying and DR	Spraying and DR	Prey	Air
Route	Contact				Oral	**Dermal**				**Oral**	Inhalation
Scenario	In-field Edge-of-the-field					**In-crop**	**In-crop Off-crop**			**In-crop**	In-crop Off-crop
Relevance	Major route	Major route	Major route	Minor route	Contamination via DR, RO or DN	Movement during daytime	Major route	Major route	Possibly major route	Possibly major route	Minor route

The **bold** characters identify the relevant exposure routes that need to be looked at.

The next step is to cross the relevant exposure routes with the pesticide applications, to detect which are the relevant scenarios for problem formulation.

		Aquatic			Terrestrial		
		Spray drift	Run-off	Drainage	Dermal In-crop	Dermal off-crop	Oral in-crop
Indoor							
professional greenhouse							
open protected structures (Walk-in tunnels, shelters)							
Spraying outdoors	Arable crops (field, vegetables)						
	Orchards						
	Vineyards						
	Ornamentals (flowers)						
	Ornamentals (shrubs, trees)						
	Forestry						
	House gardens						
	Grasslands						
	Railway tracks(1)						
Spot-application		(2)					
Granular application (in-soil)		Dust(3)				Dust(3)	
Granular application (on soil surface)		Dust(4)				Dust(3)	
Soil treatment / sterilization							
Seed treatments		Dust(3)				Dust(3)	
Pheromones dispenser							
Rodenticides / baits					(5)		(5)
Stump / wound treatment							
Dipping							
Wood stacks in forests							

(1) As long as in-crop is defined here as the ballast only, terrestrial exposure of amphibian would happen only off-crop. However, it is not always possible to adjust spray applications to the ballast section.

(2) Relevant only if a shield is not used

(3) Only preliminary calculations for dust deposition are available. A guidance is expected.

(4) Dust drift has been linked to specific FOCUS scenarios.

(5) Depends on how it is dispensed

5. Effect characterization

5.1. Acute toxicity (effects on survival)

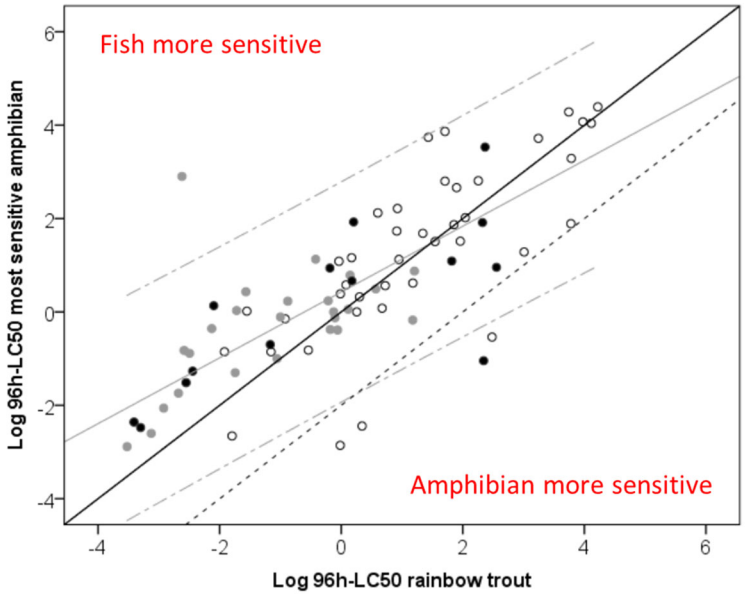
5.1.1. Aquatic stages

Current evidence points to the existence of associations between acute toxicity indicators (LC50 values) measured from fish and aquatic amphibian stages. Therefore, a 96h-LC50 value for aquatic amphibians ($LC50_{amphibian}$) can be extrapolated from the 96h-LC50 value for rainbow trout ($LC50_{fish}$) used in the application dossiers.

With the data included in the review by Ortiz-Santaliestra et al. (2018) the toxicity of a number of substances between amphibian larvae and fish, using either the most sensitive fish species tested (117 substances) or the rainbow trout (79 substances) were compared. In both cases, strongly significant correlations were found between amphibian and fish 96h-LC50 values. With the purpose of using data normally included in the application dossier, the regression model comparing amphibians and rainbow trout is proposed, as referred in Ortiz-Santaliestra et al. (2018):

$$\text{Log } LC50_{amphibian} = 0.412 + 0.700 \cdot \log LC50_{fish}$$

This regression indicates that coverage is maximized for the most toxic substances; as seen in the following figure adapted from Ortiz-Santaliestra et al. (2018), the regression line gets more into the "fish more sensitive" section as the LC50 values drop. In contrast, for substances where fish react less sensitive, amphibians are more likely to react more sensitive.



This value is representative of the acute toxicity of the substances to aquatic stages (e.g. larvae). However, as stated in section 3, survival is the endpoint used as attribute for the individual survival SPG, but this SPG does not apply to pre-metamorphic stages. It is therefore necessary to determine whether the links between acute toxicity to fish and aquatic amphibian larvae stages also apply for adult individuals while in the water. Even if most EU-native amphibians spend a significant part of their post-metamorphic lives out of the water, their presence in the water is also frequent during the breeding season, and, for some species, also along the year.

Consequently, the extrapolation from fish to adult amphibians in the water needs to be explored further. Whilst a robust relationship between sensitivity of fish and adult amphibians in the water is not determined, the calculated value for larvae can be used. According to the review conducted by Ortiz-Santaliestra et al. (2017), when comparing toxicity of the same substances using the same endpoints, larval amphibians result more sensitive than adult ones to waterborne pollutants with a higher frequency than vice versa.

5.1.2. Terrestrial stages

Acute toxicity indicator for amphibian terrestrial stages would be an LD50 value obtained from fish data following the inter-species correlation described in Weltje et al. (2017). In that work, a protocol is proposed to estimate a fish LD50 from the 96h-LC50 value for rainbow trout usually included in the application dossier, using the bioconcentration factor (BCF). The BCF should reflect a 4-day exposure period, as this is the exposure duration to which fish LC50 values in the dossiers refer. Weltje et al. (2017) proposed to follow the method by Feijtel et al. (1997) to correct the steady state BCF (BCF_{ss}) for a 4-day exposure period:

Time to reach steady state in bioconcentration should be known. If this value is not reported in the bioconcentration studies, a time to reach 95% of the steady state (t_{95}) can be calculated as a function of the depuration constant (k_d) as

$$t_{95}\ (d) = 3 / k_d$$

where k_d can be calculated from the octanol-water partition coefficient (K_{ow}) as

$$\log (k_d) = 1.47 - 0.414 \cdot \log (K_{ow})$$

If t_{95} is shorter or equal than the relevant exposure time (t, in this case, $t_{95} \leq 4$ d), the BCF does not require correction. If t_{95} is longer than the relevant exposure time ($t_{95} > 4$ d), BCF will be corrected as follows:

$$BCF_t = BCF_{ss} \cdot (1 - e^{-k_d \cdot t})$$

therefore

$$BCF_{4d} = BCF_{ss} \cdot (1 - e^{-k_d \cdot 4})$$

Once the BCF_{4d} has been obtained, the fish LD50 can be calculated as:

$$LD50_{fish}\ (mg \cdot kg\ bw^{-1}) = 96h\text{-}LC50_{fish}\ (mg \cdot l^{-1}) \times BCF_{4d}\ (l \cdot kg\ bw^{-1})$$

The inter-species correlation by Weltje et al. (2017) resulted in the following regression equation, which allows for extrapolating an amphibian LD50 from the calculated fish LD50:

$$\log (LD50_{amphibian}) = 0.852 \cdot \log (LD50_{fish}) + 0.226$$

The main limitation of this approach is that the extrapolation model has not been tested. All available dermal LD50 values for amphibians were used by Weltje et al. (2017) to obtain the regression equation. It should be a priority to generate additional data from a variety of substance in order to calibrate of validate this protocol.

Extrapolating effects on terrestrial amphibians from fish data comes associated with the uncertainty about potential under-protection against those substances that are more toxic to

amphibians than to fish. This would be the case for pyraclostrobin, some of whose formulations have been shown to cause quick and high mortality after overspray of different amphibian species at approved doses (Belden et al. 2010, Brühl et al. 2013), whereas slow-released formulations (e.g. encapsulated active substance) do not cause such a strong effect. This fast mortality could be caused by a direct disruption of the skin when entering in contact with the sprayed products, even without the intervention of toxicokinetic processes like absorption and tissue distribution. Consequently, it is important to investigate the mechanisms because of which quick mortality occurs and eventually identify substances potentially linked to similar mechanisms. Risk assessment of those substances would pay special attention to amphibians.

5.2. Long-term, chronic toxicity (effects on growth, development, reproduction, and population growth rate)

Four approaches are proposed to extrapolate a long-term, chronic toxicity value for amphibians using available data. However, these approaches are conditioned by the fact that long-term indicators in the form of NOEC/NOAEL or LOEC/LOAEL are usually determined by the choice of experimental concentrations used, which makes these indicators to be often determined by the highest tested concentration. It is strongly encouraged that long-term indicators from surrogates are referred as **benchmark values** (e.g. EC10, EC20), which can be calculated from raw data used in the experiments conducted to calculate NOEC/NOAEL or LOEC/LOAEL values.

For **effects on growth**, a correlation can be established between fish long-term toxicity indicators derived from Early Life Stage (ELS) or from the Fish Short-Term Reproductive Assay (FSTRA) test with growth endpoints from aquatic amphibians obtained from the Amphibian Metamorphosis Assay (AMA) or from ecotoxicity tests with tadpoles reported in the scientific literature. Glaberman et al. (2019) conducted this comparison of AMA-derived endpoints with ELS- or FSTRA-derived ones. They run comparison of endpoints indicative of growth, body weight and survival, and found significant associations, as revealed by Spearman correlations, in all cases between AMA and ELS or FSTRA endpoints. However, some of the correlation coefficients (Rs), especially those linking AMA and ELS endpoints, were not too high ($0.73 \geq Rs \geq 0.55$) and may raise some uncertainty relative to the potential of ELS endpoints to predict amphibian sensitivity. Authors of that paper also state that, since the reviewed studies were not designed to explore lethal effects, survival-based endpoints are frequently unbounded, which limits their predictive potential. However, for the purpose of this proposed risk assessment scheme, the interest of the study by Glaberman et al. (2019) mainly lies in endpoints associated with growth (body length or body mass). Glaberman et al. (2019) did not conduct a regression allowing for an extrapolation of amphibian data from fish test results, but an assessment of their raw data will presumably lead to a predictive model as for the acute aquatic assessment.

For **effects on development**, a correlation can be established between fish toxicity indicators derived from the Fish Life Cycle test and developmental endpoints from aquatic amphibians derived from experiments following a methodology similar to an extended AMA (i.e. prolonging AMA test until the end of the metamorphosis) or to the Larval Amphibian Growth and Development Assay (LAGDA). However, given the low number of substances having been tested under this kind of methodologies in amphibians, this approach has little potential at this moment.

For **effects on reproduction**, acute-to-chronic ratios (ACR) calculated from fish, birds and mammals can be applied to the amphibian acute indicator (LC50 or LD50, see section 5.1 above) to obtain a chronic toxicity indicator.

$$ACR_{bird} = LD50_{bird}\ (mg \cdot kg\ bw^{-1}) / NOAEL_{bird}\ (mg \cdot kg\ bw^{-1} \cdot d^{-1})$$

$$NOAEL_{amphibian\text{-}bird}\ (mg \cdot kg\ bw^{-1} \cdot d^{-1}) = LD50_{amphibian}\ (mg \cdot kg\ bw^{-1}) / ACR_{bird}$$

$$ACR_{mammal} = LD50_{mammal}\ (mg \cdot kg\ bw^{-1}) / NOAEL_{mammal}\ (mg \cdot kg\ bw^{-1} \cdot d^{-1})$$

$$NOAEL_{amphibian\text{-}mammal}\ (mg \cdot kg\ bw^{-1} \cdot d^{-1}) = LD50_{amphibian}\ (mg \cdot kg\ bw^{-1}) / ACR_{mammal}$$

$$ACR_{fish} = LD50_{fish}{}^{*}\ (mg \cdot kg\ bw^{-1}) / NOAEL_{fish}{}^{*}\ (mg \cdot kg\ bw^{-1} \cdot d^{-1})$$

$$NOAEL_{amphibian\text{-}fish}\ (mg \cdot kg\ bw^{-1} \cdot d^{-1}) = LD50_{amphibian}\ (mg \cdot kg\ bw^{-1}) / ACR_{fish}$$

*The fish values need to be extrapolated from the indicators included in the dossiers. The estimation of fish LD50 from fish 96h-LC50 is, as explained above, achieved using the protocol developed by Weltje et al. (2017). That protocol can be adapted to obtain a NOAEL from the NOEC value included in the application dossiers as follows.

$$NOAEL_{fish}\ (mg \cdot kg\ bw^{-1} \cdot d^{-1}) = NOEC_{fish}\ (mg \cdot l^{-1}) \times BCF_{t}\ (l \cdot kg\ bw^{-1})$$

With BCF_t being the bioconcentration factor calculated for the time *t*, which represents the duration in days of the test from which the fish NOEC value was obtained.

In all these calculations, exposure time is no longer considered as it is already implicit in the extrapolations to calculate the amphibian LD50 value, which, as explained in section 5.1 uses the time-corrected BCF.

For **effects on populations**, the body condition at the end of the metamorphosis is assumed to be a proxy. Numerous ecological studies on amphibian life history point to that body condition at metamorphosis can determine survival chances later in life. This value can be used when an ecotoxicity study taking animals to the end of the metamorphosis (e.g. LAGDA) is available, or can be estimated using DEB-TKTD models that are currently under development. It is proposed to use this parameter, when available, in a simple population model to determine what would be the long-term impact of reducing body condition at metamorphosis because of reduced survival to adulthood. This approach, however, needs a clear, quantitative link between reduced body condition at metamorphosis and reduced survivorship at sexual maturity or, alternatively, reduced overall breeding output because of reduced survival chances (e.g. Schmidt et al., 2012).

5.3. Toxicity tests to characterize effects

Given that the proposed risk assessment scheme is designed to get the maximum benefit from the available information, the procedures described in the previous sections aim at characterizing effects while avoiding additional testing to what is routinely included in the application dossiers. However, the possibility of providing additional data on toxicological sensitivity should be left open, not only because it can contribute to reduce the uncertainty in the effect characterization but also because it will contribute to enlarge the knowledge base on

amphibian ecotoxicology and therefore improve the different phases of the risk assessment scheme.

In case applicants want to **improve effect characterization** through the implementation of **specific toxicity tests**, the following options are provided:

- For **acute toxicity in aquatic stages** an LC50 can be obtained through a test designed as the Amphibian Metamorphosis Assay protocol (OECD Test Guideline 231) finalising it 96 hours after the beginning, assuming tadpoles will not have reached metamorphosis climax by then. Set up of test concentration will have to be adjusted to obtain dose-response curves. Testing sensitivity in tadpoles is preferred over using embryos (as it would be the case of the FETAX protocol, ASTM) given the generally higher sensitivity to chemicals that tadpoles show as compared to embryos (Ortiz-Santaliestra et al. 2017).
- For **acute toxicity in terrestrial stages**, tests should be adapted from the scientific literature to obtain LD50 values following oral, dermal or overspray exposure.
- Finally, for **chronic toxicity**, it is recommended to run extended AMA or LAGDA tests (OECD test guideline 241) if the outcome of these tests can provide an endpoint to be linked to reproductive effects. In this context, it is recommendable to replace *Xenopus laevis* by *Xenopus tropicalis* as test species, since life cycle of the latter is shorter than that of *X. laevis*, hence reproductive effects can be directly observed after only a few months. The proposed protocol can be adapted from the method developed by Berg (2019).

6. Exposure characterization

6.1. Aquatic environment

The exposure characterization in aquatic environments will follow the same procedures as described in the EFSA GD for aquatic environments (EFSA PPR Panel 2013). The Predicted Environmental Concentrations of the active substance in surface waters (PEC_{sw}) must be used as the exposure indicator value.

For the aquatic exposure, the maximum PEC_{sw} ($PEC_{sw,max}$) shall be used. For long-term exposures, EFSA PPR Panel (2013) proposed that a time-weighted average PEC ($PEC_{sw,twa}$) can be used under certain circumstances, in particular when ALL the following conditions apply:

- The effect indicator (e.g. NOEC) is derived from a study with a duration ≥ 7 days and is not expressed in terms of nominal/initially measured concentration of the active substance (i.e. it is calculated from measured concentrations over the course of the test).
- The loss of the active substance from water is <20 % of nominal at the end of the exposure period.
- The effect indicator is not based on treatment-related responses of the relevant test species early in the chronic test (e.g. mortality/immobility of animals during the initial 96-hours when exposed to the treatment level above the one from which the effect indicator is derived).
- There is no evidence for the organisms and the PPP under evaluation and/or PPP with a similar toxic mode of action (read-across information) that the following phenomena are likely to occur: (i) latency of effects due to short-term exposure; (ii) the co-occurrence of exposure and specific sensitive life stages that last a short time only.

Under the present risk assessment scheme, however, and considering the uncertainty linked to the extrapolations to calculate effect characterization, the use of $PEC_{sw,twa}$ is not recommended.

PEC_{sw} values are calculated using FOCUS_TOXSWA models. TOXSWA is a pseudo-2-dimensional model, describing pesticide behaviour in a water layer and its underlying sediment at the edge-of-field scale. FOCUS_TOXSWA is able to simulate the resulting transient flow regime with rapidly varying discharges and water levels in the ditch or small stream. FOCUS_TOXSWA is coupled to other models (i.e. MACRO and PRZM) to simulate exposure concentrations in surface water scenarios[1]. As part of PERIAMAR, a TOXSWA scenario simulating an amphibian breeding pond was developed. This scenario returned higher PEC_{sw} values than did the only previously considered pond among FOCUS scenarios (R1). Entry of pesticides in amphibian ponds during periods with shallow water depths was shown to lead to PEC_{sw} sometimes being more than a factor 10 higher than PECsw in the R1 pond having minimum water depths of 1 m (Adriaanse and Beltman, 2023).

It is therefore encouraged to use that FOCUS_TOXSWA model for amphibian ponds to calculate PEC_{sw} for use in the exposure characterization of amphibian aquatic stages.

6.2. Terrestrial environment

6.2.1. Acute exposure

Amphibians in the terrestrial environment are exposed through three routes, all of which can occur simultaneously or within very short time lapses: oral, dermal and overspray.

For the acute exposure, it is proposed to combine the three routes through the calculation of a total internal concentration ($C_{int,total}$), which would result from the summatory of internal concentrations resulting from each of the exposure routes:

$$C_{int,total}\ (mg \cdot kg\ bw^{-1}) = C_{int,oral}\ (mg \cdot kg\ bw^{-1}) + C_{int,dermal}\ (mg \cdot kg\ bw^{-1}) + C_{int,overspray}\ (mg \cdot kg\ bw^{-1})$$

Oral uptake estimation can be calculated as:

$$C_{int,oral}\ (mg \cdot kg\ bw^{-1}) = FIR \cdot \Sigma_i\ (AE_i \cdot RUD_i \cdot AR \cdot MAF_{acute}) / BW$$

This equation is derived from that used in the EFSA Guidance for Birds and Mammals (EFSA PPR Panel 2023) to calculate the acute dietary dose in the initial screening of the birds and mammal risk assessment. However, because the objective for amphibians is to calculate an internal concentration the assimilation efficiency (AE) is incorporated. The parameters of the equation are explained below:

[1] https://www.pesticidemodels.eu/toxswa/home

Parameter	Definition	Units	Source
FIR	Food Intake Rate; amount of ingested food per day	mg food · d^{-1}	Can be calculated from the animal's body weight (BW) using the equation from the USEPA's T-herps model[2]: FIR = 0.013 · $BW^{0.773}$
AE	Assimilation efficiency; factor indicative of the proportion of each ingested food type that is absorbed	-	Scientific literature. See details in Sections 7.1 and 7.2
RUD	Residue per unit dose; the initial residue of the active substance on the food item	mg a.s. · kg $food^{-1}$	EFSA Guidance for Birds and Mammals (EFSA PPR Panel et al. 2023)
AR	Application rate of the active substance per hectare. This parameter is included because the RUD values are estimated for an application rate of 1 kg a.s/ha	kg a.s. · ha^{-1}	Provided by the applicant
MAF_{acute}	Acute Multiple Application Factor, to account for accumulation of residues in the food items when multiple applications occur	-	Procedure for MAF calculations taken form the EFSA Guidance for Birds and Mammals (EFSA PPR Panel et al. 2023) and explained in Appendix 12.1
BW	Body weight	g	See section 7 for assumptions in the different risk assessment tiers

For **dermal uptake estimation** the use of the one-compartment toxicokinetic model developed by Mingo et al. (2024) to estimate anuran body burdens resulting from dermal exposure is proposed. The model assumes that uptake through the amphibian skin is the limiting toxicokinetic process because of the importance of this organ for water uptake and as a barrier against the external environment. Given the lack of metabolic information for anurans, metabolism of the pesticide is not considered, so the change in the pesticide mass inside the animal (M_{int}) is simply expressed as the difference between the in and out fluxes (ϕ_{in}, ϕ_{out}):

$$dM_{int} / dt = \phi_{in} - \phi_{out}$$

Pesticide uptake from soil is assumed to occur through three mechanisms: passive uptake from soil (ventral skin contact), active uptake from soil (result of water balance processes), and

[2] https://www.epa.gov/pesticide-science-and-assessing-pesticide-risks/t-herps-version-10-users-guide-risk-amphibians-and

rehydration following a desiccation event. Then, the total internal concentration ($C_{i,dermal}$) will result from the combination of the three processes:

$$C_{int,dermal} = C_{int(passive)} + C_{int(active)} + C_{int(rehyration)}$$

- Absorption through passive uptake:

$$C_{int(passive)} = \frac{C_{soil}}{K_{oc}} \cdot K_{fw} \cdot \left(1 - e^{\frac{[(K_p \cdot 100) \cdot (0.3 \cdot SA/H) \div BW]}{K_{fw}} \cdot t}\right)$$

Parameter	Definition	Units	Source
C_{soil}	Concentration of the pesticide in the soil	mg a.s. · kg soil^{-1}	Use a PEC_{soil} calculated for the top 1 cm
K_{oc}	Soil organic partition coefficient	-	Provided by the applicant
K_{fw}	Frog:water partitioning coefficient	-	Procedure for K_{fw} calculations as in Mingo et al. (2024) are explained in Appendix 12.2. Source data and calculation tools are available at the UFZ-LSER Database[3]
K_p	Skin permeability coefficient of the pesticide	cm · h^{-1}	Calculated from equations in Walker et al. (2003): For aliphatic alcohols: $\log K_p = 0.544 \log K_{ow} - 2.884$ For phenols: $\log K_p = -0.39 (\log K_{ow})^2 + 2.39 \log K_{ow} - 5.2$ For the rest of organic compounds: $\log K_p = -2.72 + 0.71 \log K_{ow} - 0.0061$ MW MW: molecular weight. This factor is multiplied by 100 in the equation to account for increased skin permeability in amphibians compared to mammals.
SA	Surface area of the animal	cm^2	Equations provided by Hutchinson (1968) for different amphibian groups. For anurans, the equation is: $SA = 1.131 \cdot BW^{0.579}$ The factor is multiplied by 0.3 because model considers that a 30% of the body surface is in contact with the soil.
H	Skin thickness	cm	The animal is assumed to have a cylindrical shape, for which the radius is calculated from the SA and the SVL (as the cylinder height).

[3] http://www.ufz.de/index.php?en=31698

			The model assumes that skin thickness is 5% of the radius length.
t	Exposure duration	h	The model assumes 8 hours because this is the exposure time in the majority of papers used for validation.

- Absorption through active uptake:

$$C_{int(active)} = C_{pw} \cdot K_{fw} \cdot \left(1 - e^{\frac{[(K_p \cdot 100) \cdot (0.05 \cdot SA/_H \cdot Wr) \div BW]}{K_{fw}} \cdot (t - t_h)}\right)$$

Active uptake is considered to happen via the pelvic patch, which is assumed to constitute approximately a 5% of the animal's surface area.

Parameter	Definition	Units	Source
C_{pw}	Concentration of the pesticide in the soil pore water	$\mu l \cdot l^{-1}$	Calculate according to EFSA (2018): $C_{pw} = (C_{soil} \cdot \rho) / [\varepsilon + (\rho \cdot f_{oc} \cdot K_{oc})$, with ρ (bulk density): 1500 $kg \cdot m^{-3}$ ε (fraction of soil pore volume): 30% f_{oc} (fraction of organic soil): defined from the soil to be used (see section 7 for details).
W_r	Water uptake rate	$g \cdot cm^2$	The example provided in the model uses a value of 0.6.
t_h	Time to rehydration	h	(see rehydration below)

- Absorption through rehydration process:

$$C_{int(rehydration)} = C_{pw} \cdot K_{fw} \cdot \left(1 - e^{\frac{[(K_p \cdot 100) \cdot (0.05 \cdot SA/_H \cdot W_{r,des}) \div BW]}{K_{fw}} \cdot t_h}\right)$$

Parameter	Definition	Units	Source
$W_{r,des}$	Water uptake rate of a dehydrated animal	$g \cdot cm^2$	The example provided in the model uses a value of 1.2 for animals with a 20% dehydration and 1.8 for animals with a 30% dehydration.
t_h	Time to rehydration	h	t_h = (water loss rate · BW) / ($W_{r,des}$ · 0.05 · SA) The model works under two eventual scenarios of water loss rate: 0.2 (20%) and 0.3 (30%)

Overspray uptake estimation can be calculated as:

$$C_{int,overspray}\ (mg \cdot kg\ bw^{-1}) = 0.01 \cdot AR\ (kg \cdot ha^{-1}) \cdot E_p \cdot 0.5 \cdot SA / BW$$

This equation is proposed by the EFSA opinion on amphibians and reptiles (EFSA PPR Panel 2018). The application rate (AR) is multiplied by 0.01 to express it as mg a.s. · $cm2^{-1}$. The surface area relevant for overspray is assumed to 50% of the animal.

Parameter	Definition	Units	Source
E_p	Absorption efficiency through skin for overspray	-	While no additional information is available, it can be assumed to be 100%, but see section 7.2 for possible refinement.

6.2.2. Long-term, chronic exposure

For the long-term, chronic exposure, oral uptake and dermal uptake from the soil are considered relevant. However, contrarily to the acute exposure, both exposure routes cannot be combined via the internal concentration because the exposure time has a strong influence in long-term exposures. Consequently, exposure estimates will be calculated separately for each route, to compare with toxicity estimates, and then the accumulated risk will be calculated in the risk calculation step via TER addition (see Section 7).

Oral uptake estimation can be estimated as a daily dietary dose (DDD) for the relevant exposure time, and calculated as for birds and mammals (EFSA PPR Panel et al. 2023) as:

$$DDD = FIR \cdot \Sigma_i (RUD_i \cdot AR \cdot MAF_{long\text{-}term} \cdot fTWA_i) / BW$$

Parameter	Definition	Units	Source
DDD	Daily dietary dose	mg · kg bw^{-1} · d^{-1}	
$MAF_{long\text{-}term}$	Long-term Multiple Application Factor, to account for accumulation of residues in the food items when multiple applications occur.	-	Calculate as MAF_{repro} for birds and mammals. Procedure for MAF calculations taken form the EFSA Guidance for Birds and Mammals (EFSA PPR Panel et al. 2023) and explained in Appendix 12.1
fTWA	Time-weighted average factor	-	It accounts for degradation on food item during a 21-day period (as derived from bird and mammal chronic assessments). Section 6.1.4 of the Bird and Mammal Guidance (EFSA PPR Panel et al. 2023) explains how/when this factor should be applied. However, its application is based on the outcome of toxicity shown by the substance to birds and mammals, and it is difficult to extrapolate to amphibians. Nonetheless, as chronic toxicity indicators for amphibians are to be extrapolated from bird and mammals (see section 5.2), it is recommended

			to follow the same decision as for birds and mammals relative to the fTWA application.

Dermal uptake estimation

Mingo et al. (2024) model contains a factor (Kfw) that accounts for water:tissue distribution and is used to model excretion. This can be used to model long-term exposure (the animal will reach a steady-state based on the model data). The main limitation of the model in this scenario is that it does not consider metabolism, but this would anyway leave it on the conservative side.

7. Risk assessment steps

Initial steps of the risk assessment are designed to sort out all those substances that do not represent a comparatively higher risk to amphibians than to surrogate taxa. In these steps, a toxicity to exposure ratio (TER) approach is proposed to characterize the risk (but see comment on mechanistic effect modelling below). A substance will meet specific protection goals when TER values are above assessment factors (AF) for the four assessments: risks from acute exposure in the aquatic environment, risks from long-term exposure in the aquatic environment, risks from acute exposure in the terrestrial environment, and risks from long-term exposure in the terrestrial environment.

For assessment in which different exposure routes are relevant, the combination depends on the availability of endpoints and exposure characterization values:

- Acute terrestrial: relevant routes (oral, dermal and overspray) are combined. An accumulated concentration ($C_{int,total}$) is used as exposure estimator and the acute $LD50_{amphibian}$ (see section 5.1.2).

$$TER = C_{int,total} / LD50_{amphibian}$$

- Long-term terrestrial: a TER is calculated for each relevant exposure route (oral, dermal). Then, TERs for each route are combined into a single one as follows:

$$\frac{1}{\mathrm{TER_{total}}} = \frac{1}{\mathrm{TER_{oral}}} + \frac{1}{\mathrm{TER_{dermal}}}$$

For aquatic assessment, water and sediment must be considered as relevant exposure matrices. However, obtaining different toxicity endpoints for each of these matrices is currently not possible. It is proposed that, once a $PEC_{sediment}$ can be calculated for the amphibian pond scenario in TOXSWA, the same approach as for acute terrestrial assessments (i.e. combination through an internal concentration) is followed.

Effect is characterized by default using the approach described in sections 5.1 and 5.2. However, applicants may want to provide additional data compiled from toxicity tests specifically designed to determine the toxicity of their substances to amphibians, as described in section 5.3. Ideally, the experimental evidence should refer to different species, especially if the outcome of toxicity tests differs significantly from the estimates based on extrapolations from surrogate-derived data.

7.1. Initial screening

The initial screening assumes a worst-case scenario, for which assumptions regarding the exposure characterization are simplified. As explained above a TER approach is generally used to quantify the risk for each of the assessments. The initial screening has two possible outcomes:

- $TER_{i,j}$ (= Effect indicator / Exposure estimate) > AF → SPG met for the corresponding *i* (exposure duration, i.e. acute or long-term) and *j* (habitat, i.e. aquatic or terrestrial)
- $TER_{i,j}$ < AF → SPG not met for the corresponding *i,j* combination → Go to tier 1

The following table summarizes the toxicity indicators and exposure estimates to be used in the initial screening of the assessment:

Assessment		Toxicity indicator	Exposure estimate
Acute aquatic		$LC50_{amphibian}$ (section 5.1.1)	$PEC_{sw,max}$ (section 6.1)
Long-term aquatic		NOEC (section 5.2, if a model can be obtained from Glaberman et al. 2019)	$PEC_{sw,max}$ (section 6.1)
Acute terrestrial		$LD50_{amphibian}$ (section 5.1.2)	$C_{int,total}$ [(a)] (section 6.2.1)
Long-term terrestrial	Oral	$NOAEL_{amphibian}$ [(b)] (section 5.1.2)	DDD (section 6.2.2)[(a)]
	Dermal	$NOAEL_{amphibian}$ [(b)] (section 5.1.2)	To be discussed

(a) The following considerations are taken for the initial screening when applying the models to combine exposure via different routes in a total internal concentration value ($C_{int,total}$) or to calculate the DDD:

- The T-herps model and the EFSA Scientific Opinion on amphibians and reptiles consider a minimum BW of 1.4 g. Although juveniles of some amphibian species can finish the metamorphosis with a lower body weight, this body weight is considered to cover above 95% of the cases. Consequently, the exposure indicators are calculated considering this BW, which is also used to obtain FIR and SA values for the initial screening:

$$FIR = 0.013 \cdot 1.4^{0.773} = 0.017\ g \cdot d^{-1}$$

$$SA = 1.131 \cdot 1.4^{0.579} = 1.374\ cm^2$$

- Assimilation efficiency varies depending on the prey type. For the initial screening a factor of 0.9 is proposed, as some prey items may show assimilation values of approximately 90% (e.g. mealworms, Dimmitt and Ruibal, 1980).

- Diet can be assumed as consisting of 100% of the most contaminated prey item (i.e. foliar-dwelling arthropods).
- Fraction of organic soil (f_{oc}) is established in the model by Mingo et al. (2024) between 0.018 and 0.08, because this is the range of values in the experiments that were used to validate the model. As a worst-case scenario for initial screening, the use of the lowest value of 0.018 can be used.

(b) The lowest value out of those generated according to the protocol in section 5.2 ($NOAEL_{amphibian\text{-}mammal}$, $NOAEL_{amphibian\text{-}bird}$ or $NOAEL_{amphibian\text{-}fish}$) should be taken.

7.2. Tier 1 assessment

No changes in effect characterization are defined in Tier 1 relative to the initial screening step. As explained above, effect characterization is designed to avoid additional testing, unless the applicants want to conduct toxicity testing to add data to the knowledgebase of amphibian ecotoxicological sensitivity that could be eventually used for the risk assessment.

However, the TER approach can be modified if **mechanistic effect modelling** providing data directly related to SPG is available. In this context, a DEB-TKTD model is being developed to predict individual damage during the larval and metamorphic stages of anuran amphibians when exposed to waterborne pesticides. Whereas individual survival of pre-metamorphic stages is not an SPG (see section 3), outputs of this model can be scaled-up to predict effects on long-term persistence of populations if predicted damage to larvae and metamorphic animals is clearly associated with reduced adult survival or reproductive output later in life. This DEB-TKTD model can be used to calculate an output resulting from the calculated exposure for the long-term aquatic assessment. Evidence rejecting that this model output does take to a reduction in population growth rates (PGR) could be considered as a proof for the absence of unacceptable effects from long-term; however, it needs to be determined whether this proof, based solely on the outcome of a mechanistic effect model, is enough to replace the TER approach, or whether it must be used as additional evidence.

Within the TER approach, tier 1 assessment considers some options to refine exposure characterization. In general, these options follow three different dimensions:

- Degradation of the substance if applicable.
- Combinations of traits in indicator species.
- Crop interception.

Tier 1 has three possible outcomes:

- $TER_{i,j}$ >> AF → SPG met for the corresponding *i,j* (no further action needed).
- $TER_{i,j}$ > AF → Uncertain achievement of SPG for the corresponding *i,j* → approval of the product is linked to the implementation of supporting measures (see section 10 below).
- $TER_{i,j}$ < AF → SPG not met for the corresponding *i,j* combination; in this case the exposure characterization can be modulated by the implementation of risk mitigation measures (see section 9 below). As indicated therein, RMM should provide a quantifiable reduction of the exposure, in such a way that exposure estimate to be used in the TER approach can be calculated under the influence of the proposed RMM. The application of RMM can result in:
    - $TER_{i,j}$ > AF → SPG met for the corresponding *i,j* if RMM is implemented → approval of the product is linked to the implementation of supporting measures (see section 10 below).
    - $TER_{i,j}$ < AF → SPG not met for the corresponding *i,j* combination → Go to tier 2.

For the **aquatic assessments**, exposure characterization refinement will depend on progressing in the development of amphibian-based scenarios like the TOXSWA amphibian pond. According to the Aquatic GD (EFSA PPR Panel 2013), exposure characterization in the water can be refined in step 3 of FOCUS, which constitutes a realistic worst-case for aquatic exposure. However, FOCUS step 3 is characterized using mechanistic models to calculate PEC_{sw}, hence the TOXSWA

amphibian pond model could already be assumed as a FOCUS step 3, and further refinement would be possible in this context in Tier 1.

For the **terrestrial assessments**, the following refinements can be used in the calculation of the C_{int}:

- Degradation of pesticide residues in the exposure matrix (food or soil), if information is available and clearly applicable. This refinement option applies to long-term assessments only and cannot be used to refine acute assessments.
- Mixed diet. The single food item approach is unrealistic, hence a mixed diet, obtained from a worst-case scenario considering diet composition descriptions, can be applied.
- Food item-dependent assimilation efficiency. If information is available relative to differential AE values for different prey, these can be applied to the calculation of oral uptake.
- Absorption efficiency through skin for overspray (E_p). If enough information is available to assume, considering the physico-chemical properties of the assessed substance, that the E_p value is different from the 100% assumed in the initial screening, it can be applied in Tier 1. Information like that contained in the articles by Quaranta et al. (2009) or Kaufman and Dohmen (2016) should be used or generated for this purpose.
- Crop interception is considered in the model by Mingo et al. (2024) to estimate dermal exposure from soil ($C_{int,soil}$). The same assumptions as in the Bird and Mammal guidance (EFSA PPR Panel et al., 2023) relative to crop interception for food items can be assumed in the calculation of a $C_{int,oral}$ for amphibians (see Deposition Values in section 6.2.6 of the Bird and Mammal Guidance). In the case of $C_{int,overspray}$, if the product is meant to be always applied on grown crops, interception can be considered. To do so, it needs to be confirmed that amphibians cannot occur on the upper part of the sprayed plants and that there are no bare soil areas in the treated crops (e.g. no open rows between plant lines). Crop interception values can be retrieved from Table L.1 of the Bird and Mammal Guidance.

8. Tier 2 / High tier

Tier 2 is the highest tier proposed for this risk assessment scheme. Considering how the initial steps (Screening and Tier 1) are designed, substances reaching Tier 2 are those for which, after extrapolating from data included in the dossier, the absence of unacceptable risks to amphibians cannot be discarded. Tier 2 incorporates some aspects directly applicable to the TER approach, like the assessment in realistic scenarios using focal species or the improvement of predicted exposure through substance-specific fate models. In addition, risk mitigation measures can be implemented at any time in Tier 2 to refine exposure characterization.

These approaches can be replaced by the development of field data-based, spatially explicit population models, should those models provide evidence that, under the predicted exposure conditions, no risks to the long-term persistence of populations are foreseen. For consistency with other risk assessment schemes, where population models are incorporated at higher tiers (not at Tier 2), the denomination ‘High tier’ is included here.

Tier 2 has two possible outcomes:

- ($TER_{i,j}$ > AF) OR (Population model reveals no significant effects) → approval of the product is linked to the implementation of supporting measures (see section 10 below).

- ($TER_{i,j}$ < AF) AND (Population model does not evidence the absence of effects) → SPG not met for the corresponding *i,j* combination → No options to meet SPG

8.1. Focal species for exposure characterization

Focal species must be selected from all species present in the relevant scenarios of application. For focal species selection, considerations about temporal and spatial overlap with the intended pesticide applications should be considered. This are cut-off criteria, but should not be used for refining exposure calculations; ecological aspects shall be considered for population modelling approaches.

When using focal species, the following parameters can be looked at for refining C_{int}, assuming a combination of worst-case options for the listed parameters cannot concur within the same species.

- Body weight. If demonstrated that body weight of the relevant exposed organism is higher than 1.4 g, the lowest body weight for the focal species can be used to calculate internal doses, FIR and SA values.
- Specific equations to calculate the surface area of the animal (SA). The equation used to calculate SA presented in section 6.2 is the one proposed for all anurans. However, Hutchinson et al. (1968) calculated equations for three anuran families: Bufonidae ($SA = 0.966 \cdot BW^{0.645}$), Hylidae ($SA = 1.129 \cdot BW^{0.610}$), and Ranidae ($SA = 1.056 \cdot BW^{0.688}$). SA values of species belonging to these families can be recalculated in accordance.
- Diet composition. If demonstrated that the diet of the relevant exposed species results in an estimate of overall RUD which is lower than the worst-case scenarios calculated in Tier 1, it can be replaced.
- Food item-dependent assimilation efficiency. If information is available relative to differential AE values for different prey, these can be applied to the calculation of oral uptake.

The focal species approach must consider all the above parameters together. Estimations should be done for all possible species to ensure that the selected focal species leads to an exposure estimate that covers all the other species. To support the selection of focal species, PERIAMAR has elaborated a database with European species present in agricultural areas, which includes life history traits relevant to determine the susceptibility on the basis of the parameters described above[4].

8.2. Substance specific fate models

If fate models are available that can predict substance-specific residue decline per environmental matrix, these can be applied to refine the exposure calculation in the relevant assessment. This could be the case, for instance, of substances whose degradation differs among different prey types. These data could be used to refine the estimates of oral uptake.

8.3. Field data-based, spatially explicit population models

Population models to be used in this step of the assessment must be spatially explicit integrate the available information on field biology and ecology of the selected focal species. A possible

[4] PERIAMAR Deliverable 9
https://docs.google.com/spreadsheets/d/1jJmvwrDC59ZwQkcd8GFRuYC4E6xwsVVb/edit?usp=sharing&ouid=116390621908614043827&rtpof=true&sd=true

example is given in the EFSA SO, where a developing model for the great crested newt (*Triturus cristatus*), based on the modelling environment ALMaSS (Topping et al. 2003), is shown.

A review has been conducted in PERIAMAR to compile those aspects from amphibian (and reptilian) field ecology that can be useful to incorporate to spatially explicit population models[5]. The review addresses parameters that can modulate exposure of animals and populations to pesticides in the wild, hence they can be used in high tier assessments. However, given the uncertainty associated to the risk characterization in general, it is considered that these field-based information is acceptable only if it contributes as an input for population models, whereas its direct use to refine exposure estimates within TER approaches is discouraged.

Model species should be selected from the basis of susceptibility criteria. In this high tier, aspects to consider can include ecological (habitat use, seasonal and daily activity patterns, etc.) and biological (life cycle duration, mean productivity, lifespan, etc.) features.

The spatially explicit population models shall apply to:

- Refinement of the exposure.
- A more realistic characterization of risk (e.g. considering multiple applications…) and quantification of the margin of safety.
- Consideration of landscape structure in the outcome of the risk assessment and prediction of the supporting measure efficacy.

9. Risk mitigation measures (RMM)

Mitigation measures are imposed as part of the authorization process of specific PPP uses to reduce exposure in a quantifiable manner to achieve acceptable levels of PPP in the environment. However, as efficiency studies are lacking, we assessed the reduction in a semi-quantitative manner.

symbol	Effectiveness estimated on percentage reduction
+	<50%
++	~50%
++	>50%

Measures that may also reduce exposure, such as a reduced rate, number of applications or application time (in terms of crop growth stage), are part of the risk assessment and are therefore considered a management issue and not a risk reduction measure. In general, it is estimated that these measures can reduce the risk by less than 50%. It is important that the use is not reduced below the minimum necessary to achieve the desired effects also as sufficient efficacy is part of the authorisation requirements. These measures reduce the risk but may not be sufficient to achieve acceptable levels.

Mitigation measures relate to spatial or temporal aspects of the application. One way of increasing effectiveness is to restrict the application of the RMM to specific areas of high concern

[5] PERIAMAR Deliverable 4+6 https://periamar.com/assets/Uploads/482e81020a/Deliverable_04+06.pdf

as part of a local case-by-case management. In the following, possible RMM are listed, their effectiveness assessed, and their suitability described. Several RMM are familiar to the farmers as they are also implemented to protect other NTO. However, with regards to the vegetated buffer strip to reduce run-off into surface waters it is pointed out here that clover and alfalfa could both represent a barrier to amphibians due to the density of the vegetation. On the other hand, flower strips are not considered to pose a barrier and have additional benefits by providing food (insects).

Measure	Effectiveness	Suitable to mitigate identified risk by (list exposure route, compartment)	Comment
Locally no application of PPPs during the main migration season.	Useful for one species at a time	Only suitable for areas which are used by species with overlapping migration times or only suitable for one species. Ideally this RMM should thus be linked to a conservation program which protects certain species as it is not feasible to protect all species. Easier to implement in crops with fewer number of applications.	Implementation only suitable on a case-by-case basis with the help of advisors; not suitable as a national RMM
No application of PPPs during twilight hours	+	For terrestrial amphibian stages	Counterproductive with potential bee RMM in flowering crops
No application of PPP before or after rain	+	Generally, PPP are not applied before and after rain, but there are certain PPP that have to be applied during that time. As residues would remain, it reduces primarily overspray exposure and would be most effective during migration.	As migration is highest close to water bodies, the RMM could be restricted spatially, e.g. 100 m radius to water bodies
Temporal restriction of PPP application during low crop interception	+++	The prohibition of the application between March and April would benefit terrestrial amphibian stages. No exposure during the breeding period is considered very effective.	Not really a RMM but a management issue
No application of PPPs next to protected sites for amphibian species	++/+++ (depends on definition of protected site)	If a higher protection level is required for protected sites.	Suitability depends on the definition of protected spawning sites
Temporal buffer strips and no-spray zones to ponds during the spawning season	+++	Suitable to reduce drift and run-off for aquatic stages especially between March-June to cover most amphibian species for all of Europe; Member States may narrow the period.	
Buffer strips to water bodies	+++	All life stages.	Definition of pond is required, farmers need to recognize relevant water bodies
Drift reducing nozzles	+++	Suitable to reduce off-crop exposure of aquatic and terrestrial amphibians	Consider funding and regular checks to ensure effectiveness
Spraying last row to the inside of permanent crops, using a spraying shield or drift	+++	Suitable to reduce off-crop risk in stone walls.	In the absence of a risk assessment scheme for reptiles this RMM should always be implemented if the tier 2 RA requires

reducing nozzles next to stone walls			RMM for terrestrial life-stages of amphibians.
Promotion of low-loss application techniques (precision farming)/ Limit the area (x%) that can be treated	+++	Suitable for terrestrial and aquatic amphibians	Funding needed for the acquisition of the technology. Implementation requires that PPP cannot be applied if more than x% of crop is affected and needs to be treated

10. Supporting measures for the populations of amphibians and reptiles

There are measures suitable to manage the impact of PPP on non-target organisms (NTO), as amphibians and reptiles, which are currently not subsumed under the term "risk mitigation measures, RMM". These measures have the goal to reduce the overall PPP effects on the population of NTO by creating as far as possible optimal environments in or nearby the fields and allowing for (a part of) the non-target population to be less affected by the PPP use in these areas.

The naming of such measures is currently open, possibly due to their wide application domain. There are suggestions for e.g., "compensation measures" for specific identified risks arising from PPP use, "ecological supporting measures", or "landscape management for biodiversity support" which would possibly apply also to wider measures not directly linked to identified PPP risks for NTO.

What is common to this type of measures and discriminates them from RMM is the focus on an effective support of the populations of NTO and not primarily the numerically quantifiable reduction of the exposure of the organisms. Currently, the consideration of such additional areas that support the thriving of NTO populations is implemented in e.g., Germany, where the existence of so called "small structures" decides if drift RMM need to be applied or not.

Due to the high uncertainty because of the lack of ecotoxicological studies, the proposed scheme does not follow the current paradigm of the risk regulation for chemicals: avoid → reduce → compensate. Rather, "supporting measures" are sometimes implemented prior to RMM.

Being defined in this way, "supporting measures" can be considered in the risk assessment schemes at any time and not only after having implemented exposure reduction measures (RMM). They can be implemented in- or off-field.

Since the exact quantification of the risk reduction extent is not the focus of these supporting measures, it is a matter of debate how the threshold indicating the need for their implementation can be defined. In the proposed scheme, we linked the need for supporting measures to the occurrence of high uncertainties in the effect and/or exposure characterization for amphibians and opted for a precautionary approach in case the tier 1 of the assessment would indicate that the safety margins to risk are small.

In the opinion of PERIAMAR, such measures are a prerequisite for the use of a particularly risky PPP and would need to be already in place or be implemented together with the authorization

of that PPP. Such enforcement mechanisms are already available in the risk assessment scheme for NTO exposed to PPP, for instance regarding the existence of buffer strips to reduce run-off in case the slope of the field exceeds a certain value as a prerequisite for the use of some PPP. It is of central importance that similar implementation steps would be available when performing the ERA for amphibians exposed to PPP, so that supporting measures can be taken into account in the procedure.

Therefore, we suggest an approach able to consider supporting measures by indicating if needed as one outcome of the risk assessment that "*the use of the PPP (containing active substance X) is allowed in cases where xxx supporting measures for the population of amphibians are in place*".

The choice of the/an array of supporting measures to be implemented would lie in the responsibility of the farmer, who best knows the specific environment and the situation of the NTO population on site. It should be clarified that a certain extent of supporting measures need to be in place, also by possibly considering / combining different measures, considering their primary target, their effectiveness, and their suitability to be combined with other (RMM) measures.

The advantages of supporting measures for a given population are manifold. Firstly, the populations of NTO in the field are supported by considering / creating areas adapted to specific needs (shelter, migration, breeding habitat, etc,). Secondly, the use of a PPP that is linked to high uncertainties regarding the margin of safety for NTO would be still possible, in cases in which the supporting measures are in place. The creation of these supporting measures provides benefits not only for single authorised PPP, but for the use of all PPP further supporting biodiversity.

The steps for implementations imply a labelling of the product (see above) and a comprehensive information of the farmer about the possible supporting measures. Before this, it would be very favourable to link i) supporting measures to financial schemes at Member State level, which are then implemented locally; ii) to supporting measures for other NTO, so to maximize the set-up of such areas or structure for more than one group of organisms if relevant; and iii) to an overall assessment of the individual farm situation.

Two approaches are introduced on how to implement supporting measures.

- The first, simple approach consists of fallow strips covering a minimum area of 10 % of the area where PPPs have been applied.
- The second approach is a point-based system where credits can be earned for various measures to compensate for PPP use.

These measures can be directly related to agricultural production. The point-based system and adequate measures is presented in detail. The point-based system can be implemented into existing current measures within agri-environmental schemes and measures to facilitate climate adaptation.

Measures that create additional pesticide-free habitats and refugia in the agricultural landscape have a positive impact on amphibian populations, especially as a higher proportion of insects and other invertebrates in these structures increases food availability and should therefore enable amphibians, especially juvenile stages, to survive longer (Berger et al. 2011). As part of a DBU project, a detailed problem analysis was carried out for amphibian conservation in German

agricultural landscapes rich in small bodies of water, and various implementation options were proposed and also their effectiveness checked in the field (Berger et al. 2011).

In addition to measures that can be carried out in the field, an enhancement of field margin structures through the creation of cairns piled up with stones from the fields, a general better linking of water bodies through wide field margins and hedges, as well as the establishment of 30 m wide margins surrounding the spawning waters are recommended.

It should be noted that rainwater retention basins are also important spawning ground waters for amphibians in cleared agricultural landscapes and should be included in the analysis (Lenhardt et al. 2017). In addition, from an amphibian conservation perspective, a general ban on the use of pesticides within a radius of 300 metres around spawning waters and wetlands is called for (Plötner and Matschke 2012).

The following tables describes the proposed supporting measures for amphibians:

Measure	Suitable to support…	Effectiveness	Steps for implementation	Agri economic feasibility Pros/cons	Final score
Off-field /off-crop					
Partly uncontaminated terrestrial habitats next to breeding ponds to avoid migrations though fields - off -field / next to ponds - (partly) unsprayed	Terrestrial stages (and aquatic)	High if properly arranged +++	Monetary compensation needed and possibly sufficient	Feasible. Could be also set asides. Very good combination affinity to supporting measures for other NTO (set asides, see below).	++++
Set-aside of marginal land, - off -field - (party) unsprayed	Terrestrial stages (and aquatic)	High if properly arranged +++	Monetary compensation needed and possibly sufficient	Feasible. Could be also set asides. Very good combination affinity to supporting measures specifically for amphibians (if next to ponds, see above).	++++
Site-appropriate creation of small structures (e.g., stone or woody piles) - off crop, off field - unsprayed (drift?)	Terrestrial stages	High if properly arranged +++	Monetary compensation not necessarily needed, information possibly sufficient	Feasible. Only small areas needed, possibly already there.	+++
Corridors for migration - off -field / edge to the field - unsprayed (drift?)	Terrestrial stages	High if properly arranged +++	Monetary compensation needed and possibly sufficient	Feasibility higher than for same measure in field. Medium combination affinity to supporting measures (linear structures) for other NTO. To be properly arranged, multi-year implementation needed.	+++
Hedges as corridors for migration - off -field / edge to the field - unsprayed (drift?)	Terrestrial stages	High if properly arranged +++	Monetary compensation needed but possibly not sufficient	Feasible. Very high combination affinity to supporting measures (linear structures) for other NTO.	++

				To be properly arranged, multi-year implementation needed, long term projects.	
Upgrading of forest edges adjacent to cultivated land - off -field / edge to the field - unsprayed (drift?)	Terrestrial stages	High if properly arranged +++	Monetary compensation needed but possibly not sufficient	Feasible. Very high combination affinity to supporting measures (linear structures) for other NTO. To be properly arranged, multi-year implementation needed, long term projects. Can only be planned if forest is there.	++
(extended) zones to spawning areas, spatially arranged measures additionally to edge of the field - off -field / edge to the field - unsprayed (drift?)	Aquatic and terrestrial	High if properly arranged +++	Monetary compensation needed but possibly not sufficient	More feasible with bigger ponds, but smaller ponds might be crucial in some regions. Difficult implementation for temporary ponds.	+++
In field					
Corridors for migration - in-field - unsprayed (drift?)	Terrestrial stages	High if properly arranged. Reduces the exposure of amphibians in field if accepted +++	Monetary compensation needed but possibly not sufficient	Difficult implementation. Medium combination affinity to supporting measures (linear structures) for other NTO. Might separate fields in different parts. Planning and spatial arrangement needed. Some in-field area lost.	++
Greening tramlines / undersowing - in-field - sprayed	Terrestrial stages	Exposure reduced via interception	Monetary compensation needed but possibly not sufficient	Facilitates migration. Potentially full exposure reduced by interception.	+/-
Cereals planted in wide rows (for birds) - in-field - sprayed	Terrestrial stages	Unknown	Monetary compensation needed and possibly sufficient	Could facilitate migration. Potentially full exposure reduced by interception.	-
"skylark windows" - in-field - drift?	Terrestrial stages	Habitat in field, appropriateness for amphibians questionable +	Monetary compensation needed and possibly sufficient	Less contaminated habitat in field. Potential exposure. Need to be combined to amphibian habitats.	+
Site-appropriate small structures (e.g. 3x3m) as supporting measures (stone piles, wood piles) - in-field	Terrestrial stages	Medium if properly arranged, Reduces the exposure of	Monetary compensation needed but possibly not sufficient	If created in very large fields could be the only off-crop available.	+/-

- drift?		amphibians in field if accepted e.g. daytime shelter +++		If the area is already used by amphibians, then the exposure is reduced. If not well connected could be a trap for attracted animals from the off-field.	
Creation of (extended) buffer zones to spawning areas additionally - in -crop - no-spray (drift?)	Aquatic and terrestrial stages	Medium if properly arranged ++	Monetary compensation needed but possibly not sufficient	Most feasible with bigger aquatic bodies. Difference to RMM unclear.	++
Bigger Landscape planning projects					
Creation of new spawning areas, building ponds	Amphibian populations	Need to be arranged properly	Monetary compensation needed but possibly not sufficient	See above for protection of ponds.	
Integrated water management/ drainage abandoned?	Amphibian populations		Monetary compensation needed but possibly not sufficient		
Planting of wet rice fields e.g., instead of drainage	Amphibian populations		Monetary compensation needed but possibly not sufficient		
Spatial arrangement of supporting areas/connectivity	Amphibian populations		Monetary compensation needed but possibly not sufficient		
Creation of pasture land, conversion to less pesticide intensive agriculture	Amphibian populations		Monetary compensation needed but possibly not sufficient		
Awareness raising					
Raising awareness among farmers and agricultural advisors	Amphibian populations	Extremely high	Mandatory	Prerequisite for all ecological supporting measures.	

The success of any ERA scheme succeeds or falls with the implementation by the farmer. Due to the poor inspection of actions taken by farmers, it is important to develop communication strategies. Events and workshops to inform farmers should be mandatory for farmers to buy and use a product, not only to protect amphibians but biodiversity. The implementation of supporting measures, and their efficacy, will be linked to an appropriate monitoring programme[6].

[6] PERIAMAR Deliverable 14 https://periamar.com/assets/Uploads/09a0fbd0e0/Deliverable_14-v2.pdf

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12. Appendix

12.1. MAF calculation

The procedure for calculation of acute (MAF_{acute}) and reproductive ($MAF_{long\text{-}term}$) multiple application factors is described in the EFSA Guidance for Birds and Mammals (EFSA PPR Panel 2023). For the present risk assessment, $MAF_{long\text{-}term}$ replaced what in the Bird and Mammal Guidance is referred as MAF_{repro}.

$$MAF_{long-term} = \frac{1 - e^{(-nki)}}{1 - e^{(-ki)}}$$

with:

n = number of applications
k = ln2 / DT50 (rate constant)
i = application interval (d)

$$MAF_{acute} = \frac{MAF_{long-term} \cdot RUDm + f_{90} \cdot \sqrt{MAF_{var} \cdot \sigma^2}}{RUD_{90}}$$

with:

RUDm = geometric mean of RUD value (Table J.1 in EFSA PPR Panel et al. 2023)
f_{90} = 1.28 (90th percentile for standard normal distribution)
σ^2 = variance of RUD data set
RUD_{90} = 90th percentile of RUD value (Table J.1 in EFSA PPR Panel et al. 2023)
MAF_{var} = MAF variance. Can be calculated as:

$$MAF_{var} = \frac{1 - e^{(-2nki)}}{1 - e^{(-2ki)}}$$

12.2. K_{fw} calculation

The K_{fw} is calculated using Polyparameter Linear Free Energy Relationships (ppLFERs), which are multiple regression models that use several solute- or sorbate-specific descriptors as independent variables (Endo and Goss, 2014). Out of the different equations available for ppLFERs to calculate logarithmic partition coefficients (log K), the following one has a wide applicability and used in the amphibian body burden model by Mingo et al. (2024):

$$\log K = c + sS + aA + bB + vV + lL$$

The uppercase letters on the right-hand side of the equation are the solute descriptors: E, excess molar refraction; S, dipolarity/polarizability parameter; A, H-bond donating property; B, H-bond accepting property and L, logarithmic hexadecane-air partition coefficient (Hammer et al., 2021).

The lowercase letters s, a, b, v, and l are system parameters for phases 1 and 2 and are regression coefficients. Each term quantitatively describes the energetic contribution of a molecular interaction to log K (Hammer et al., 2021). Mingo et al. (2024) used parameters for storage lipid-water (Endo and Goss, 2014), muscle protein-water (Endo et al., 2012) and Phospholipid-water (Endo et al., 2011) partitioning were used.

Experimentally derived solute descriptors for a wide variety of chemicals have been compiled and are available in a public database at http://www.ufz.de/index.php?en=31698 (UFZ-LSER Database). For chemicals for which multiple solute descriptors were described, the arithmetic mean will be used. In case experimentally derived descriptors are not available, they can be calculated based on the chemical structure (SMILES format) of the pesticide using the UFZ-LSER databases calculator.

The selected ppLFER equation is applied to calculate three partitioning coefficients of the chemical relative to different tissues: $K_{storage\ lipid}$ (storage lipid:water partitioning coefficient), $K_{membrane\ lipid}$ (membrane lipid:water partitioning coefficient) and $K_{muscle\ protein}$ (muscle protein:water partitioning coefficient). Then, the K_{fw} is calculated as follows:

$$K_{fw} = (K_{storage\ lipid} \cdot K_{storage\ lipid} / D_{storage\ lipid}) + (K_{membrane\ lipid} \cdot f_{membrane\ lipid} / D_{membrane\ lipid}) + (K_{muscle\ protein} \cdot f_{muscle\ protein} / D_{muscle\ protein}) + (f_{water} / D_{water})$$

Where “f” values are volume fractions of each tissue and “D” is the corresponding density. The “f” values can also be calculated from the UFZ-LSER Database using the biopartitioning tool based on information about tissue composition described for amphibians. In particular, Mingo et al. (2024) used data from marhsfrogs (*Peophylax ridibudus*) retrieved from Çağiltay et al. (2014). For tissue density, Mingo et al. (2024) used 0.93, 1, 1.4 and 1 kg / l for storage lipid, membrane lipid, muscle protein and water, respectively, as reported by Endo et al. (2013).