Deliverable 14 – Proposals for retrospective assessment, future research steps, and possible coordinated actions

CA18221 – PERIAMAR
PEsticide RIsk AssessMent for Amphibians and Reptiles

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Funded by the European Union

Contents

1. INTRODUCTION

This document constitutes the deliverable #14 of PERIAMAR, which summarizes three main topics that were discussed and agreed during the Final Meeting, celebrated in Dessaru (Germany) on March 20-22, 2024.

The three topics refer to further activities to be conducted after the finalisation of PERIAMAR. Experts gathering in the final meeting conducted several workshop to formulated these needs in three specific directions:

- Uncertainties linked to the risk assessment proposal. This consists on the identification and categorization of the uncertainties associated with each step, procedure of assumption included in the proposal for amphibian risk assessment that has been elaborated during the Action (see deliverable #13). A column is incorporated, as a result of the uncertainty analysis, to list the necessities to reduced the identified uncertainty. Priority should be given to actions to address those aspects with highest influence on the risk assessment outcome and with highest chances of resulting in an under- or over-protective scheme.
- Post-registration monitoring. This is a kwy element of the newly designed risk assessment. As detailed in the uncertainty table, the long list of assumptions on which the risk assessment is based fosters the needs of a post-registration assessment that serves to control the efficacy of the assessment. However, given that monitoring linked to a single substance is complicated, the PERIAMAR proposal is to link the monitoring to the efficacy of supporting measures introduced to reinforce the assessment outcome. This generates an integrated approach where the focus is placed on the specific protection goal instead of on the pesticide, and can be additionally adapted to other taxa.
- Future actions. The main research needs and opportunities for funding to address them were discussed during the final PERIAMAR meeting. The last part of this deliverable summarizes the main conclusions agreed by participants in that meeting.

2. UNCERTAINTIES LINKED TO THE RISK ASSESSMENT PROPOSAL

Parameter or assumption	**Sources of uncertainty**	**Sensitivity of the parameter in the TER**	**Uncertainty for under-protection in the risk assessment**	**Uncertainty for over-protection in the risk assessment**	**Actions to reduce uncertainty**
Fish LC50 extrapolated to amphibian adults LC50	• Extrapolation based on limited data set and old pesticide substances (no longer approved). • Extrapolation from fish to amphibian larvae. • Extrapolation from amphibian larvae to adult.	High	Medium	Medium	• Studies on coverage of aquatic adults by larvae. • Toxicity data to compare with fish for current-use pesticides.
Amphibian LD50 extrapolated from fish LC50	• Limited data set, mostly linked to a few types of substances (organochlorines, organophosphates). • No validation possible due to limited data. • Body surface area in contact with treated substrate not confirmed. • 100% skin absorption assumed.	High	High (except skin absorption)	High	• Increase amphibian LD50 dataset to improve model and offer validation chances. • Studies on body surface area in contact with treated substrate. • Studies on skin absorption for substances with different chemical properties.
Amphibian larval growth extrapolated from fish growth-based LOEC (from ELS and FSTRA tests)	• AMA-based data (not designed to measure growth, not to be used as a dose-response test) • Extrapolation based on limited data set.	High	Low (to cover larval growth) High (to meet population persistence SPG)	Low (to cover larval growth) High (to meet population persistence SPG)	• Link larval growth to population persistence. • Toxicity data for under-represented substances in the comparison.

Use of ACR to derive a reproductive endpoint for amphibians from fish, birds or mammals	• High frequency of open-ended toxicity estimators. • Little consistency across taxa (need to test patterns related to chemical types) • Endpoints on which NOECs are based are not always the same.	High	High (especially if the long-term toxicity estimator is open-ended)	High (especially if the lowest calculated amphibian NOAEL is used, or acute toxicity estimators are open-ended)	• Test inter-taxonomical patterns for specific chemical types. • Run an impact assessment of using this extrapolation approach. • Depending on the results of the two previous actions, consider increasing available dataset.
Use of benchmark values as alternative to NOECs to calculate ACR	• Same as the for the previous row.	High	Lower than above, but still high	Lower than above, but still high	• Same as the for the previous row. • Increased dataset needed to estimate benchmark values compared to NOECs.
Condition at the metamorphosis to extrapolate population level effects (link to adult survival or reproduction)	• Little empirical data to link both parameters (despite evidence showing little survival of recently metamorphosed individuals with poorer conditions).	Does not apply to TER. High impact in the RA outcome	Unknown	Unknown	• Data to link condition at metamorphosis to population persistence.
Use of long-term mechanistic effect models to estimate long-term effects (toxicity testing in tadpoles as a replacement method for	• No models available so far. • Data to develop and validate those models is not too large.	Medium	Undefined, would depend on the model developing conditions	Undefined, would depend on the model developing conditions	• Build models. • If no data are available to build models, generate specific data.

toxicity testing with adults)					
Use of PECsw and PECsed from amphibian pond TOXSWA model	• Estimates are affected by the scenario definition (high fluctuations depending on the water depth) • Climatic conditions behind the scenarios are not considered (general for the aquatic RA)	High	Low	Medium (considering that models are on the conservative side)	• Apply models locally, and possibly validate with measured data.
Oral uptake (acute and long-term, all tiers): FIR estimated from T-Herps	• Taken from the USEPA model (check source for uncertainty assessment).	Medium	Low	Low	
Oral uptake (acute, all tiers): assimilation efficiency (AE) estimates	• Very little information, in general, especially for variability among food items.	Low	Low	Medium at all tiers, as the most conservative value is taken	• Increase data set for AE, especially for its application in tier 1.
Oral uptake (acute and long-term, all tiers): RUD, MAF and fTWA from EFSA bird and mammal guidance	• Taken from the EFSA bird and mammal guidance (check source for uncertainty assessment). • fTWA based on the outcome of toxicity testing for birds and mammals.	High	Medium	Medium	• Studies on the applicability of fTWA to the case of amphibians.
Oral uptake (acute and long-term, tier 1): mixed diet	• The preliminary database compiled during the action includes 81 records from 21 different EU species 21; little is known about site- or season-dependent variations.	Low	Low	Low	• Increase data set for diet composition to build a more realistic and generalizable mixed diet composition.
Dermal uptake (acute and long-term, all tiers): PEC_{soil}	• Unknown whether 1 cm is a relevant depth to determine the	Low	Low	Low	• Improve knowledge on the relevant soil layer depth

assumed for a 1 cm layer	contact of amphibians with soil.				in contact with amphibians.
Dermal uptake (acute and long-term, all tiers): K_{fw} calculation procedure	• Tissue composition in amphibians collected from a single paper.	Low	Low	Low	• Improve knowledge on the composition of amphibians to refine Kfw calculations.
Dermal uptake (acute and long-term, all tiers): skin permeability	• Skin absorption equations are corrected by a factor for increased permeability through amphibian skin.	Medium	Low	High	• Studies on skin absorption for substances with different chemical properties (see above) can be used to validate this factor.
Dermal uptake (acute and long-term, all tiers): skin thickness assumed as 5% of the radius length	• Unclear source	Low	Medium	Low	• Studies on skin thickness.
Dermal uptake (acute and long-term, all tiers): exposure duration	• Decision for acute exposure duration (8h) is assumed, but not justified. • Application for long-term exposure is done by changing the exposure duration, but it is not validated	Low (acute) High (long-term)	Medium (acute) High (long-term)	Medium (acute) High (long-term)	• Validate the model for long-term exposures. • Explore further what would be a relevant acute exposure time for dermal uptake
Dermal uptake (acute and long-term, all tiers): water uptake rate and time to rehydration	• Decisions for default parameters are assumed, but not justified.	Low	Medium	Medium	• Explore further what would be a relevant water uptake rate and time to rehydratione
Dermal uptake (acute and long-term, all tiers): soil organic fraction	• Assumed within the range of soils from the experiments used to develop the model	Low	Low	Low	• Increase the range of organic fraction in the soil to make it relevant to

					amphibians under all scenarios.
Overspray uptake (acute, all tiers): skin permeability	• Assumed 100% in the screening, refined according to two studies in tier 1.	High	Low	Medium	• Studies on skin absorption for substances with different chemical properties (see above).
Use of focal species in tier 2: combination of parameters (body weight, surface area, diet composition, AE)	• Uncertainty linked to all the parameters individually is addressed above as part of low tiers. The need to have them combined for a given species increases the overall uncertainty, as chances for the available information to come from the same taxon are reduced.	High	High	High	• Same actions as for each of the parameters when treated individually (see lower tiers above).
Field data to support population modelling in tier 2 / high tier	• Limitation of input parameters. • Difficulties in validation, as they need to be spatially explicit.	High	High	High	• Increase dataset for selected species. • Develop models that can be validated as easily as possible.
Mitigation and supporting measures	• Lack of data to determine effectiveness	High	High	High	Monitor their implementation in relation to amphibian exposure to pesticides (for RMM) or to population persistence (for supporting measures)

3. POST-REGISTRATION MONITORING

The general goal of ecological monitoring is to describe how biodiversity changes. A secondary goal is to understand why changes occur. In the context of the regulation of the plant protection products, the idea is that a pesticide is approved based on a prospective risk assessment, but then there must be a post-approval monitoring to support a retrospective risk assessment. Monitoring could be designed with the purpose of testing the efficacy of risk compensation measures. This way, the monitoring is not linked to a specific pesticide, but is still linked to the risk assessment. The protection goal is population persistence, so the monitoring should be designed in a way that it serves to test whether the compensation measures linked to the approval of a product are enough to maintain the stability of amphibian populations in a geographic area.

When designing a monitoring programme, three questions have to be answered (Yoccoz et al. 2001): why, what and how? The "why" question is crucial and has to be answered first. One has to be clear what the objectives of a monitoring programme are. What does one wish to learn and how will be the data be used? In most cases, results of a monitoring programme should inform conservation practice, species management, or pesticide regulation. There is no general answer to the "why" question. In the context of pesticide regulation, the answer to the "why" question is fairly straightforward: Is the protection goal met? That is, do populations persist in a landscape where (novel) pesticides are used? The question might be rephrased as "do populations in a landscape persist where (novel) pesticides because compensation actions work?".

The second question, "what", can be answered once there is a clear answer to the "why" question. "What" essentially means which state variable or variables should be measured. State variables could be biochemical markers, individual growth and health, population-level parameters (e.g., abundance), characteristics of the metapopulation, or many other variables. In the case of pesticide regulation, the protection goal, and therefore the answer to the "what" question, is population persistence. However, population persistence can be defined and measured in many ways. For example, it could be defined as population viability (in the sense of a population viability analysis) or as a non-negative trend of a time series of population size estimates. The answer to the "what" question can also depend on how quickly the monitoring programme should deliver results. For example, it can take many years until one can test for a trend with enough statistical power in a time series analysis. In contrast, early warning signals such as malformations can be detected quickly (Böll et al. 2013).

This leads us to the third question, "how". Once the "why" and "what" questions are answered, this question is often fairly straightforward to answer, at least when there are no financial constraints and man-power is not limiting. There are many books, manuals and guidelines on field methods in herpetology and there is also a vast literature on the analysis of monitoring data and demographic and population analysis. If the goal of a monitoring is to assess the effect of compensation measures, then good data on the compensation measures is necessary as well and such data may have to be collected as part of the monitoring programme.

We emphasize that it is useful to plan both goals, hypotheses, field work, data analysis, and possible interpretation of the results in advance. This facilitates conflict resolution (Anderson et al. 1999).

Given the complexity of a monitoring programme, no final answer can be given. A case-by-case approach will be necessary. This chapter can be used as a guideline for monitoring amphibians; a proposal for monitoring the effects of pesticides on amphibians was published by Böll et al. (2013). We focus on amphibians but the general principles for reptiles are the same.

3.1. 'WHY'

In this section, we assume that the "why" question is the following: "do populations in a landscape persist where (novel) pesticides because compensation actions work?".

3.2. 'WHAT'

There are many way how the protection goal "persistence of populations" could be quantified. This begins with the question over which time period a population should persist. Consequently, monitoring would have to be carried out over this period. However, it may be preferable to monitor early warning signals rather than to conclude after, say, ten years that the population went extinct. The time needed to arrive a conclusion should perhaps better be measured in terms of generations rather than years. As some amphibians can be short-lived (e.g., treefrogs) and others long-lived (e.g., salamanders), generation can vary among species. It is not straightforward to predict whether one would see negative effects of pesticides quicker in short- or long-lived species.

An early warning signal might be tadpole survival. Estimates of tadpole survival, or other vital rates, might be used in a population model to predict population growth rate and therefore population viability (e.g., a Leslie matrix model). Böll et al. (2013) suggested a mixed approach where both short-term and long-term effects are monitored.

The complex life cycle of most European amphibians, which is often associated with seasonal migrations between different aquatic and terrestrial habitat types, can be a challenge and an opportunity. It can be a challenge because it may mean that amphibians have to be monitored in multiple habitats. At the same time, the life cycle offers the possibility to focus on life history stages which are easy to monitor.

An important point to consider is whether one would like to monitor a single species (which might serve as a sentinel for pesticide effects) or entire amphibian communities (i.e., multiple species). The general principles are the same for single species and communities but the methods to be used in the field depend on the species.

We recommend that all data are analysed using robust statistical methods for monitoring data. In particular, the data should account for imperfect detection (Kéry and Schmidt 2008).

One needs a benchmark or control against which persistence can be compared. For example, if one would like to know whether compensation works, then one needs to compare populations or metapopulations with and without compensation.

3.2.1. POPULATION PERSISTENCE

Population persistence cannot be monitored directly. It is inferred from an analysis of data on, for example, abundance. A population viability analysis means that population growth rate or extinction risk is quantified. For this, demographic data are necessary. One might also estimate persistence of an metapulation at a landscape scale. For this, occurrence data are required.

3.2.2. ABUNDANCE

Persistence can be inferred if there is no change in abundance. Therefore, abundance can be a good state variable to monitor. Abundance can be monitored at a single or multiple sites.

Abundance can be quantified for different life history stages (egg masses, adults, larvae).

3.2.3. DISTRIBUTION

The distribution of a species in a larger geographic area could also be used to monitor persistence. Such an approach may have advantages because populations can go extinct due to stochastic processes. Distribution of amphibians is usually presence/absence in ponds or wetlands but one could also quantify abundance at multiple sites.

Numbers can give you quantitative information in also small area or just a pond. The difficulty with absolute numbers is that the variety of life stadia of amphibian is different. The amount of egg (clutches) won't directly say something about the population. By amphibian is the combination of egg or larvae and adults important. Egg-larvae combination is about the ratio of breeding success. If data of adults can joint to the egg-larvae you have information about the breeding success or breeding area quality and the land habitat. Data of the different life stages, composition of stages of life, distribution of species in the different biotopes says much more than a present or absence. The frequency of monitoring during a year determines the usability and reliability.

3.2.4. DEMOGRAPHY

It can be useful to collect data on the demography in a monitoring programm. Demography allows to better understand the ecological processes that lead to changes in abundance and occupancy (e.g., survival of different life history stages, recruitment, dispersal).

3.2.5. OTHER POPULATION PARAMETERS

Other parameters can be useful as well. For example, one might quantify malformations and correlate them to pesticide use and compensation measures.

3.2.6. ENVIRONMENTAL PARAMETERS

Environmental parameters are an important addition to monitoring. It contributes to interpreting the collected data. In the case of pesticide regulation and the goal to understand the effects of compensation, data on compensation is necessary as well. More environmental parameter can be useful because there are generally many drivers of amphibian occurrence, abundance and demography. It may be the case that the effects of pesticides and compensation can only be detected and described if another variables (such as pond size, pond hydroperiod, amount and quality of terrestrial habitat, etc.) are taken into account. are an important addition to monitoring. It contributes to interpreting the collected data. Parameters can be useful to exclude or include influences on the collected data. In the diagram is a list of parameters that can be used by amphibians.

The minimum information in addition of the data must consist of the observer, location, time and method. Water quality sampling is also a parameter what is useful in some cases. A disadvantage with many of the parameters is that the moment of recording and in certain cases the subjectivity can influence the assessment. It is therefore important that this is described correctly in the protocol. The choice of the moment and the frequency determines the application in the dataset.

Diagram after example of RAVON monitoring and habitat analysis crested newt.

name colleague									
date	.... / ... /								
time									
locatie coordinaat (x-y)									
Research area									
km square									
trajectnr. / waternumber									
watertype	pond	clay pit	ditch	loam pit	bayou	whirpool	pingo	ven	others
substrate	sand	loam	clay	peat	others				
picture									
lenght pond in meters									
wide pond in meters									
inclination	>45 degree	1:1	1:2	1:5	1:10				
waterdepth (cm) 1 meter off shore									
waterdepth (cm) op 3 meter off shore									
change on drying out in breeding season	none	small	moderate	large					
clarity water	helder	turbid	very turbid						
thickness organisch sludge (cm)									
% shadow	0-10%	10-25%	25-50%	50-75%	75-100%				
presence of fish?	uncertain	probably	yes	no					
presence water bird (duck/goose etc)	yes	no							
% submerged vegetation	0%	1-5%	5-10%	10-25%	25-50%	50-75%	75-100%		
% coverage alg.flab	0%	1-5%	5-10%	10-25%	25-50%	50-75%	75-100%		
% coverage helophyte	0%	1-5%	5-10%	10-25%	25-50%	50-75%	75-100%		
% coverage floating leaf (no duckweed)	0%	1-5%	5-10%	10-25%	25-50%	50-75%	75-100%		
% coverage duckweed	0%	1-5%	5-10%	10-25%	25-50%	50-75%	75-100%		
% totale coverage vegetation	0%	1-5%	5-10%	10-25%	25-50%	50-75%	75-100%		
% coverage indication plants seepage indication	0%	1-5%	5-10%	10-25%	25-50%	50-75%	75-100%		
other seepage indicators	n.v.t.	Ironoxide (orange)	white/grey	bubbling water					
pH value									
Are there new ponds	yes	no							
Trophicity	very nutrient poor / strongly acidified		oligotrophic		mesotrophic		eutrophic	hypertrophic	
Are there more potential ponds in the area?	yes, similar to measurement trajectories		yes, less suitable than measurement trajectories		no, other waters are unsuitable		different or additional explanation		
comments									

3.3. 'HOW'

There are many possibilities to monitor amphibian occupancy, abundance, demography, or other parameters. It is important to choose a method that is not harmful for the animals (e.g., toe clipping for individual identification). The method must be reliable, enforceable and repeatable and should allow to control for imperfect detection. Standardisation is important for monitoring. The procedures of the monitoring is usually recorded in a monitoring protocol. People doing field work must follow the guidelines. The 'why, 'what' and 'how' is described in detail. The monitoring protocol should describe how field work has to be done but it should also deal with the safety of field workers and biosafety (in order to avoid the spread of pathogens). It should also be clear how data are collected (on paper or digital form?), how and where data is stored. Protocols for data analysis should also be planned ahead of data collection.

The choice and number of sites (or populations) depends on the "why" and "what" questions. The choice of sites should be representative for the study area and species.

As there are many different species with different natural histories in a wide range of environmental settings, it is advisable to consult with a local amphibian specialist. The specialist can provide advice on how to monitor species once the state variables have been defined. There is a vast number of books, manuals and guidelines on field methodology (e.g., Heyer et al. 1994, Dodd 2009). One should also consult with a person who has a good knowledge of the methods to analyse monitoring data. The combined advice will lead to a strong monitoring programme, particularly if monitoring and decision-making are planned at the same time (Nichols and Williams 2006).

The monitoring has to be done by professional herpetogists or ecologists but sometimes it is possible to use citizen scientists or well trained volunteers.

The monitoring has to be done by professional herpeto- or ecologists but sometimes it is possible to use citizen science or well trained volunteers.

3.3.1.LISTEN, SEE AND CATCH

Call surveys (listen), visual encounter surveys (see) and captures are the most common methods common in monitoring. They can be used sequentially. If you reach the field site you can start at a distance with the method of listening. After that, one can search amphibians visually or through captures (using a net or, if you plant to visit a site again, using traps). A combination of the method gives you a complete picture.

Call survey

Listing can be done by ear, (long range) microphone or hydrophone. You can listen to chorus activity of amphibians but also you can count the splashes/plops on track when frogs jumping into the water if you walk by. The long range microphone can be used in terrain that's difficult to reach. Some species like natterjack toad and tree frog are good to monitor with this microphone. For common spadefoot the use of a hydrophone is also a useful tool for monitoring.

Visual encounter survey

With a torch during you can find easily amphibians in the dark. Not only adults and larvae but also eggs or clutches can be seen in the water.

Captures

Use the net for catching amphibian is common in the field. You can catch different species in different stadia. In some areas the use of net is not appropriate. The option to use a trap is than a better option. Actually it requires a greater effort.

3.3.2.E-DNA

eDNA is a method to detect species. A water sample is collected and later analysed in the laboratory. The focus can be on single species or communities. eDNA provides information on the presence and absence of species.

3.4. REFERENCES

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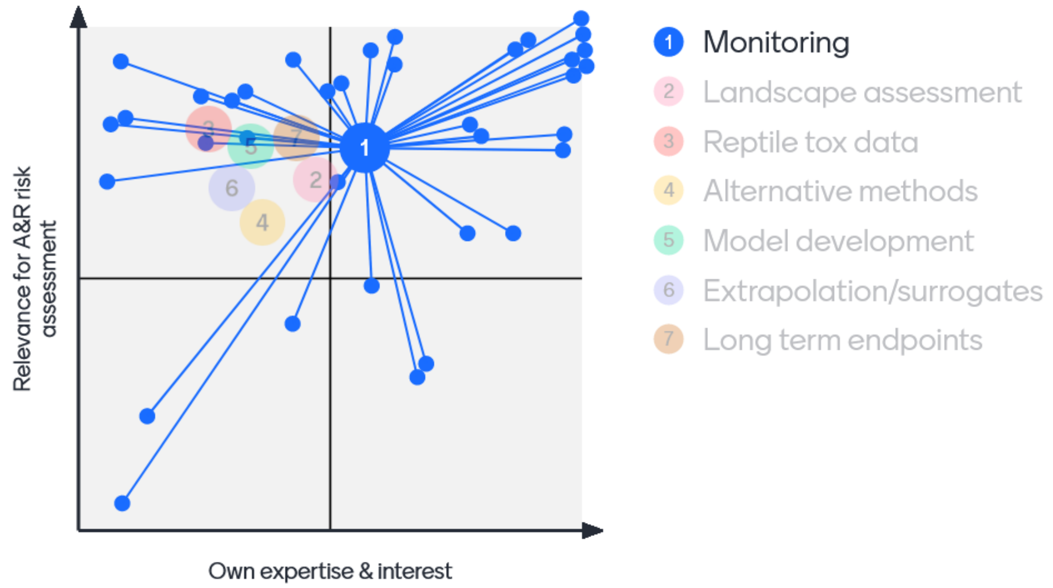
4. FUTURE ACTIONS

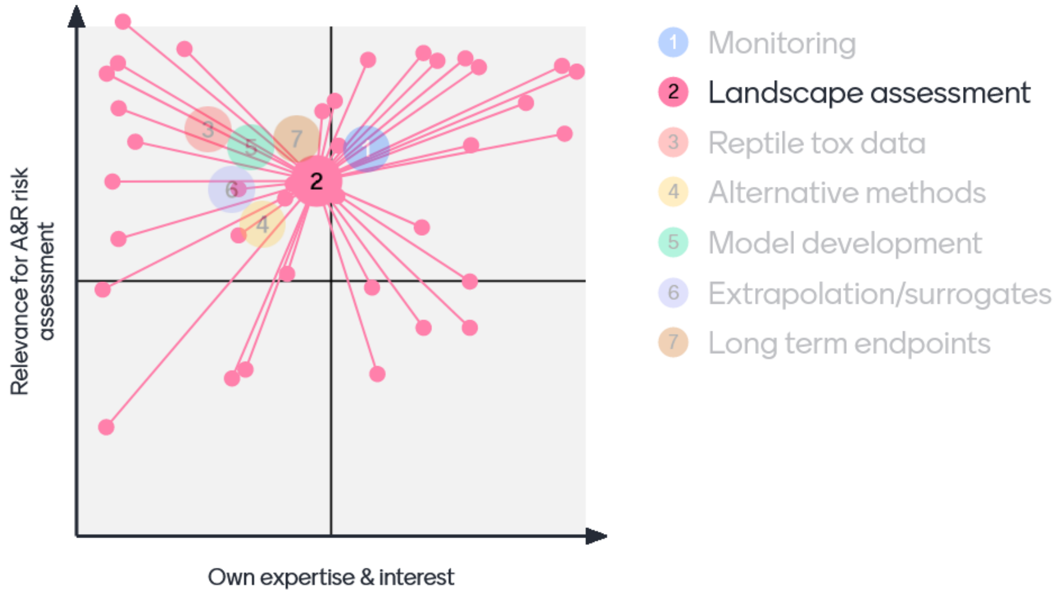
The exercise consisted of two parts. In the first part, participants identified relevant research project calls that could offer an opportinuti to submit proposals driven to address the open questions, like those included in the right column of the ucertainty table above, or focus on developing and validating post-registration monitoring schemes. Then, they were asked to distribute 100 points among the identified calls, points representing the degree of effort that they would put in preparing a proposal for that specific call. The following table presents the averaged points given to each call:

Choices	Score
EU Framework Programme: Standard Action Projects for nature and biodiversity. Keywords of the call: Conservation of biodiversity, habitats and species	19.90
EU Biodiversa+	14.93
COST Actions	14.38
EFSA - tendering of relevant topics for the ERA for amphibians & reptiles	14.19
EU: Interrreg-SUDOE - Promote the protection and conservation of the environment, biodiversity, & reduce all forms of contamination	13.38
EU Framework Programme: MSCA Doctoral Networks	9.40
EU Agroecology- Enhancing agroecology at the farm level. Implementing agroecology at the landscape level.	7.81
Interreg various	3.76
Cyted - networking	2.25

During the second part of the exercise, the participants wrote the tree topics that, in their opinion, should be prioritized as topics to conduct further research. After compiling all answers, they were summarized in seven categories: monitoring, landscape assessment, toxicity data for reptiles, alternative methods, model development, extrapolation/surrogates and long-term endpoints. Then the participants were asked to participate in a poll during which they should rank, from 1 to 10, (i) their self-percieved expertise in each of the topic, and (ii) the importance of that topic for risk assessment of amphibians and reptiles.

The following figures show the results relative to each of the topics.


Relevance for A&R risk assessment
Own expertise & interest
1 Monitoring
2 Landscape assessment
3 Reptile tox data
4 Alternative methods
5 Model development
6 Extrapolation/surrogates
7 Long term endpoints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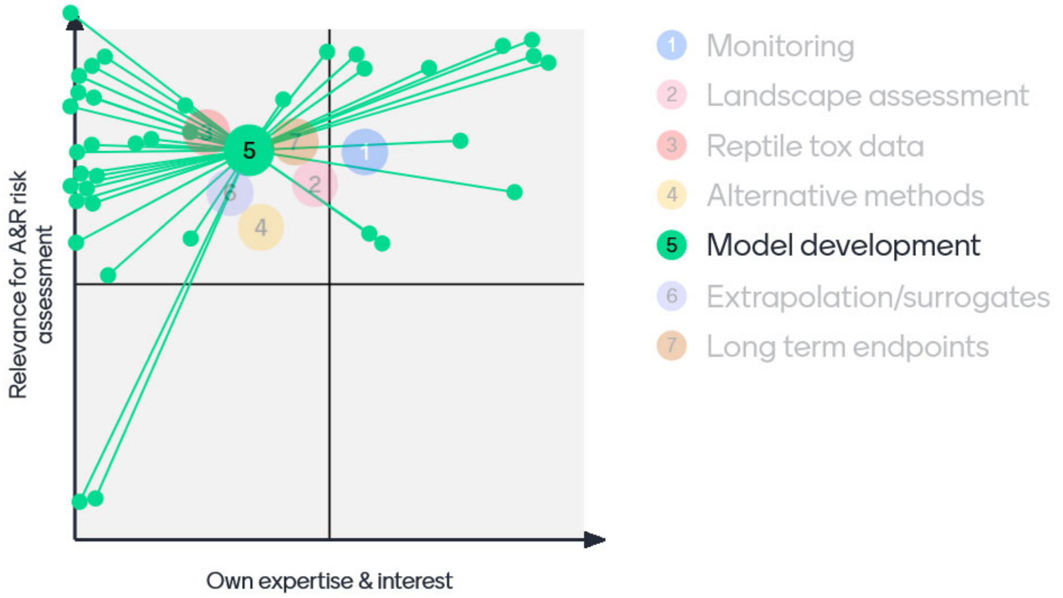
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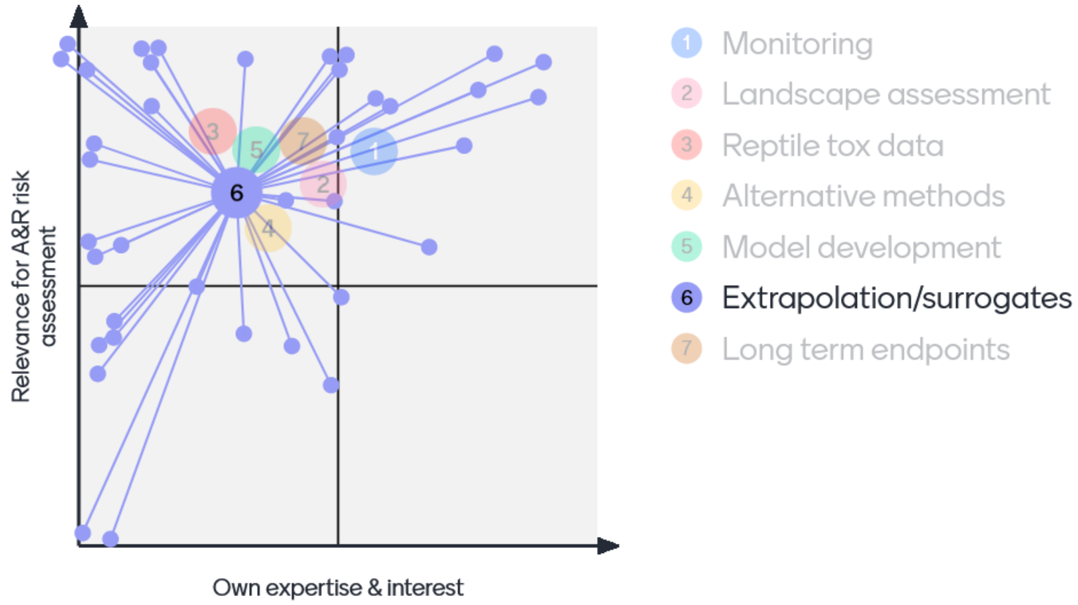
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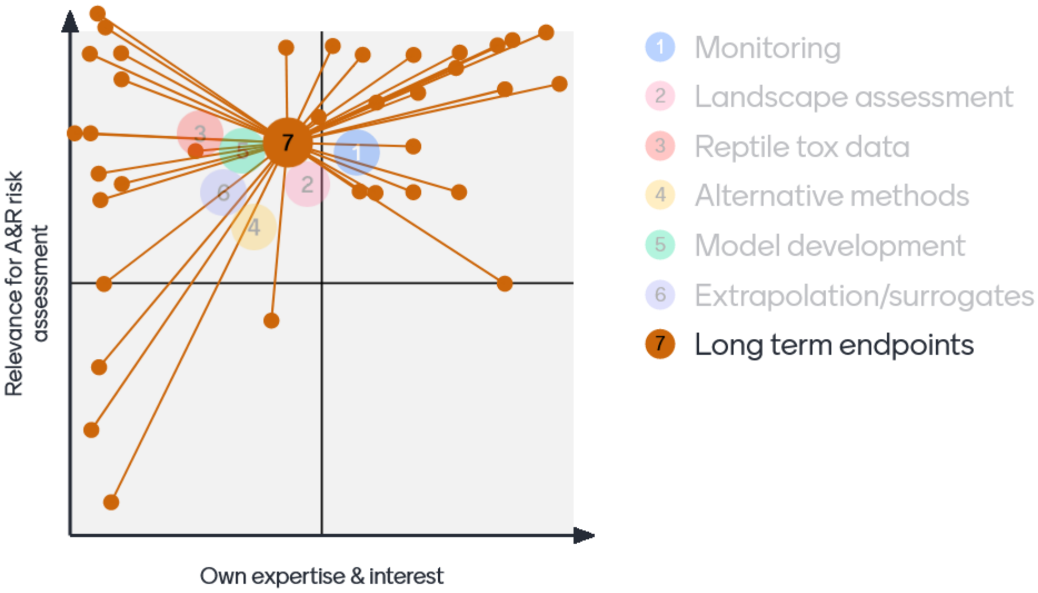

All topics were ginven a high relevance for risk assessment, which is expected as they compiled the list of three provided by participants. The gradient in the self-perceived expertise is larger, from low to high but normally avoiding extremes. This perception supports the capacity building of the network, since, despite meeting participants coming from a very diverse range of research fields, the majority of them indicated some degree of expertise in all the topics. There was no correlation between expertise and relevance given to the topic, which also proofs how action participants have assumed the general interest of the matter instead of giving a higher preference to those topics they are more familiar with. The exercise does not clearly show any specific topic to which highest attention should be given; perhaps the most challenging ones, simply because the generally lower expertise existing in the network, would be generation of reptilian toxicity data, model development and extrapolation.

In general, none of these topics by itself was seen as broad enough to justify by itself the preparation of a project proposal to be submitted to one of the calls above. Rather, the network supported a broader proposal comprising the seven topics. In this context, there was a general agreement that the integration of all (or the majority) of the identified topics could be supported by a specific call within one of the Clusters of the upcoming Work Programmes within Horizon Europe framework.