DRAFT SCIENTIFIC OPINION

Coverage of endangered species in environmental risk assessments at EFSA[1]

Scientific Committee[2, 3]

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ABSTRACT

EFSA performs environmental risk assessment (ERA) for single potential stressors such as plant protection products, genetically modified organisms and feed additives, and for invasive alien species that are harmful to plant health. The ERA focusses primarily on the use or spread of such potential stressors in an agricultural context, but also considers the impact on the wider environment. This opinion explores to what extent endangered species are covered in these current ERA schemes. First, the legal basis and the relevant ecological and biological features used to classify a species as endangered were analysed. The characteristics that determine vulnerability of endangered species are reviewed and whether or not endangered species can suffer more than non-endangered species from potential stressors in an agricultural context. It is not the intention of this opinion to indicate where, when or for how long endangered species have to be protected. Due to their legal status, a lack of effect and exposure data can be assumed for endangered species, and the reliability of using data from other species is a key issue for their ERA. This issue and other possible limitations, such as the use of assessment factors or lack of certain exposure routes, are discussed when reviewing the current ERA schemes. The main conclusion is that there is variability among EFSA ERA schemes to which degree (implicit or explicit) endangered species are covered and how they are covered. Potential tools, such as population and landscape modelling and trait based approaches, for extending the coverage of endangered species in current ERA schemes are explored and reported. © European Food Safety Authority, 20YY

KEY WORDS

Endangered species, environmental risk assessment, ERA, plant protection products, genetically modified organisms, feed additives, invasive alien species.

[1] On request from EFSA, Question No EFSA-Q-2013-00901, adopted on DD Month YYYY.

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[3] Acknowledgement: The Scientific Committee wishes to thank the members and the *chair of the Working Group on overarching elements of Environmental Risk Assessment: Michael Bonsall, Theo Brock, Gianni Gilioli, Christer Hogstrand, Jonathan Jeschke, Mira Kattwinkel, *Robert Luttik, Ad Ragas, Paulo Sousa, Claus Svendsen, and Wopke Van Der Werf for the preparatory work on this scientific opinion and EFSA staff Fernando Alvarez, Yann Devos, Jean-Lou Dorne, Angelo Maggiore, Agnes Rortais, Reinhilde Schoonjans, Franz Streissl, Jose Tarazona, Sara Tramontini and Maria Vittoria Vettori for the support provided to this scientific opinion.

SUMMARY

Plant protection products (PPPs), feed additives (FAs) and genetically modified organisms (GMOs) are subject to a risk analysis and regulatory approval before being placed on the market, released into the environment, spread or used in agriculture. In this process, the role of European Food Safety Authority (EFSA) is to independently assess and provide scientific advice to risk managers on possible risks that PPPs, GMOs and Fas may pose to the environment. EFSA also assesses the environmental risks related to the entry and spread of invasive alien species (IAS) that are harmful to plant health and the effects of their management. PPP, GMOs, FAs and IAS are herein commonly denominated as potential stressors.

At EFSA's 10th anniversary conference (EFSA, 2012), it became apparent that EFSA's environmental risk assessment (ERA) schemes have evolved independently in the different areas within its remit (see EFSA, 2011), and that further harmonisation is possible on specific topics. EFSA therefore mandated (under mandate M-2013-0098) the Scientific Committee (SC) to harmonise EFSA's environmental risk assessment (ERA) schemes with regard to: (1) accounting for biodiversity and ecosystem services to define protection goals for ERA; (2) coverage of endangered species as non-target organisms in single-stressor ERA; and (3) temporal and spatial recovery of non-target organisms for environmental risk assessments. The SC therefore prepared three separate scientific documents to address the abovementioned issues.

The current opinion focuses on the coverage of endangered non-target species in ERA and considered common approaches across the EFSA areas of responsibility. The specific questions to be addressed include the following Terms of Reference (ToR):

ToR1: Are endangered species more vulnerable than other species

- based on their toxicological sensitivity, probability/possibility of exposure, specific potential for recovery, low genetic diversity, or
- because of other stressors, e.g. limited, marginal or fragmented habitat?

ToR2: Do the current ERA schemes appropriately cover endangered species?

ToR3: If not, what risk mitigation measures can be envisaged to prevent endangered species being put at risk from potential stressors?

ToR4: Is monitoring needed to check the efficacy of risk mitigation measures for the occurrence of endangered species?

No generally accepted definition is available for endangered species since endangerment is related to spatio-temporal scales. In this opinion, an endangered species is defined as a species that is either:

1. listed in one or more "red lists" as threatened (i.e. vulnerable, endangered, or critically endangered, or variants thereof), where the considered red lists are: (i) the European Red List, (ii) the global IUCN Red List of Threatened Species, and (iii) national and other regional red lists within Europe that follow the IUCN or another suitable classification scheme; or
2. rare based on the classification of Rabinowitz's seven classes of rarity (including "endemics", "classic rarity", "habitat specialists" and "truly sparse" species).

Regarding question *(ToR 1) Are endangered species more vulnerable than other species?*, endangered species differ from other species in being endangered, and this might be because they are more vulnerable than other species due to particular traits related to (i) exposure, (ii) recovery and/or (iii) sensitivity to the potential stressor, directly or via indirect effects. No convincing scientific evidence was found that endangered species have in general a higher exposure than other species of the same group. It appears that not the potential stressor or the endangered species *per se* may be decisive for ecological recovery from impact, but their interaction with (the properties of) the environments impacted by stressors, in which endangered species (temporarily) dwell. However, it seems that endangered species more often exhibit traits that are related to a decreased ability for recovery (e.g. they often have a slow life history). With respect to sensitivity against toxicological stressors, there is no evidence that endangered species are *per se* more sensitive towards regulated chemicals. However, since many of the endangered species are highly specialised, e.g. in their food or habitats, they may

only have been exposed to a restricted range of natural chemicals, therefore resulting in the phylogenetic loss of certain detoxifying pathways relevant for assessed chemicals. Some endangered species appear to suffer more from indirect effects than many non-endangered species. In conclusion, question (1) cannot be answered in general, but anecdotal examples illustrate why, where and when endangered species can be more vulnerable. Hence, endangered species can indeed be more vulnerable than the species or the vulnerable taxa currently considered in ERAs. It is therefore important to identify these more vulnerable endangered species and to explicitly consider them in ERAs.

Regarding question *(ToR2) Do the current ERA schemes appropriately cover endangered species?*, there are four types of potential stressors undergoing ERA within EFSA's remit and (mainly) in an agricultural context: PPPs, GMOs, IAS and FAs. For GMO and IAS, the protection of endangered species is explicitly dealt with during the problem formulation phase in the respective ERA schemes. These ERA schemes allow a tailor made-assessment and the selection of one or more relevant endangered species. For PPPs, the PPR Panel adopted an approach to species selection for prospective risk assessment of an individually assessed pesticide using (or leaving the option for) the concept of vulnerable species. Endangered species are assumed to be partly covered by the vulnerable species approach. While the vulnerable species concept takes account of exposure, sensitivity and/or recoverability, then it does not usually consider that the conservational state of a species can already be unfavourable. For FAs, the ERA does not tolerate population effects on any species in the environment and, thus, endangered species are implicitly included by the assumption that no FA is allowed on the market should a species be at risk. Thus, it currently varies among EFSA ERA schemes to which degree (implicit or explicit) endangered species are covered and how they are covered. The level of protection afforded by these four ERA schemes for endangered species seems to vary. However, the limited data availability does not allow to draw a firm conclusion and also does not allow an assessment of the level of protection achieved (regardless whether endangered species are implicitly or explicitly covered). Hence, risk assessment is conducted via selected (test) species, with assessment factors and extrapolations to endangered species (bottom-up approach). There is, however, a growing need for a landscape assessment (for each potential stressor addressed in this opinion, i.e. PPPs, GMOs, IAS, FAs) plus population modelling (top down approach).

Regarding question *(ToR3) What risk mitigation measures can be envisaged to prevent endangered species being put at risk from stressors resulting from the application of a regulated product?*, the mitigation for and monitoring of endangered species can often be best addressed in a site-specific manner (e.g. by specific conservation areas for weeds or hamsters in specific crops; financial compensation of farmers to implement specific land-use requirements that favour the red list species) rather than generically. Two objectives of mitigation can be distinguished: (i) to achieve a safe use of the product under assessment; (ii) specific risk mitigation measures that can be proposed as a result of observations from monitoring schemes. Very often, farmers will be the in-field risk managers, hence their education and training should be supported. The importance of risk mitigation measures should be well communicated to farmers and emphasised.

Finally, regarding question *(ToR4) Is monitoring needed to check the efficacy of risk mitigation measures for the occurrence of endangered species?*, the Scientific Committee considers it important to monitor the level of protection achieved by all management measures or mitigation measurements taken to protect endangered species (either compliance or supplementary monitoring). At present, only the GMO Panel is actively involved in regulated monitoring of the potential stressor. For invasive species, surveillance and monitoring is advisable in any case. For PPPs and FAs, EFSA is currently not involved in monitoring. At the member state level information on chemical and biological monitoring, for instance conducted within the context of the Water Framework Directive (WFD), may be used in the re-registration of the PPP.

During the scientific analysis for *ToR2*, scientific knowledge was collected on diverse approaches that are available to risk assessors. Without judging their necessity or implementation, potential approaches for extending the coverage of endangered species in ERA schemes include the following:

Explicit inclusion of endangered species in ERA schemes requires a detailed specification of the protection goals for endangered species, particularly in terms of what species (groups) should be protected where and when, to what level and with what level of certainty. The establishment of these specific protection goals for endangered species requires a joint coordinated effort involving risk managers, risk assessors, scientists and other stakeholders.

Different approaches can be followed to cover endangered species in ERA schemes for EFSA's remit. There is, however, not one single approach that suits all EFSA sectors (i.e. PPR, GMO, PLH and FEEDAP). For example, the surrogate species concept is frequently applied to assess GMOs, whereas a generic protection level in combination with a species-specific trait-based assessment (the vulnerable species concept) is more often used to assess PPPs.

Trait-based approaches, in which species traits are being used as indicators of potential (increased) risk, provide promising opportunities for including endangered species in ERA schemes. Further exploration and elaboration of the potential of this type of approaches is recommended, i.e.:

- Identification and validation of species traits that drive the ecological vulnerability of endangered species for different types of stressors, i.e. traits related to exposure, stressor sensitivity, recovery and sensitivity to indirect effects;
- Development of a systematic procedure in which species traits are being used to obtain a qualitative and/or quantitative estimate of the environmental risk of stressors for endangered species;
- Construction of a species trait database that can be used as a basis to assess the ecological vulnerability of endangered species for different types of stressors.

The rapid advancements in "omics" and "in silico" techniques are resulting in large amounts of data which provide information about the molecular mechanisms and species traits that drive the sensitivity of organisms to stressors. Current practical and ethical limitations involved in testing endangered species in the field or the laboratory can be overcome if this type of information can be applied in a predictive way, i.e. to predict the sensitivity of species based on molecular traits regarding the phylogenetic relationships between endangered and non-endangered species of the same group.

Mathematical models linking individual species traits and behaviours to populations, communities and landscapes provide a promising tool that can aid the risk assessment of potential stressors for endangered (if information on the actual impairment of the population is available at the ecologically relevant spatial scale) and other species.

Because the coverage of endangered species in ERA schemes cannot *a priori* be limited to one particular spatial scale, risk assessment might need be conducted at different spatial scales. This also depends on the overlap between the sphere of influence of the potential stressor and the occurrence of the endangered species.

At the end of this opinion recommendations for future studies are formulated.

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1. INTRODUCTION

Maintaining a healthy environment and conserving biodiversity are major goals of environmental protection. Legal frameworks therefore require the protection of human, animal and plant health, and the environment (including biodiversity). In the context of protecting biodiversity, impacts of agricultural practices also need to be considered. The European Food Safety Authority (EFSA), within its remit to ensure the safety of the food/feed chain, conducts environmental risk assessment (ERA) for potential stressors such as plant protection products (PPPs), genetically modified organisms (GMOs), invasive alien species (IAS) and feed additives (FAs). These PPPs, GMOs, IAS and FAs that fall under the remit of EFSA, may impact non-target populations, including endangered species. The current opinion explores how far the current ERA schemes adequately cover endangered species.

The Habitat Directive (Council Directive 92/43/EEC on the conservation of natural habitats and of wild fauna and flora, EC 1992) defines which species should be protected on what protection level and by which measures (e.g. by conservation areas or by strict protection). Such species of Community interest are in need of protection because they are endangered, vulnerable, rare and/or endemic (Article 1(g)). For strictly protected species, among other things, the deliberate killing or disturbance (animal species) or destruction (plant species) is prohibited (Articles 12 and 13). This provision could be interpreted as laying the foundation for the legal status of endangered species in the current opinion (see Appendix A for an analysis of the Habitat Directive; see Section 3 for a definition of endangered species). However, first, one precondition laid out by the Habitat Directive is the deliberate action, hence the negative consequences of an action need to be known and accepted. Therefore, prohibitions are based on a case by case basis depending on the knowledge on the occurrence of a protected species in a certain area and the anticipated effects of the deliberate action (although also incidental killing should be monitored). Second, the Habitat Directive allows member states to derogate from its provisions, e.g. to prevent serious damage to crops and livestock, if there are no satisfactory alternatives and if the species maintain a favourable conservation status in their natural range (Article 16). The question remains in how far the potential stressors dealt with in the current opinion fall under this derogation, which alternative approaches need to be taken into account and if any endangered species can be considered to be and be maintained in a favourable conservation status[4]. Hence, directly linking the Habitat Directive to the current opinion is not readily possible nor is it further pursued because the focus here is on the scientific knowledge about endangered species and EFSA's sectorial legislative framework.

The sectorial legislation in EFSA's remit and also the current practice in other countries outside of the EU account for endangered species to various extents. EFSA's regulatory frameworks are not designed for risk assessment for individual (endangered) species or local scales. In Section 4 it will be explored what characteristics make endangered species more vulnerable and on this basis it will be investigated in Section 5 whether and how endangered species are sufficiently addressed in current ERA schemes, implicitly or explicitly.

One example how endangered species are considered in the authorisation of potential stressors in other countries is the RA for the registration of PPPs in the US. There it needs to be ensured explicitly that there are no effects on listed endangered species (which are clearly interpreted as being part of the

[4] According to Council Directive 92/43/EEC conservation status of a species means the sum of the influences acting on the species concerned that may affect the long-term distribution and abundance of its populations within the territory referred to in Article 2; The conservation status will be taken as 'favourable' when: − population dynamics data on the species concerned indicate that it is maintaining itself on a long-term basis as a viable component of its natural habitats, and − the natural range of the species is neither being reduced nor is likely to be reduced for the foreseeable future, and − there is, and will probably continue to be, a sufficiently large habitat to maintain its populations on a long-term basis.

environment on which unreasonable adverse effects are to be prevented). The involved authorities that coordinate how to assess the risk of PPPs to endangered species are the Fish and Wildlife Service (FWS), the National Marine Fisheries Service (NMFS), which are the authorities responsible for listing endangered species – and the US Environmental Protection Agency (EPA), which is the authority responsible for the registration of PPPs. Recently, the US National Academy of Science (NAS) published a report trying to bridge the different domains and giving guidance on the RA for protected species (NAS, 2013). In this report, a framework is developed based on three steps with *step 1* assessing whether a PPP "may affect" a listed species, *step 2* whether it is "likely to adversely affect" a listed species, and *step 3* whether it is likely to jeopardise the continued existence of a listed species. However, it is important to note that the protection goal in the US legislation seems to be to prevent the extinction of listed species, while e.g. the EU Habitat Directive clearly aims for an improvement of the status of protected species and the protection goals in the sectorial legislations may aim at higher levels of protection.

1.1. Background and Terms of Reference as provided by requestor

In EFSA's context, environmental risk assessment (ERA) considers the impact on the environment caused by, for example, the use of certain substances in food and feed, the application of plant protection products (PPPs), the introduction and spread of non-native invasive alien species (IAS) or the introduction of genetically modified organisms (GMOs).

For those products falling within its remit, the European Food Safety Authority (EFSA) is responsible for ERA in accordance with the various relevant legislations (EFSA, 2011). More detailed descriptions of ERA have been developed in a number of guidance documents from individual EFSA Scientific Panels: e.g. Panel on Plant Protection Products and their Residues (PPR) (EFSA PPR Panel, 2009 and 2013); Panel on Plant Health (PLH) (EFSA PLH Panel, 2010 and 2011); Panel on Genetically Modified Organisms (GMO), (EFSA GMO Panel, 2010 and 2013), Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) (EFSA FEEDAP Panel, 2008); Panel on Biological Hazards (BIOHAZ) (EFSA BIOHAZ Panel, 2010a,b); and it is envisaged that other Panels (e.g. the Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (CEF)) will perform ERA on applications submitted to EFSA.

To keep up with new regulatory and scientific developments, such guidance documents require updating as appropriate and are therefore considered as "living documents" (EFSA SC, 2015). Against this background, the EFSA Scientific Committee (SC) continues to identify opportunities to harmonise best practices for ERA.

From the EFSA 10th anniversary conference (EFSA, 2012), it was evident that there is a clear need for making protection goals operational for use in ERA. A need for more harmonised environmental risk assessments was also recently pointed out in a letter titled "*Environmental health crucial to food safety*" to the editors of Science (Hulme, 2013).

Protection goals are just briefly mentioned in the respective legislative frameworks of the different Panels and could be further specified e.g. by the use of the ecosystem services concept (EFSA PPR Panel, 2010; Nienstedt et al., 2010). Moreover, following a harmonised approach across ERAs of different potential stressors would ensure that environmental protection goals are considered consistently, irrespective of the type of innovation (EFSA SC, 2016a).

Many of the overarching elements that exist in ERA of respective EFSA areas are related to protection goals. Guidance is needed on methodologies to implement biodiversity and ecosystem services as protection goals. Two specific items have been identified recently as requiring more detailed scientific considerations from a working group of the SC: coverage of endangered non-target species and recovery of non-target species. Such specific considerations could further complement the currently existing practises for RA, as described in the existing EFSA guidance documents.

The European Food Safety Authority therefore requested the Scientific Committee (SC) to establish a working group, including experts from the relevant EFSA Panels, to provide separate opinions on harmonising the approach to setting protection goals and the two specific elements of environmental risk assessment (ERA) within the remit of EFSA, i.e. "Coverage of endangered non-target species " and "Recovery of non-target species".

EFSA requested to consider and involve during the preparation of the opinions the experience and guidance developed by other EU and MS agencies and scientific bodies (e.g. Scientific Committee for Environmental Health Risks, European Environmental Agency, European Medicines Agency, European Chemicals Agency, JRC), international bodies (e.g. WHO/IPCS, OECD) and other international agencies (e.g. US Environmental Protection Agency).

For the task of developing an opinion on endangered species, the working group was requested to consider common approaches and the specific questions including the following Terms of Reference (ToR):

ToR1: Are endangered species more vulnerable than other species:

- based on their toxicological sensitivity, probability/possibility of exposure, specific potential for recovery, low genetic diversity, or
- because of other stressors e.g. limited, marginal or fragmented habitat?

ToR2: Do the current ERA schemes appropriately cover endangered species?

ToR3: If not, what risk mitigation measures can be envisaged to prevent endangered species being put at risk from potential stressors?

ToR4: Is monitoring needed to check the efficacy of risk mitigation measures for the occurrence of endangered species?

For the two other opinions to be developed by the working group, the ToR are specified in the respective parallel opinions (EFSA SC, 2016a,b).

1.2. Interpretation of the Terms of Reference

"Potential *Stressor*" is used herein as "*environmental potential stressor*" and meaning any physical, chemical, or biological entity resulting from the use of a regulated product or the introduction of an invasive alien plant species related to the food/feed chain that is assessed in any area of EFSA's remit and that *can* induce an adverse response in the environment. Potential stressors *may* adversely affect specific natural resources or entire ecosystems, including plants and animals, as well as the environment with which they interact (http://www.epa.gov/risk_assessment/basicinformation.htm).

The concept *"regulated products"* as used herein means "claims, materials, organisms, products, substances and processes" submitted to EFSA for evaluation in the context of market approvals/authorisation procedures[5].

In line with EFSA's responsibilities regarding the food and feed chain, the scope of this opinion includes the ERA of regulated products and invasive alien species. "*Other stressors*" include non-regulated products, habitat destruction, environmental contamination or products covered by other regulations, such

[5] For an official list of the relevant legal acts identifying all the "products" subject to EFSA's scientific evaluation see: http://www.efsa.europa.eu/en/apdesk/docs/apdeskhow.pdf

as those on pharmaceuticals, biocides or the "Registration, Evaluation, Authorisation and Restriction of Chemicals" (REACH). "*Other stressors*" are outside EFSA's remit and therefore beyond the scope of this opinion, even if these other stressors may be the main threats or main drivers for endangerment. However, there are significant commonalities in the ERA of agents released into the environment independently of the origin and release process. Hence, this opinion may be useful not only for the consideration of the ERAs under EFSA's responsibility but may provide potentially useful insights for other ERA's conducted by other EU risk assessment bodies, such as ECHA and EMA.

The interpretation of "*endangered non-target species*" and of "*other species*" is given in Section 3 and 4, respectively.

"*Vulnerable*" is used herein since the EFSA Panels (PPR, GMO and PLH) adopted an approach to species selection for prospective risk assessment using (or leaving the option for) the concept of vulnerable species. For PPP this concept is explicitly based on their exposure, (toxicological) sensitivity and potential for recovery. Furthermore across all Panels, the application of the vulnerable species concept requires a pre-information on the actual viability of the population of an endangered species at the ecologically relevant spatial scale. This is of particular importance in an ERA when addressing the recovery potential of a species that could be already impaired by other stressors than the potential stressor under assessment.

"C*urrent risk assessment schemes*" refer to the EFSA Panels' ERA schemes and corresponding applicable sectorial legislations as reviewed in the "*Review of current practices of environmental risk assessment within EFSA*" (EFSA, 2011). EFSA performs individual prospective ERA for single PPPs, GMOs, and FAs before they are placed on the market. For IAS, EFSA's ERA can be both prospective and retrospective. The protection of the environment is also envisaged by the risk assessment of certain biological hazards in certain products (e.g. animal by-products) and can be envisaged for more products of relevance to EFSA Scientific Panels (e.g. for food contact materials). Therefore, the scope of this opinion covers risk assessment schemes corresponding to EFSA's legal framework, and extends to checking how comprehensive these schemes are to predict if one or more endangered species could be at risk by the use/spread of the potential stressor under assessment.

"*Appropriately*" as mentioned in ToR2 is interpreted as "*scientifically appropriate*". To answer this ToR2, it was recorded what was done under the current ERA schemes and what could be possible options for the future. The interpretation of "*appropriate*" is not linked to legal provisions or requirements, since this would require agreed specific protection goals that are a result of a dialogue between risk assessors and risk managers.

The scope of this opinion covers single-products and invasive species risk assessments as currently foreseen in the specific regulatory frameworks. Therefore, "*coverage of endangered species in current ERA schemes*" is not taken as a whole, which would mean taking account of multiple stressors. The SC recognises, however, that a more holistic assessment considering multiple stressors *(in and outside of the remit of EFSA, regulated and non-regulated)* is essential for ensuring the viability and protection of the environment (incl. endangered species) in the long-term. In this sense, the opinion could be also of interest for other parties with a focus on environmental quality such as European Environmental Agency (EEA). For bees, EFSA has initiated work towards the "development of Holistic Approaches to the Risk Assessment of Multiple Stressors in Bees", published on EFSA's website (EFSA, 2013c; EFSA, 2014d) and more recently, EFSA embarked on a multi-annual work program MUST-B on this complex issue (i.e. taking account of multiple stressors and aspects of the landscape) (MUST-B EFSA mandate M-2014-0331, EFSA-Q-201400880).

The ownership for problems related to endangered species is not clearly defined, and it remains unclear which legislation is covering which aspect. In the Netherlands there is however one example to protect hamster species occurring in agricultural land and this is initiated under the Habitat Directive (http://edepot.wur.nl/163477). EFSA's regulatory frameworks are also not designed for risk assessment for individual (endangered) species or at local scales. When there is a suspicion or retrospective observation that an endangered species suffers from one or more of the stressors assessed by EFSA, it could be decided at a national level to conduct a local assessment, as the concrete protection of endangered species is often to be addressed by local managers.

"Agricultural context": In line with EFSA's responsibilities regarding the food and feed chain, the scope of this opinion includes the risk assessment of products for use in, or threatening, plant and animal production, including their impact on the wider environment, as well as invasive alien species threatening crop and non-crop plant health. This opinion, however, does not cover the intentional introduction of PPPs, GMOs and FAs outside of agriculture, aquaculture or forestry[6]. From the exposure point of view, quite a number of endangered species occur in areas used for or influenced by agriculture (e.g. Liess et al., 2010; Jahn et al., 2014). Therefore, endangered species are also considered as potential non-target organisms in EFSA's prospective ERAs for the agricultural context.

The ERA, however, also considers the impact of the potential stressor on the wider environment (managed or non-managed) since their impact may extend beyond the area of application. This becomes clear e.g. from the accumulation of persistent PPPs in aquatic and terrestrial food webs, and the propagation of (indirect) effects beyond the site where the potential stressor is applied or its "*sphere of influence*" (including action at a distance). Assessments of indirect effects and further away effects are considered during current ERA schemes and will be discussed for their appropriateness regarding endangered species and any species in general. Also, assessment of bioaccumulation is considered during existing ERA schemes and is therefore within the scope of this opinion.

In managed areas, such as agricultural areas (and also, where relevant, aquaculture areas), typically a whole range of PGs can be set and one has to prioritise what to achieve and what to protect. Regarding such managed areas, and the biodiversity therein, trade-off decisions have to be made as one cannot protect everything, everywhere, at the same time in agriculture and aquaculture. Biodiversity is a common and prominent legal PG for all ERAs performed by EFSA and it is noted that agricultural systems are highly disturbed habitats with food production as one main goal[7]. However it is also noted that agricultural areas can form quite large proportions of the area of some Member States and therefore protection of the biodiversity as another common good might strongly depend on the implementation of biodiversity goals in these areas (e.g. farmland birds as one prominent systematic group). EFSA is not responsible for trade-off discussions, as this falls under the domain of risk management.

1.3. Aim of the opinion

The aim of this opinion is to present information on whether endangered species are appropriately covered under current single-stressor ERA schemes at EFSA. Scientific options that could extend the coverage of endangered species in current ERA schemes (at EFSA) are provided. Also, directions are given for future research needed to answer the ToR. This is not a guidance document, but a document to

[6] Uses that are not considered are, for example, domestic uses of GM pets (e.g. glow fish) or GM insects.

[7] This baseline is heavily impacting on biodiversity through necessary agricultural management practices such as tillage, ploughing, and harvesting. Greenhouse gas emissions are also stressors related to agricultural practices, which are not further discussed herein.

promote a dialogue between risk assessors and risk managers responsible for the food and feed chain, and dialogue with other agencies that are involved in the protection of endangered species.

While the current EFSA ERA schemes are generic for the EU, this opinion also comprises to a certain extent suggestions for location-specific ERA schemes.

One additional aim of this opinion is to provide risk assessors with a number of suggestions (e.g. a checklist with traits relevant for endangered species (see Section 8.4)) that can help determining whether endangered species are covered in a particular risk assessment or determine the spatial scale for ERA.

2. Data and Methodologies

2.1. Data

The evidence used for the current opinion consists of:

- Established approaches as described in existing EFSA guidance documents and opinions (on dossiers submitted for assessment) from the PPR, GMO, PLH, and FEEDAP Panels (e.g.; EFSA PPR Panel, 2009, 2013; EFSA GMO Panel, 2010, 2013; EFSA PLH Panel, 2011, 2014 and EFSA FEEDAP Panel, 2008); and to a certain extent the respective regulatory frameworks (reviewed in EFSA, 2011);
- Expert knowledge through the EFSA WG dedicated to draft this opinion, consultations with members of the EFSA PPR, GMO, PLH and FEEDAP Panels, and data retrieved from the literature. The scarce scientific evidence relevant for the coverage of endangered species was fragmented; there was no prior comprehensive overview of the relevant literature. Where possible, the WG strived to make general statements, but examples and anecdotes play an important role in this opinion. These serve as "proofs of principle" where generic statements cannot yet be made.
- Experimental data used for Sections 5.1.2.1 and 5.1.2.2: numeric data were used in the form of existing Excel databases and statistical calculations were outsourced to the Durham University, United Kingdom. For aquatic species, the information sources used were described in Hickey et al. (2012) and are freely available as Supplemental Data to this publication. It concerned a database for insects, crustaceans and fish. For birds, the data were previously used and described by Luttik et al. (2011). The test results in these databases are either EC_{50}s (aquatic, counting LC_{50} as a form of EC_{50}) or LD_{50}s (birds). Appendix B shows the number of compounds and species available for each organism group.

Note: In this opinion, examples were sought for each of the four involved EFSA Panels, but for PPPs many more examples are available than for the other three Panels. This is creating an unbalance between the PPPs and the other potential stressors, but it reflects the amount of data and knowledge available.

2.2. Methodologies

The methodology used for this opinion was to aggregate the information from the diverse EFSA areas and external experts, discuss draft answers to the ToR in working group meetings and extract from such discussions principles and proposals for adoption by the SC. Remaining uncertainties related to coverage of endangered species in prospective risk assessment were reported, in line with the principles described in the SC guidance document on uncertainties in dietary exposure (EFSA SC, 2006), and in line with the principles described in the SC guidance on transparency Part 2 (EFSA SC, 2009).

EFSA followed its specific Standard Operating Procedure (SOP) detailing the steps necessary for establishing, updating or closing the scientific WG that prepared this opinion for the SC. This SOP implements the Decision of the Executive Director on the selection of experts of the Scientific Committee, Panels and Working Groups[8].

The following consultations took place on the prepared draft prior to adoption: Prior to the first operational meeting of the working group, the topics of the mandate were openly discussed with experts representing a wide variety of stakeholders. The summaries and outcomes of the discussions from the 19th EFSA Scientific Colloquium on “Biodiversity as Protection Goal in Environmental Risk Assessment for EU agro-ecosystems” are published on EFSA’s website (EFSA, 2014a).

- Letters of invitation to participate in this activity were sent to other EU RA bodies (ECHA, EEA, EMA, JRC, SCENIHR and SCHER), to WHO, OECD and US-EPA. All invited RA bodies and the OECD have appointed a contact point or an observer to the WG meetings.
- Public consultations (including the above international Institutions) were held online between Mid-June til Mid September 2015. The report of this public consultation will be published together with this opinion.

[8] See http://www.efsa.europa.eu/en/keydocs/docs/expertselection.pdf

3. What is meant by "endangered species"

"*Endangered non-target species*" as mentioned in the original ToR will be hereafter referred to as "endangered species". One critical aspect for the present opinion is which species should be considered "endangered" and if those cover only species that are under threat or disappearing at a given spatio-temporal scale. In this Section, the term "endangered species" will be defined for the purposes of this document.

A widely used and straightforward approach for defining endangered species is to take a red list and define endangered species as those species that are listed therein as threatened. Red lists also report threats to endangered species, i.e. they report *why* these species are endangered. Such threats include, for example: (1) residential and commercial development, (2) agriculture and aquaculture, (3) energy production and mining, (4) transportation and service corridors, (5) biological resource use, (6) human intrusions and disturbance, (7) natural system modifications, (8) invasive and other problematic species and genes, (9) pollution, (10) geological events, or (11) climate change and severe weather (IUCN, 2014a). As outlined in the following paragraphs, different red lists exist for different spatial scales, e.g. at regional, national, European or global scale.

Since EFSA primarily focuses on the European level, it seems reasonable in principle to define endangered species based on the European Red List[9]. Species listed therein as threatened – i.e. with the status vulnerable (VU), endangered (EN), or critically endangered (CR) – are threatened with extinction at the European level. Hence, such red-listed species on the European level could be called "endangered species" for the purposes of this document. The list is prepared by the International Union for Conservation of Nature (IUCN) for the European level, so a species listed there as threatened is not necessarily threatened globally, and a species threatened in a member state is not necessarily threatened at the European level. The European list currently includes ca. 6000 species of a selected range of organism groups (mammals, reptiles, amphibians, freshwater fishes, butterflies, dragonflies, and selected groups of beetles, molluscs, and vascular plants). Therefore, the European Red List must be considered incomplete at the moment (and implies a responsibility for member states/regions/communities). Nonetheless, some taxa (mammals, reptiles, amphibians, fresh water fishes, butterflies and dragonflies) are well covered, hence for these taxonomic groups, investigating whether endangered species are more vulnerable or sensitive than non-endangered species against potential stressors in agriculture, the European Red List might be used.

The global IUCN Red List of Threatened Species (IUCN, 2014b, http://www.iucnredlist.org) covers more species than the European list (>70,000 species) and more taxonomic groups, although it also cannot provide a full coverage of all taxa: while terrestrial vertebrates are relatively well covered, most other taxa are only covered by a fraction of their known species. Still, the coverage is good overall; however the problem of using the global list as a basis for defining endangered species for the purposes of the current document is that species threatened on the European level are not necessarily threatened on the global level[10]. For example, the wolverine (*Gulo gulo*) is classified as vulnerable (i.e. threatened) according to the European list but as least concern (i.e. not threatened) according to the global list. Another example is the western barbastelle (*Barbastella barbastellus*), a bat that is classified as vulnerable (i.e. threatened) according to the European list but as near threatened (i.e. not threatened) according to the global list. As

[9] (http://ec.europa.eu/environment/nature/conservation/species/redlist/index_en.htm

[10] For instance, if a species is endangered globally according to the IUCN Red List of Threatened Species (http://www.iucnredlist.org), it was classified as such based on criteria at the global level, e.g. because it has experienced a reduction in its global population size (criterion A for being red-listed; IUCN, 2012).

these examples for mammals illustrate, defining endangered species only on the basis of the global list would miss species that are endangered in Europe but not globally.

There are also national or other regional red lists within Europe. Again, species that are threatened nationally within Europe are not necessarily threatened for Europe as a whole. Species such as the brown bear (*Ursus arctos*) or wolf (*Canis lupus*) are relatively abundant in some parts of Europe but extinct in or slowly re-entering other parts. A drawback of using national or other regional red lists is that the different parts of Europe are not evenly covered by such lists, and the amount of resources and effort behind the lists varies substantially. Thus, defining endangered species only on the basis of national and other regional red lists would produce a regionally biased definition. However, if MS and local authorities use this document for developing national or local approaches, it can make sense to define "endangered species" at this national or local level. In general, the approaches developed in this opinion for the EU level can in principle be followed on the MS and local level.

For the general purposes of this opinion, it seems unreasonable to restrict it to a given red list for defining endangered species. Aside from the substantial differences in taxonomic coverage and detail among red lists, each red list represents a different spatial scale. Importantly, the spatial scale of interest will highly differ among different stressors; hence we should not exclude a given spatial scale by excluding a given red list a priori but consider all red lists for defining endangered species. For example, in the UK, the high brown fritillary (*Argynnis adippe*) is classified as critically endangered (Fox et al., 2011). Yet, at the broader continental scale (Europe, EU28), this butterfly is listed as locally common (EU Red list). ERA schemes need to appropriately define the spatial scale to ensure that appropriate levels of rarity and endangerment are covered.

For some localities, especially at small spatial scales, no red list might exist for defining endangered species. Particularly under such circumstances, alternative definitions of endangered species based on ecological principles may be applied. In ERAs, it is important to understand why species are endangered. Rabinowitz (1981) suggests that the pathways by which species become rare and endangered are diverse but can be usefully summarised in terms of geographic distribution, habitat specificity, and local population size (Table 1). Under Rabinowitz's classification, species are only classified as 'common' if (and only if) they are widely distributed, have broad habitat usage, and large population sizes. All other classifications, seven in total, are a manifestation of rarity and provide a way to classify endangered species based on geographic distribution (spatial scale), local population size (temporal scale) and specific species' characteristics. This is considered a paradigm for understanding species rarity. Classic rarity occurs when species have small population sizes, narrow geographic distributions and are habitat specialists. Other forms of rarity emerge from the ecological processes (effects on population abundance, spatial extent and habitat utilisation) operating on species.

The important consequences of Rabinowitz's classification is that by classifying rarity in this way leads to a consideration of the temporal and spatial scale on which to define species endangerment. These ecological definitions of rarity should focus the prospective ERA at the appropriate spatial and temporal scale.

Species are not equally abundant in ecosystems. In fact the majority of species are likely to be rare. Species rarity can be defined in terms of spatial scale, habitat use, and local population size (Table 1). Levels of rarity are also dependent on the time over which assessments are made. Measuring the attributes of rarity (highlighted in Table 1) might be best achieved in terms of characterising ecological species diversity (numbers of individuals and number of species) (Magurran, 2004). One of the simplest distributions is that species are equally or evenly represented in samples. A simple way to represent the differences in the number of species is through the use of species rank (most common to least common) – abundance relationships which will highlight rare species in a landscape/ecosystem (e.g. Fisher et al., 1943; Preston, 1948).

Table 1: Classifications of rarity and endangered species (following Rabinowitz, 1981) based on geographic distribution, population size and habitat specificity

Geographic distribution		***Wide***		***Narrow***	
Habitat Specificity		***Broad***	***Narrow***	***Broad***	***Narrow***
Local Population Size	***Somewhere large***	Common	Habitat Specialist	Rare	Endemics
	Everywhere small	Truly Sparse	Rare	Rare	Classic Rarity

In summary, an endangered species is here defined as a species that is either:

1) listed in one or more "red lists" as threatened (i.e. vulnerable, endangered, or critically endangered, or variants thereof), where the considered red lists are: (i) the European Red List, (ii) the global IUCN Red List of Threatened Species, and (iii) national and other regional red lists within Europe that follow the IUCN or another suitable classification scheme; or

2) rare based on Rabinowitz's (1981) seven classes of rarity (including "endemics", "classic rarity", "habitat specialists" and "truly sparse" species).

4. ARE ENDANGERED SPECIES MORE VULNERABLE TO POTENTIAL STRESSORS THAN OTHER SPECIES?

This Section addresses the first term of reference "*Are endangered species more vulnerable than other species (1) based on their toxicological sensitivity, exposure, specific potential for recovery, low genetic diversity, or (2) because of other stressors e.g. limited, marginal or fragmented habitat*?". "*Other species*" as mentioned in the above ToR1 mean non-endangered species, which is *de facto* equal to "*all species except endangered species*".

4.1. General characteristics of endangered species that may influence their vulnerability

This Section considers characteristics of endangered species such as life-history traits and geographical distribution that go beyond the potential stressors under assessment. The purpose of this Section is to provide a general picture for endangered species, although information is rather scattered, not fully understood and sometimes speculative or even conflicting.

Most studies on the characteristics of endangered species have been conducted for vertebrates. Many of these studies have found that endangered species tend to have a large adult body size, start reproducing late in life, and have few offspring per year (Reynolds, 2003; Jeschke & Strayer 2008; and references therein). These results are sometimes generalised to the statement that species with a slow life history tend to be more frequently endangered than species with a fast life history (Reynolds, 2003; Jeschke & Strayer 2008; Jeschke & Kokko, 2009; and references therein). A relatively new finding is that endangered species tend to have a low intraspecific variability in their life-history traits as compared to other species. In particular, mammalian species with low variability in litter size, sexual maturity age, and adult body mass have been shown to be more frequently endangered than species with higher variability in these traits (González-Suárez & Revilla, 2013). An intuitive explanation for these results is that low intraspecific variability (which might, at least partly, come from low genetic variability, see Section 4.2.6) may increase species vulnerability, as it reduces the possibility to respond appropriately to new stressors (regulated as well as non-regulated ones).

In addition, endangered species tend to be habitat specialists and have small geographic ranges (Fisher & Owens, 2004; Jeschke & Strayer 2008; and references therein). Geographic range is sometimes used as a criterion for classifying species as being endangered (see e.g. the IUCN (2014a,b) criteria for red-listed species, http://www.iucnredlist.org), so one has to be aware of a potential circularity here. Those studies that only analysed species that were not classified as endangered based on their geographic range still often (but not always) found that species with small ranges are more frequently endangered than other species (Fisher & Owens, 2004; Jeschke & Strayer, 2008; and references therein). Diet specialists are also more frequently endangered than other species (e.g. McKinney, 1997; van Valkenburgh et al., 2004). Finally, top predators tend to be more often endangered than other species as well (e.g. Ripple et al., 2014).

These findings for vertebrates are largely in line with those for invertebrates where a slow life history and high degree of specialisation are typically related to being endangered (Kotze & O'Hara, 2003; Reynolds 2003; Koh et al., 2004; Kotiaho et al., 2005). In addition, invertebrates that are less mobile than others tend to be more frequently endangered (Reynolds, 2003; Collen et al., 2012; and references therein). With the exception of butterflies, however, much less is known about the characteristics of endangered invertebrates than vertebrates (Collen et al., 2012).

In conclusion, although there are knowledge gaps, researchers have identified some general characteristics of endangered species, and these can be linked to their potentially higher ecological vulnerability to potential stressors under assessment, as outlined in Section 4.2.

4.2. Ecological vulnerability in the light of potential stressors under assessment

The concept of ecological vulnerability (reviewed by De Lange et al., 2010) is essential for answering the question whether endangered species are sufficiently protected by current regulations and risk assessment practices. Van Straalen (1994) defined ecological vulnerability to toxic stressors as consisting of three elements, i.e. (1) exposure, (2) sensitivity and (3) recovery. According to this definition, a vulnerable species is a species with a relatively high sensitivity for the stressor at hand, high exposure and/or a poor potential for population recovery. Other direct effects are related to behavioural change resulting e.g. in decreased predator avoidance or decreased competitive strength due to toxicant stress. It should be noted that this definition of vulnerability is limited to the direct effects of toxic stressors.

Vulnerability to indirect effects, e.g. propagated through disturbed predator-prey or competitive relationships, cannot be characterised by the triad of exposure, sensitivity and recovery. Vulnerability to indirect effects is related to dependability, i.e. whether a species depends, either directly or indirectly, on a species that is affected by the stressor at hand.

The following Sections will address the traits that can contribute to higher vulnerability of endangered species to a potential stressor compared with other species.

4.2.1. Are endangered species likely to have a higher exposure?

The question "*Do endangered species have a higher exposure than the exposure regimes applied in current ERA schemes*?" will be addressed in detail in Section 5 (e.g. Section 5.1.2.4 for the PPP ERA scheme). The current Section merely provides some examples of endangered and non-endangered species which have been shown to be at risk due to specific exposure routes and levels, with the aim to learn lessons from these incidents.

Exposure has been defined as the concentration or amount of a particular agent that reaches a target organism, system, or (sub)population in a specific frequency for a defined duration (WHO, 2004). This definition makes clear that the level of exposure depends on the spatiotemporal distribution of the potential stressor on the one hand and that of the target organism on the other. Here, we concentrate on external exposure, while Section 4.2.2 on toxicological sensitivity includes internal exposure. External exposure is defined as the concentration in the exposure medium, e.g. water, soil, sediment, air or food since most ERA schemes express the PEC and PNEC as concentrations in exposure media. Bioavailability is included in external exposure, which means that external exposure can be more specifically defined as the bioavailable fraction of the potential stressor in the exposure medium.

A well-known pollution incident where exposure played an important role is the large-scale death of Indian vultures due to diclofenac poisoning after eating the carcasses of free wandering cattle preventatively treated with this anti-inflammatory drug (Oaks et al., 2004). This incident can be explained by the concurrence of a specific exposure route (i.e. eating death carcasses with high diclofenac levels) and the toxicological sensitivity of the vultures. This exposure route is very specific for vultures living in remote areas, i.e. areas where the carcases are not removed from the field and thus eaten by the vultures. Another well-known example of unexpected high exposure is the exposure of honeybees to neonicotinoids. Potential risks were highlighted via different routes of exposure: via consumption of nectar and pollen from treated plants on which bees forage, for some crops via consumption of guttation fluid, and via contact to dust of treated seeds during sowing operations (EFSA, 2013a and b). Examples of exposure routes so far not addressed in ERA schemes include risks from the exposure of wild birds via the dermal and inhalation routes after PPP application in orchards (Vyas et al., 2007), and frogs exposed to land and not only to water: terrestrial exposure of the European common frog *(Rana temporaria)* to several PPPs (Brühl et al., 2013).

Some of these examples relate to specific exposure routes involving exposure media in which the contaminant accumulates in relatively high levels. They also stress the importance of knowledge about exposure routes and the accumulation of stressors in different exposure media. Van Straalen (1994) points to transition layers as media contributing to potentially high exposures because many substances tend to accumulate in transition layers, such as the interfaces between water and air, between soil and air, and between air and vegetation (Simkiss, 1990). Organisms that specifically live on these transition layers are expected to be exposed to a higher dose than would be expected on the basis of a homogeneous distribution of the substance. Van Straalen provides examples such as surface-active spiders in agricultural fields (very sensitive to pyrethroid insecticides: Jagers op Akkerhuis, 1993), surface-feeding freshwater snails (very sensitive to organotin compounds: Stebbing, 1985) and surface-rooting grasses (development of resistance to zinc, not observed in deep rooting plants: Dueck et al., 1984).

A very specific route of exposure, particularly for predatory animals, is the accumulation of potential stressors in food webs, often referred to as biomagnification. Biomagnification typically is an issue for persistent chemicals with a high affinity for fat tissue and/or that are poorly metabolised or excreted. Examples include mercury in polar bears (Dietz et al., 2011) and orcas (Endo et al., 2006), PCBs in eels (De Boer et al., 2010), PBDEs in birds of prey (Chen et al., 2010) and DDT in marine birds (Risebrou et al., 1967). Biomagnification is particularly relevant for endangered species since the animals at the top of the food web will experience highest exposure due to biomagnification and many of these animals are typically endangered.

Besides exposure routes and concentration levels in the exposure media, exposure duration is an important driver of exposure. It is generally recognised that in the field time-variable exposures are more the rule than the exception. In aquatic systems, the time-variable exposure pattern typically depends on the mobility of the aquatic organism (e.g. fish) and that of the pollutant. In terrestrial and sediment systems, the pollutant tends to be immobile while the organism moves around (in case of animals). In both cases, organisms may show avoidance behaviour, e.g. fishes avoiding discharge plumes (e.g. Maynard & Weber, 1981; Larrick et al., 1978; Tierney et al., 2010) or earthworms moving to non-polluted soil patches (e.g. Stephenson et al., 1998). It has been shown in several studies that seasonal changes in exposure factors such as food availability and activity patterns can have a profound impact on the exposure. For example, the blood levels of methylmercury in breeding Rusty Blackbirds from the Acadian forest has been shown to be an order of magnitude higher than that of wintering birds (Edmonds et al., 2010). If such high seasonal exposures occur during critical life stages, this may have a profound effect on the viability of the population (e.g. Biga et al., 2013).

Another potential reason for high external exposure is an increased release rate of the contaminant from the exposure medium. Species with a preference for habitats where a larger fraction of the toxic stressor is bioavailable are likely to have a higher exposure than other organisms. The aforementioned increased exposure of breeding rusty blackbirds in the North American Acadian ecoregion has been attributed to the high bioavailability of MeHg within this region (associated with parameters such as dissolved oxygen, pH and dissolved organic matter) compared to other wetlands and water bodies in combination with the high trophic position of the birds (Edmonds et al., 2010, 2012).

The key question addressed here is whether high external exposures are typical for endangered species. No convincing scientific evidence was found indicating that endangered species in general have a higher exposure than other species. However, since endangered species tend to show a high degree of food and habitat specialisation compared to non-endangered generalist species, the variety among different endangered species in exposure routes and levels will also be higher. As such, it can be argued that there is a substantial likelihood that some endangered species will experience a higher external exposure than other species. Identification of these species will require a detailed analysis of all relevant exposure routes, covering factors such as concentration levels, contact duration and bioavailability.

4.2.2. Are endangered species likely to have a higher (toxicological) sensitivity?

The sensitivity of a species to a potential stressor can be defined as the degree of response by the species after exposure to a standardised amount of the potential stressor. Sensitivity to chemicals is referred to in this opinion as toxicological sensitivity which is typically quantified by metrics such as a lethal concentration (LC_{50}), no observed effect concentration (NOEC) or a dose-response relationship. The current Section focuses on toxicological sensitivity of endangered species, while sensitivity to IAS is discussed in Section 4.2.5.

Assessing toxicological sensitivity based on toxicity tests

An important question at stake is whether endangered species are generally more toxicologically sensitive to potential stressors than frequently tested species. If there was a higher toxicological sensitivity, endangered species are expected to be found at the left hand site of species sensitivity distributions (SSDs), which are often used in chemical risk assessment. This hypothesis is difficult to test because endangered species are rarely tested in toxicity experiments.

The insecticide chlorpyrifos is an exception because it is a benchmark compound for which many acute laboratory toxicity data are available (e.g. Giddings et al., 2014; Rubach et al., 2010; van Wijngaarden et al., 1993). The available 48-96h LC_{50} or EC_{50} values for aquatic insects and the 96h LC_{50} values for aquatic vertebrates (fish and amphibians) were placed in species sensitivity distributions (SSDs) (see Figure 1). In this example, the acute toxicity value for the endangered insects *Ephemerella* spp (with two species listed as threatened in the Czech Republic and Germany, and in the Czech Republic, Germany and Great Britain, respectively) and of two related taxa of the same genus as a listed species (*Molanna* and *Caenis*) are positioned over the whole range of the SSD curve (Figure 1, A). This suggests that endangered species are not *per se* more sensitive to chlorpyrifos than the other species tested. Likewise, the endangered fish species *Leuciscus idus* (listed in the Netherlands) is positioned more or less in the middle of the SSD curve (Figure 1, B). Additionally, aquatic vertebrate taxa of the same genus as some listed species are not positioned in the tail of the SSD curve. Although the question in how far related species can act as surrogate species for endangered ones is still to be answered, this also seems to suggest that endangered species are not more sensitive than non-listed species.

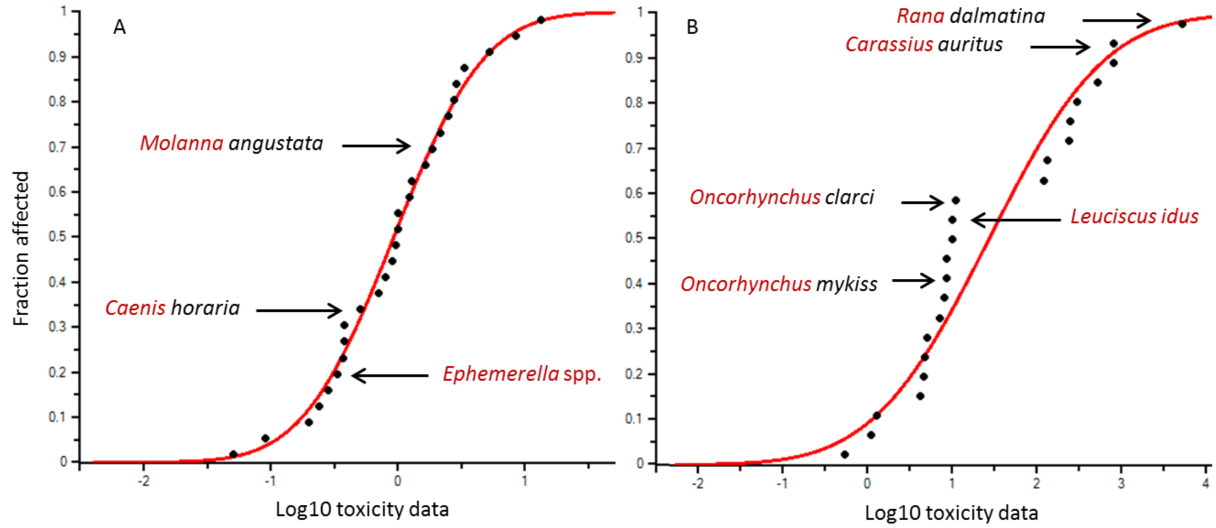


Figure 1: Species sensitivity distribution (SSD) for chlorpyrifos, for aquatic insects (A) and aquatic vertebrates (B). Endangered species are indicated at the right hand side of the curve, related taxa of the same genus as an endangered species are indicated at the left hand side of the curve.

A similar picture is obtained when evaluating the toxicity data for other insecticides characterised by a large acute toxicity database for aquatic organisms (e.g. lindane, endosulfan, malathion, esfenvalerate) (Theo Brock, personal communications). Raimondo et al. (2008) compared species sensitivity distributions (SSDs) based on acute LC_{50} values of standard test species with more than 20 LC_{50} values of endangered species. Likewise, they showed for SSDs including 20 endangered fish and four mussels, that acute LC_{50}s for endangered fish and mussels were in 97% and 99.5% of all recorded cases greater than the HC_5s and HC_1s (hazardous concentrations to 5% and 1% of species tested), respectively. Hence, the endangered species seem to be not *per se* amongst the more sensitive for a specific potential stressor.This indicates that the SSD approach is at least as protective for endangered species as for non-endangered species. Nonetheless, it should be realised that application of the HC_5 values as "safe exposure value", would still result in 3 out of every 100 endangered species to be exposed above their acute LC_{50} value while that this is 5 out of 100 when considering all species.

Another example demonstrates the position of raptorial birds in SSDs for birds for a number of compounds (see Table 2). For a few compounds LD_{50} tests with raptorial birds have been carried out in the past (see Luttik et al., 2011 for a description of the data used). These tests had fewer individuals than in a standard avian toxicity test. Therefore, the uncertainty around the outcome is larger than for standard tests. For sodium cyanide and monocrotophos raptorial birds (American kestrel and Golden eagle) are the most sensitive species tested. On the other hand for Starlicide and EPN there are also raptorial birds among the least sensitive species (Cooper's hawk and Eastern screech-owl). On average, in this data set there is a slight indication that raptorial birds could be more sensitive on average.

Table 2: Rank order of raptorial bird species amongst all tests carried out with bird species for a number of compounds. Test outcomes are ranked from more to less sensitive (note that some species are tested more than one time).

Compound	***Species***	***Place in rank order***	***Tests available***	***Number of species tested***
4-Aminopyridine	American Kestrel	23	44	36
Carbofuran	American Kestrel	9	33	18
	Eastern Screech-Owl	20	33	18
EPN	American Kestrel	4	20	14
	Eastern Screech-Owl	20	20	14
Fenthion	American Kestrel	2	50	25
	American Kestrel	4	50	25
	Eastern Screech-Owl	18	50	25
Monocrotophos	Golden Eagle	1	48	24
	American Kestrel	14	48	24
Sodium cyanide	American Kestrel	1	8	7
	Black Vulture	2	8	7
	Eastern Screech-Owl	4	8	7
Starlicide	Barn Owl	12	37	31
	Cooper's Hawk	37	37	31
Zinc Phosphate	Golden Eagle	6	13	8

Although the results presented above seem to indicate that endangered species are not more toxicological sensitive *per se*, it is important to realise that the scientific basis for this conclusion is weak. First of all, the available data on endangered species are insufficient to allow any conclusion that is statistically robust. Furthermore, it should be realised that the position of the same test species in the SSD may be different for different chemicals. Craig and Hickey (2012) showed for fish that the sensitivity of a particular test species is not exchangeable between test substances. This means that showing that endangered species have a comparable sensitivity for some substances, does not automatically imply that endangered species have a comparable sensitivity for all substances.

Assessing toxicological sensitivity based on toxicokinetic and toxicodynamic markers

In addition to the position of endangered species in SSDs, a closer look at the mechanisms that result in a toxicological effect can be helpful to assess whether endangered species have a higher toxicological sensitivity. Once the organism is in contact with the exposure medium that contains the potential stressor, several factors determine its toxicological sensitivity, namely the transfer and fate of the chemical in the organism (toxicokinetics, TK) and the toxicological consequences once the chemical has reached its target site (toxicodynamics, TD). Toxicokinetics covers the processes that relate external and internal exposure, such as absorption, distribution, metabolism and excretion (often abbreviated as ADME). Toxicodynamics covers the processes that relate the internal exposure to the occurrence of adverse effects, such as receptor interactions and the propagation of effects through molecular networks and over different levels of biological organisation (e.g. cell, tissue, organ, and individual). In the context of endangered species, taxa-specific traits, interspecies differences, and inter-taxa differences in both TK and TD processes are important aspects to determine and quantify toxicological sensitivity to chemicals. Over the last decade, the understanding of these processes in different taxa has increased considerably and there is a vast amount of literature describing these processes.

The following text discusses how differences in certain TK/TD traits can lead to differences in effects between species that may be relevant for endangered species. Where available, examples are provided to illustrate these principles, preferably on endangered species.

From a theoretical perspective, a high release rate of a toxicant from exposure media may occur after intake of the exposure medium, such as the consumption of food or the ventilation of water. However, contaminant uptake from such media is the resultant of a complex interplay between substance, media and species characteristics, such as the partitioning coefficient between octanol and water (K_{ow}) of the substance, the energy or oxygen content of the exposure medium, and the lipophylicity and persistence of the substance. The fat content of the species is also relevant but rather for the risk of bioaccumulation. This interplay takes place at the borderline between external and internal exposure. A relatively high exposure can be expected if: (i) the intake rate of the exposure medium is relatively high and/or (ii) the contaminant is easily released from the exposure medium. Intake rates of exposure media such as food, water and air depend on: (1) the activity level of the organism, (2) the energy or oxygen content of the exposure medium, (3) assimilation efficiency of the exposure medium (i.e. for food) and (4) body size (small species tend to eat more to maintain their body temperature). A relatively high intake rate can be expected for species that (1) demonstrate a relatively high activity level in the field, (2) consume contaminated food with low energy levels or digested with low assimilation efficiency, (3) breath contaminated air with low oxygen levels, (4) ventilate contaminated water with low oxygen levels, or (5) have a small body size. This mainly holds for relatively short external exposures, i.e. before a steady state is reached. Under steady state conditions (chronic exposures), internal exposure often remains unaltered since higher intake rates are generally compensated by faster elimination rates.

In practice, it is not easy to find specific examples of species at risk due to high intake rates or high contaminant release rates from exposure media. A potential explanation is that high intake rates often coincide with low release rates. For example, herbivores typically have higher food intake rates than carnivores, but often the contaminants in the food are less easily released due to lower food assimilation efficiency. Another potential explanation is the aforementioned compensation of a high uptake rate by a high elimination rate, which eventually may result in a similar steady-state concentration as the combination of a low uptake rate with a low elimination rate. It can be concluded that it is difficult to assess the influence of intake and release rates on the ultimate exposure in isolation. It is the complex interplay between intake, release, absorption and elimination rates that determines the ultimate internal exposure.

Besides intake, metabolism is an important factor governing internal exposure. There can be profound differences in detoxification potential among species. For instance, permethrin is commonly found in pet flea treatments, ant killers and fly sprays, and is used there as it is more than 1000 times more toxic to insects than to most mammals. Permethrin can, however, be deadly for cats because they are lacking the enzyme glucuronyl transferase which detoxifies permethrin and other synthetic pyrethroids (see Appendix C). As a consequence, permethrin remains much longer in cats than in dogs or other mammals. A study of 286 cases in which canine spot-on permethrin preparations had been used on cats found that 97% showed signs of poisoning (Campbell, 2007).

Organo-chlorines in sparrow hawks and kestrels serves as an example of where the toxicokinetics of several species in a food chain interplay to affect two endangered species in different ways, and clearly illustrates how important detailed ecological knowledge is in assessing the risk for endangered species and when using data from other species to “read across”. When comparing data among the raptors monitored by the UK Predatory Bird Monitoring Scheme, Newton et al. (1993) found that the sparrow hawk had higher concentrations of most pollutants than the kestrel, with the latter also showing less decline in levels during the study period. There were probably three reasons for this difference. First, the sparrow hawk eats other bird-species (herbivores and insectivores), and hence feeds higher in the food chain than does the kestrel, which eats mainly herbivorous voles. This may be seen as an example of increased external exposure as covered above under Section 4.2.1. However, the higher internal concentrations also arise from toxicokinetic differences in the prey items, with birds in general being less able to metabolise organochlorines and some other pollutants than mammals (Walker, 1983). Hence, also for this second reason the bird-eating sparrow hawk would tend to accumulate higher levels than the mammal-eating kestrel. Third, sparrow hawks themselves are less able than kestrels to metabolise (by oxidation) organochlorines (Walker et al., 1987). So here several toxicokinetic driven factors together all played against the sparrow hawks who suffered a more marked and widespread population decline than kestrels.

Another example where ecological behaviours of certain species may lead to higher internal concentration long after exposure occurred was reported by J.H. Koeman (oral communication at his farewell lecture from the University of Wageningen in 2000 (http://edepot.wur.nl/234508)). The early 60s emission of so called “drins” (e.g. dieldrin and telodrin) had effects on the populations of a number of bird species in the Wadden Sea. Those effects were caused by an additive effect of dieldrin and telodrin. Predominantly female Eider ducks died immediately after the breeding period. Female Eider ducks hardly eat during the breeding period and loose up to 30% of their body weight. This resulted in internal concentrations of the two compounds (not being eliminated) above the critical lethal concentration and in massive mortality in the years 1964 to 1966. After limiting emission and stopping manufacturing of telodrin the populations recovered.

An example of how differences in enzyme isoforms result in big differences in toxicological sensitivity, even between closely related species, can be found in earthworms. When investigating the toxicity of cholinesterase (ChE) inhibiting PPPs towards the earthworm species *Eisenia andrei*, *E. fetida* and *E. veneta* Stenersen et al. (1992) found that *E. veneta* was much more sensitive to carbaryl compared to the other two species. The results are partly explained by the fact that in the two less sensitive species one isoform of the ChEs was completely resistant to inhibition by carbaryl, while two other isoforms were very sensitive.

Another study showing the complexities of comparing both TK and TD differences between species was undertaken by Kretschmann et al. (2012). The effects of the organothiophosphate insecticide diazinon were investigated using the standard test species *Daphnia magna* and a second crustacean species of a different order and with a much larger body plan, *Gammarus pulex*, for which *D. magna* data may be used in ERA. While the bioconcentration factors (BCF) for the two species were comparable the enzyme activities of the P450 system responsible for elimination of diazinon both by activation to its toxic metabolite diazoxon and detoxification where higher in *G. pulex* than in *D. magna*. The contrasting comparison revealed that although the activation step to diazoxon is two times faster in *G. pulex*, it is less sensitive because of a six times faster detoxification of diazinon and diazoxon and an approximately 400 times lower rate for damage accrual. This example shows clearly how TK and TD parameters act together in a complex interplay, and that misinterpretations are possible if a simple approach analysing only one aspect is taken when trying to use data from one species to address sensitivity in another.

The combination of these experiments shows that making general statements about sensitivities across species with slight variations in their enzyme forms is precarious and that reading across from one chemical to another is not simple. Judging the suitability of ERAs based on standard species for affording protection to endangered species therefore comes down to knowing how comparable their biochemistry is. With second generation genetic sequencing and post genomic techniques this is becoming more and more feasible (see Appendix C).

In conclusion, the SSD examples and the TK/TD considerations presented in this Section do not provide conclusive evidence that endangered species are *per se* more sensitive towards potential stressors than other species. However, the anecdotal examples presented illustrate that species differences in toxicological sensitivity can, at least partly, be explained by differences in TK/TD mechanisms and traits. It was shown that some highly dietary specialised species lack certain detoxifying pathways or isoforms of enzymes, leading to a higher toxicological sensitivity (Shrestha et al., 2011). Since many of the endangered species are highly specialised, e.g. in their food or habitats, they may only have been exposed to a restricted range of natural chemicals and toxins, resulting in the phylogenetic loss of certain detoxifying pathways relevant for assessed chemicals. As a consequence they may be more toxicologically sensitive than generalists. It is recommended to further explore this line of reasoning, e.g. in an explorative study in which the TK and TD traits of endangered species are compared with the TK and TD traits of other species, such as the standard species used in toxicity tests.

4.2.3. Are endangered species likely to have a poorer recovery potential?

The term recovery is used in a number of different ways in ERA. For example, these include physiological recovery with the focus on the individual level, and population recovery focused at the population level. To protect endangered species, population recovery is of particular interest. Population recovery is here defined as the return of population abundance (in terms of numbers or biomass) after a disturbance to a defined reference state (e.g. return to its pre-disturbance state or its normal operating range).

A full description and the conditions required for ecological recovery can be found in the parallel SC Opinion on ecological recovery (EFSA SC, 2016b). According to that opinion, the potential for ecological recovery of an affected population from potential stressors may be disfavoured depending on one or more of the following interrelated conditions:

- Long duration of exposure or repeated exposures relative to the life-cycle of the species
- Large spatial scale exposure relative to the spatial characteristics (e.g. home range) of the species
- High probability of exposure of sensitive life-stage of the species
- Lack of exposure avoidance behaviour of species
- Lack of physiological ability to reduce the sensitivity to the potential stressor
- High probability of suffering indirect effects
- Low fecundity and long generation time
- Low re-colonisation ability
- Lack of, or inadequately connected, source populations
- Population viability already threatened by other (potential) stressors.

In particular the latter point is often generally applicable for endangered species as they are often endangered due to the effects of one or several other stressors.

A comparison of demographic and re-colonisation traits between endangered species and vulnerable non-endangered species should shed some light on the question whether endangered species exhibit traits that influence population growth rate relevant for both internal and external ecological recovery. Important demographic traits are life span, survival to reproduction, generation time (the average time between two consecutive generations), voltinism (the number of generations per year) and number of offspring per reproductive event. Re-colonisation traits are traits that govern the ability of an organism to reach a new habitat and consequently may be crucial for external ecological recovery. Important recolonisation traits are for example dispersal capacity (the ability of a species to disperse to a new area, including timing of dispersal periods), dispersal mode (active or passive), territorial behaviour and diet specialisation.

As outlined in Section 4.1 (on general characteristics) and also described in Liess et al. (2010) for freshwater macro-invertebrates, endangered species are often characterised by traits that do not facilitate a fast internal or external ecological recovery, since they often have a long life span, start reproducing late in life, have few offspring and are characterised by low intraspecific variability in their life-history traits. In addition, they often are habitat specialists highly vulnerable to disturbances related to habitat destruction and shifts in land-use.

The distribution of endangered species in a particular landscape is governed by both niche-assembly and dispersal-assembly rules. Since the spatial distribution of both endangered species and (potential) stressors tends to be patchy and aggregated, particularly in agricultural landscapes, external population recovery cannot be evaluated without considering the landscape context. In other words, the spatio-temporal arrangement of habitats, resources and exposures to potential stressors is critical for the evaluation of population dynamics. Only for endangered species that do not move beyond the treated field, the traditional approach of separating in-field and off-field assessment of recovery is useful. This is not the case for the vast majority of endangered species (exceptions may be some endangered weed species).

Whether or not the potential for ecological recovery of endangered species differs from that of the vulnerable species already considered in the higher-Tier ERA of potential stressors remains an open question. The vulnerable species currently considered in ERA schemes that allow to address the recovery option (for example (semi-)field experiments and/or mechanistic effect models) have been selected based on traits affecting internal and external ecological recovery (see e.g. EFSA PPR Panel, 2013; EFSA PPR Panel, 2015).

It appears that not the potential stressor or the endangered species *per se* may be decisive for ecological recovery from impact, but their interaction with (the properties of) the environments impacted by stressors, in which endangered species (temporarily) dwell. However, it seems that endangered species more often exhibit traits that are related to a decreased ability for recovery. In addition to the fact that information on the actual state of the population (viability) of an endangered species at the ecologically relevant spatial scale is not available at EU level, the ecological threshold option (ETO), allowing negligible population effects only, is a plausible option to consider at EU level. The alternative ecological recovery option (ERO), allowing some population level effects when recovery takes place within an acceptable period of time, might be more relevant for refinements in species-specific ERA at the relevant spatial scale.

4.2.4. Are endangered species suffering more from indirect effects?

Compared to direct effects, indirect effects of a stressor include a much wider set of considerations: any organism that is directly affected by the stressor can in turn affect other organisms. To look at such effects, it is helpful to consider the different types of direct and indirect interactions that species can in principle be involved in. Such interactions can be classified according to their effects on the interacting partners which can be either positive, neutral, or negative (Begon et al., 1996; EFSA GMO Panel, 2013).

Direct interactions between species can be classified as follows: In case of predator-prey, host-parasite, and other consumer-resource interactions, one species (the predator, parasite, or consumer) benefits from the interaction, whereas the other species (the prey, host, or resource species) is negatively affected by the interaction (+ - interactions). In case of mutualistic interactions, both species benefit from the interaction (+ +). In case of commensalism, one species benefits from the interactions, whereas the other species is not affected (+ 0). And finally, in case of amensalism, one species is negatively affected by the interaction, whereas the other species is not affected (- 0) (Begon et al., 1996).

These are direct interactions between *species*; hence if a stressor directly affects a species that is, in turn, directly interacting with an endangered species, the stressor itself indirectly affects the endangered species. Examples have been reviewed by Freemark & Boutin (1995), particularly direct effects of herbicides on arable weeds, and associated indirect effects on insects and birds that use weeds as a food source. In a recent report for the German Federal Environment Agency (UBA), the role of indirect effects of PPPs on birds and mammals (among other aspects) was reviewed (Jahn et al., 2014). Two examples were found that showed consistent indirect effects of PPPs on the populations of endangered farmland birds (grey partridge *Perdix perdix* and corn bunting *Emberiza calandra*).

Indirect effects between species can also be positive, neutral, or negative. For example, in case of predator-prey and other consumer-resource interactions, density-mediated indirect effects are discriminated from trait-mediated indirect effects (Trussell et al., 2006). Density-mediated indirect effects result from two or more direct consumer-resource interactions. For example, competition between two consumers that share a common resource is an important indirect density-mediated interaction. This is an indirect interaction where both interacting partners are negatively affected (- -). Other important examples of density-mediated indirect effects are trophic cascades in food chains (Begon et al., 1996; Eisenberg 2010; Terborgh and Estes, 2010). In case of trait-mediated indirect effects, the presence of a third species modifies the strength of interaction between two species by altering the behaviour, morphology, or physiology of one or both of the interacting species. For example, a stressor might not only cause direct mortality in species populations, but potential prey species of an endangered species may reduce their activity in open habitats and spend more time in refuges in order to avoid encounters with the endangered species. Such a reduced activity may lead to reduced food consumption of the endangered species. The effects of such trait-mediated interactions can exceed those of density-mediated interactions (Trussell et al., 2006).

There are many other possible indirect effects (Begon et al., 1996). All species in a food web are connected to each other, hence a stressor affecting any species in a food web can indirectly affect any other species in the food web (although biomagnification is mediated through food webs, the effect of the toxicant on the species is direct and thus treated above in Section 4.2.1 on exposure). A food web is just one representation of the interactions in species communities. Other ecological networks include host-parasite or plant-pollinator networks.

Stressors can affect the state and functioning of ecosystems and in this way indirectly affect many or most species in the system. This is, for instance, the case for some invasive species (Gaertner et al., 2014). The golden apple snail (*Pomacea canaliculata*) is a particular example of such an invader, as it has been shown to alter the state and functioning of wetlands it has invaded (Carlsson et al., 2004; Carlsson 2006). Of course, *abiotic* stressors can also lead to regime shifts. Well-known examples are lake ecosystem shifts caused by eutrophication. Another example is the reduction of photosynthesis rates due to toxic substances, which can also affect the whole ecosystem and thereby cause indirect effects on species in the system.

With respect to spatial scale, a distinction can be made between indirect effects that can be observed at the location of exposure and indirect effects occurring at a distance. The concept of “action at a distance” was put forward by Spromberg et al. (1998) in a (metapopulation) modelling study showing adverse effects in populations living at distance from the dosed patch. Brock et al. (2010) showed direct and indirect action-at-a-distance effects of spraying (parts of) ditches with lufenuron.

Due to the outlined complex nature of indirect effects, a full consideration of all different types of indirect effects is beyond the scope of this opinion. It is currently unclear whether endangered species generally suffer more from indirect effects than other species. However, it is likely that some endangered species are strongly affected by indirect effects, e.g. food specialists cannot easily switch to alternative food sources if the population of the species they are specialised to consume collapses due to a stressor (see Appendix C). In general, indirect effects for endangered species might be better evaluated from a case-by-case perspective. The SC thus considers the possibility of a case-by-case assessment for particularly relevant endangered species, asking whether they are more vulnerable than other species.

In conclusion, some endangered species appear to suffer more from indirect effects than many non-endangered species. Due to their complex nature, indirect effects can be better evaluated from a case-by-case perspective.

4.2.5. Vulnerability to IAS: traits that render native species more vulnerable to invasion

Further to the above discussions on vulnerability against chemical stressors, this Section explores traits that render native species more vulnerable to invasive alien species (IAS). In the context of plant health, the Plant Health Panel only considers invasive species that are pests of cultivated plants (i.e. herbivores, pathogens and competitors). Because cultivated plants cannot be considered vulnerable, the vulnerability due to direct effects refers to other species that are alternative (non-cultivated) hosts of the plant pest and that are endangered species. The direct interaction between endangered species and IAS always occurs in a dynamical and systemic context, therefore the indirect effects mediated by trophic or competitive interactions are important, and the possibility to generalise (deriving general patterns on the most important traits of the endangered species and the IAS) are limited. Relevant to the indirect effects are recent attempts to explain biological invasions on the basis of the invasive traits of successful IAS (invasiveness), or characterizing the susceptibility of the receiving ecosystems to an IAS (invasibility). The interaction between traits facilitating invasiveness and invasibility is poorly considered as for the contribution of other factors (e.g. time since introduction that influences both the IAS population pressure and the system reaction) (Barney and Whitlow, 2008).

Hypotheses on general patterns explaining invasiveness and invasibility have been proposed. Examples are the Enemy Release Hypothesis (Keane and Crawley, 2002), the Evolution of Increased Competitive Ability Hypothesis (Blossey and Nötzold, 1995), and the Novel Weapons Hypothesis (Callaway and Aschehoug, 2000). However, a substantial fraction of current invasion hypotheses seems to be poorly supported by empirical evidence (Jeschke et al., 2012; Jeschke, 2014), and more research is needed in this relatively young field to discriminate useful from less useful hypotheses.

The effects of introduced herbivores, pathogens, and competitors are difficult to foresee (e.g. how they cascade across higher levels of organisation), as these effects frequently depend on the introduced species in question and the systems to which they are introduced. The development of a predictive approach for the indirect effects is a complex task, because among other things the resistance and resilience properties of communities are involved. The recent concept on “eco-evolutionary experience” (Saul et al., 2013; Saul & Jeschke 2015) may prove helpful for addressing which resident species are particularly affected by which invasive species with which traits. This concept suggests that if the invader is totally different to other species than the native species has interacted with before, the impacts of the invader can be quite strong.

The role of context-dependence of the impact on endangered species is also emphasised by considering how humans modify ecosystems changing their susceptibility to invasion. This further shifts the attention from the dominant focus on the properties of invading organisms to how anthropogenic changes in ecosystems facilitate invasions, and how they in turn can affect endangered species (Simberloff et al., 2013).

In conclusion, most studies in invasion biology focused on the traits of IAS (specifically, traits related to their invasion success) or those of ecosystems (specifically, traits related to their vulnerability against IAS), whereas studies looking at traits of native species related to their vulnerability against IAS are rare. Regarding the latter, it is likely the type of interaction with IAS that makes them vulnerable and the lack of "eco-evolutionary experience" they have in interacting with such species.

4.2.6. Population size, genetic diversity and habitat

In addition to Sections 4.2.1 – 4.2.5, the following influences can also cause endangered species to react more strongly on stressors and may make them more vulnerable to one or more potential stressors under assessment.

Endangered species often occur in small populations because they typically experienced a reduction in their population size, at least at the spatial scale on which they are classified as "endangered" (see Section 3 for a definition of endangered species). For instance, if a species is endangered globally according to the IUCN Red List of Threatened Species (http://www.iucnredlist.org), it was classified as such based on criteria at the global level, e.g. because it has experienced a reduction in its global population size (criterion A for being red-listed; IUCN, 2012). In turn, small populations run a high risk of extinction due to stochastic events, including demographic and environmental stochasticity, inbreeding and natural catastrophes (Schaffer, 1981; Lande 1993). Potential stressors can be regarded as additional environmental or demographic stochastic factors, resulting in increased vulnerability of endangered species. For prospective risk assessment, one can try to estimate minimum viable population sizes (see Nunney & Campbell, 1993; Beissinger & McCullough, 2002). In conservation management of endangered species, population viability analysis (PVA) is a common tool to investigate species extinction risk or recovery potential (Lindenmayer et al., 1993), although the actual implementation of PVA has been criticised and could be improved (Beissinger & Westphal, 1998; Zeigler et al., 2013). Even if not resulting in extinction, effects of potential stressors on small populations might be more severe compared to larger populations. For instance, it was shown in a mesocosm experiment that sensitivity to a PPP was higher for invertebrate species with declining populations compared to ones with growing populations (Liess & Beketov, 2011).

Furthermore, as a general statement, a relatively low genetic diversity is one of the characteristics of endangered species (e.g. Leimu et al., 2006; Hirai et al., 2012; Furches et al., 2013; Verhaegen et al., 2013). Genetic diversity (as mentioned in the ToR1), describes the genetic variation between and within species. This can be characterised by the proportion of polymorphic loci (different genes whose product performs the same function within the organism), or by the heterozygous individuals in a population (Frankham and Briscoe, 2002). Therefore, the assumption can be made that endangered species are less capable of coping with additional stressors. Importantly, in a parallel opinion it will be explored to which degree low genetic diversity forms a bottle neck for recovery and whether this can be connected to the prospective ERA of potential stressors (EFSA SC, 2016b). This is also of relevance for endangered species, as recovery is one of the three components of vulnerability to direct effects.

Furthermore, the reasons for the endangerment of species are manifold, often including habitat destruction and fragmentation. In areas characterised by intensive agriculture, many endangered species are currently restricted to small areas of marginal habitat quality. Hence, endangered species are *per se* likely to be species under physiological stress due to other stressors i.e. the factors leading to their endangered status. Biological constraints (e.g. food deficiency, predatory stress) and environmental stressors (e.g. unfavourable temperature, low oxygen, high UV radiation) can increase toxicological sensitivity (Liess et al., 2010 and references therein). This might be caused by less resources available to devote to detoxification and damage repair, or by a higher cost of having to make such allocations of resources (Baas et al., 2010). It was also shown, that often multiple stressors (regulated and non-regulated ones) might play an important role for the decline of endangered species (e.g. pesticides and predation by invasive fish in the case of *Rana muscosa*; Davidson & Knapp, 2007). Hence, while under ideal conditions (= non physiological stress), endangered species might not *per se* be expected to have different (toxicological) sensitivity (see the Section 4.2.2 on SSDs and TK/TD considerations), under additional physiological stress conditions they are, as all species, more sensitive to chemical or other extra stressors (Holmstrup et al., 2010). As endangered species are more often under such additional stress, both the physiological (individual) level and the population level vulnerability is likely to be increased compared to other species.

Moreover, in addition to direct and indirect toxic effects, PPPs (and GMOs, FAs) may cause secondary effects due to enabling farming practice harmful to endangered species (Jahn et al., 2014). For instance, autumn sown wheat is only possible with intensive fungicide application leading to very dense stands that are unfavourable for farmland birds and therefore might additionally impair endangered species' populations.

In conclusion, it is likely that populations of endangered species are more vulnerable to potential stressors compared to other species in risk assessment as they usually exist in smaller, less viable populations than other species.

4.3. Conclusion for ToR1

For the questions posed in Sections 4.2.1 – 4.2.6, no general answers can be given but anecdotal examples illustrate why, where and when endangered species may be more vulnerable. Together with the general influences described in Sections 4.1 and 4.2.6, there is theoretical evidence that some endangered species are likely to be more vulnerable than the standard species or the vulnerable taxa currently considered in ERAs. However, there is too little data to make empirically-based conclusions on whether endangered species are generally more vulnerable.

The extent to which vulnerability is increased is difficult to quantify and a number of aspects remain unknown. However, it is likely that endangered species are more vulnerable due to their often higher level of specialisation compared to other species, resulting in (1) higher exposure due to food and habitat specialisation, (2) missing or less effective detoxification mechanisms, and (3) stronger vulnerability to indirect effects (by both toxic substances and invasive species) due to food specialisation. It also appears that the concept of ecological vulnerability is context dependent. For example the ability of a potentially sensitive and mobile species to avoid exposure depends on the spatio-temporal scale of the exposure to the potential stressor and the presence and connectivity to patches of habitat with less stressful exposure conditions. Sensitivity of organisms of a certain population may not only be influenced by life cycle properties (e.g. smaller and younger organisms often are more sensitive) but also by the fitness of the organisms such as determined by the availability of good quality food in stressed habitat. External recovery for a large part is dependent on landscape factors like the connectivity of sink and source populations within the larger metapopulation. Vulnerability to indirect effects may be larger when key species (e.g. ecological engineers) are affected, since many other species may depend on them. In addition, in structurally less complex communities indirect effects may be more pronounced than in structurally more complex communities, e.g. determined by the degree of availability of alternative food sources/prey organisms. Endangered species may be particularly susceptible to ecosystem changes driven by invasive species.

5. DO THE CURRENT ERA SCHEMES APPROPRIATELY COVER ENDANGERED SPECIES?

The key issues characterising endangered species and the questions as reviewed in previous Section 4, were considered for the evaluations of the current ERA schemes for PPPs, GMOs, IAS and FAs. The general risk assessment paradigm of problem formulation, exposure assessment, hazard characterisation and risk characterisation was followed (optionally mitigation measures and post-market environmental monitoring (PMEM)), when investigating whether the EFSA guidance documents (and/or their corresponding sectorial legislation or resulting opinions) have mentioned endangered species explicitly. If not mentioned explicitly, the evaluation continued for assumed implicit coverage of endangered species taking account of the characteristics and questions reviewed in Section 4.

The level of certainty for any assumed implicit coverage is, however, to be considered. This Section 5 will only report those aspects of the current ERA schemes for which there is a high concern of not covering for endangered species or areas with high uncertainty in this regard. Where there is potential to extend the coverage of endangered species in the current ERA schemes, suggestions were made in Section 8.

A general limitation of all evaluated ERA schemes is the scarcity of data and tests with endangered species. Therefore, for both effect and exposure assessment, there is a lack of data, even if data from taxonomically or ecologically related species is also considered. It is standard practice to establish a plausible link (also referred to as exposure pathway) between the stressor under assessment and its potential to adversely affect human, animal or plant health or the environment. However, for endangered species this approach might be rather difficult to follow. As outlined above (Section 4.2.1) it is unknown if and to what extent external exposure to potential stressors is higher for certain endangered species than anticipated in the ERA. Moreover, owing to their protection status, endangered species cannot be generally subjected to testing and therefore, limited or no data is available on their sensitivity to the potential stressor under assessment.

Despite the protection status of endangered species, some studies have been carried out e.g. with aquatic stages of amphibians and listed fish species (noting that rainbow trout is a listed species in some EU countries). Additionally, non-invasive study methods allow for measuring endpoints useful for risk assessment (e.g. taking blood/feathers for study) and afterwards releasing the animals undamaged. Endangered species of larger mammals and birds for example have been studied in the field at the individual level, e.g. by tagging them with a transmitter. This allows observations on their behaviour and provides information how long they spend in treated fields and in off-crop areas. Likewise, bee foraging behaviour and carabid/staphilinid beetle dispersal behaviour and their potential exposure both at local and landscape scales can be studied in this way. As an example for vertebrates, bats (of which 22% of the species are considered threatened) are widely studied in the field to identify risk factors to their population decline. Post-registration monitoring studies could also provide valuable information for related stressors. However, for species that occur in low numbers, which is often the case for endangered species, field and semi-field studies may have a low statistical power.

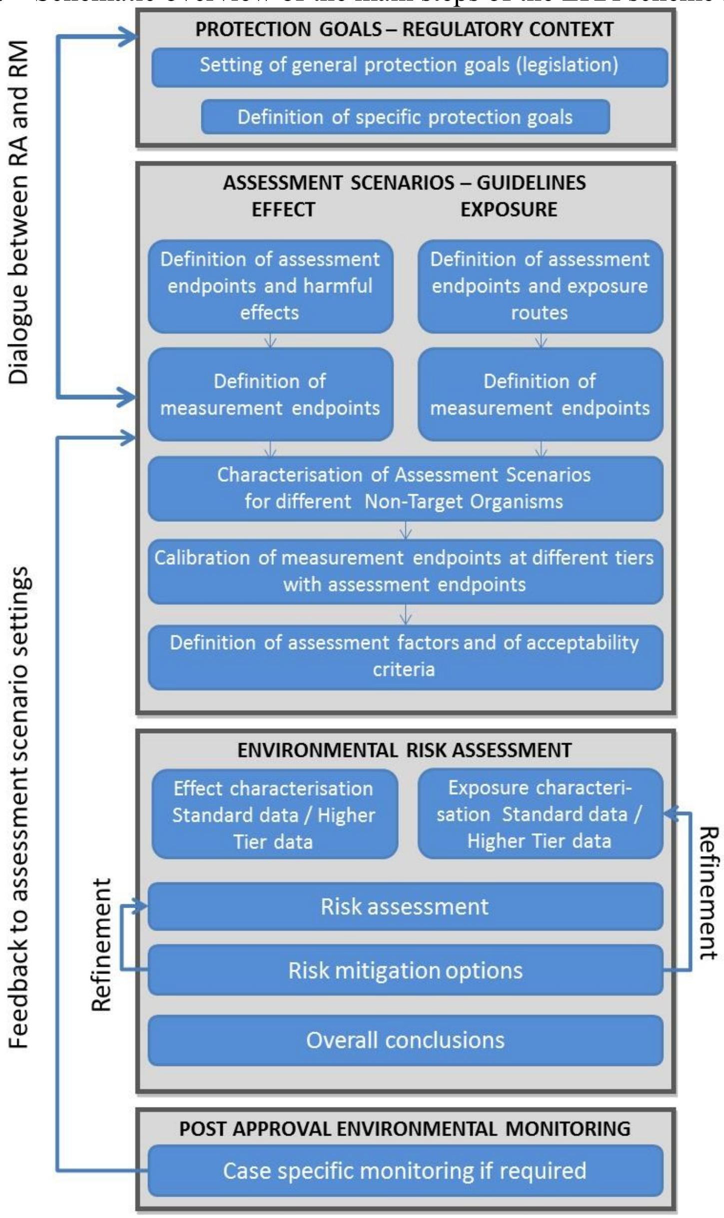
5.1. Coverage in ERA of plant protection products

5.1.1. Overview ERA of PPP

The current risk assessment of PPPs is done based on dossiers prepared by applicants and comprises different schemes for terrestrial and aquatic organism groups. A schematic overview of the current RA for PPPs is presented in Figure 2. The current schemes aim at protecting the large array of species from each organism group (aquatic organisms: freshwater invertebrates, plants and fish; terrestrial organisms: in-soil invertebrates, plants, non-target arthropods including bees, birds and mammals) working with data from tests with representative species from each group. These schemes evaluate mainly direct effects of the potential stressor, focusing on lethal and sub-lethal (e.g. behavioural, reproduction) endpoints. Indirect and cumulative effects are rarely taken into consideration, the former only when performing higher-Tier mesocosm or field tests. However, the upcoming schemes, being developed under the EFSA mandate to revise both Guidance Documents on Aquatic and Terrestrial Ecotoxicology (EFSA mandates EFSA-Q-2009-00001 and EFSA-Q-2009-00002, respectively) to attend the demands of the new PPP Regulation (EC) No 1107/20094, have the same general protection goal (protecting the sustainability and diversity of non-target organisms, especially those involved in the delivery of ecosystem services in the agricultural context), but take several steps forward, e.g. by (i) defining specific protection goals for each group (with more ecologically sound thresholds for acceptable effects/risks and, under certain conditions, also considering the recovery option), (ii) by attaining a better link between effects and exposure, (iii) by expanding the spatial boundaries of risk assessment at the landscape scale (using mechanistic effect modelling of populations and communities in pre-defined landscape scenarios), (iv) by attaining a better refinement of both exposure and effects using more meaningful assessment factors or modelling approaches (e.g. SSDs for refining effects), and (v) by considering not only direct effects (focusing mainly on sub-lethal parameters affecting the growth and reproduction of organisms), but also indirect and cumulative effects (mainly through semi-field and field tests and complex population and foodweb modelling). This is the case for the recent *EFSA Guidance Document on the risk assessment of plant protection products on bees (Apis mellifera, Bombus spp. and solitary bees)* (EFSA, 2013a) and the *Guidance on tiered risk assessment for plant protection products for aquatic organisms in edge-of-field surface water* (EFSA PPR Panel, 2013), the *Scientific Opinion addressing the state of the science on risk assessment of plant protection products for non-target terrestrial plants* (EFSA PPR Panel, 2014a) and the *Scientific Opinion addressing the state of the science on risk assessment of plant protection products for non-target arthropods* (EFSA PPR Panel, 2015).

Following a tiered approach, these schemes assume that Tier 1, which is the simplest one, should be more conservative when compared to other tiers. Tests species adopted in Tier 1, following the legal framework established by the current data requirements (EC, 2013), in combination with the respective assessment factor must address the specific protection of a certain organism group. However, since the array of species used in single-species laboratory tests do not depict the biological diversity in a particular ecosystem, nor the intra- and inter-species interactions, and neither the interactions between species and the environment (including the potential stressor), threshold levels for accepted effects in this Tier should be validated using data from a “reference Tier”, usually a mesocosm or a field test or a model. This validation should be done on a broader evidence basis and for each specific protection goal by comparing lower-Tier threshold concentrations for acceptable effects (regulatory acceptable concentrations) with regulatory acceptable concentrations derived from the most sensitive measurement endpoint as observed in semi-field studies that contain a sufficient number of populations of the sensitive and vulnerable taxonomic group(s). By this way Tier 1 regulatory acceptable concentrations may embrace the protection of the large array of species, including vulnerable species that are a particular focus of these schemes when defining the specific protection goals for each organism group. This is currently done for aquatic organisms and pulsed exposure regimens (see e.g. van Wijngaarden et al., 2014), but the framework for this validation for other organism groups must be established and more semi-field and field data (especially for terrestrial systems) must be gathered to accomplish this goal.

Figure 2: Schematic overview of the main steps of the ERA scheme for PPPs



5.1.2. Coverage of endangered species in ERA of PPP

Both in current and most of the upcoming PPP risk assessment schemes, endangered species or species of conservation concern are not explicitly taken into consideration during the problem formulation. The exceptions, up to now, are (1) the risk assessment of higher plants as stated in the *Scientific Opinion addressing the state of the science on risk assessment of plant protection products for non-target terrestrial plants* (EFSA PPR Panel, 2014a), where the protection of rare plant species is clearly addressed, and (2) the risk assessment towards larval stages of amphibian species, where a comparison with fish acute toxicity data is presented in the *Guidance on tiered risk assessment for plant protection products for aquatic organisms in edge-of-field surface waters* (EFSA PPR Panel, 2013). However, as mentioned earlier, in all the upcoming schemes vulnerable species (in terms of sensitivity, probability of exposure and recovery potential) are considered in the risk assessment. Therefore, it is assumed that by addressing vulnerable species in ERA, endangered species are at least partially addressed. However, it is also noted that the standard ERA usually does not consider the specific situation of endangered species for which an impairment of the population at the ecologically relevant spatial scale can be assumed. When applying the vulnerable species concept including aspects such as the recovery potential, this specific situation at time of exposure should be considered appropriately. Also, by calibrating Tier 1 assessment factors with data from the “reference Tier”, their use in combination with tests addressing realistic worst-case exposure regimes and containing a sufficient number of populations of potentially sensitive (ecological threshold option) and/or vulnerable non-target organisms (ecological recovery option) may implicitly cover endangered species if not already impaired by other stressors. However, since for terrestrial systems there is a shortage of mesocosm or field data for many compounds, the coverage of endangered species may be less adequate than in aquatic ERA. Note that for the spatio-temporal extrapolation of a regulatory acceptable concentration derived from an experimental (semi)field study and assessment factor (AF) is used as well.

One of the obvious problems related to assess risks towards endangered species is that these usually cannot be tested not only due to their conservation status, but also due to other logistic issues related to their biology (e.g. complicated life cycles, difficult to maintain in laboratory cultures). So, how to extrapolate the risk towards these species from data obtained with test species is the big question. While being complicated, surrogacy could be an option, but even so, some endangered species or species with conservation status (e.g. reptiles) do not have a related taxonomic species that could act as surrogate. In the following Sections different approaches are analysed.

5.1.2.1 Coverage based on toxicological sensitivity: The use of assessment factors

In risk (and hazard) assessment often assessment factors[11] (AF) are used. The general idea is that the uncertainty in an assessment is accounted for by imposing a certain safety margin between exposure and hazard. The larger the uncertainty in an assessment the larger the assessment factor will be. Thus, e.g. a certain endpoint is multiplied with or divided by an AF to extrapolate from a laboratory study with for instance a bobwhite quail to a fish eating bird or from single-species laboratory data to a multi-species ecosystem (“to the real world”). In the literature (e.g. EC, 2003a, a Technical Guidance Document on Risk Assessment) a number of uncertainties are identified which should be included in the assessment factor:

- Intra- and inter-laboratory variation of toxicity data;
- Intra- and inter-species variation (biological variance);
- Short-term to long-term toxicity extrapolation;
- Laboratory data to field impact extrapolation (additive, synergistic and antagonistic effects from the presence of other substances may also play a role here).

Sometimes the overall uncertainty factor is then derived by multiplying the single assessment factors.

For deciding whether a certain overall AF is also applicable for risk assessment of endangered species, it is necessary to assess to which extent the standard AFs provide an adequate coverage of the above mentioned sources of variability and uncertainty.

For a number of groups of organisms, large enough databases with acute toxicity data are available to analyse some of the above mentioned sources of variability and uncertainty: aquatic insects, aquatic crustaceans, fish and birds (see Appendix B for number of compounds and species available for each

[11] Often different names are used for an assessment factor, e.g. extrapolation factor, safety factor, uncertainty factor, trigger value, etc. In this document the term assessment factor (AF) is used.

topic mentioned below and for more detailed analysis). Using these databases, it was tested in how many cases a species would have a lower LC_{50} compared to the test species using a certain assessment factor:

- when a random test species would be used,
- when a standard test species would be used.

In both cases, inter-test variation per chemical was not taken into account (see Appendix B for different weighting schemes).

Two approaches were followed:

- Random species; all ratios between each pair of species within a species group were calculated and thereafter the percentages of outcomes not covered by the default AF were determined.
- Standard test species; all ratios between the standard test species and other test species were calculated and thereafter the percentages of outcomes not covered by the AF were determined.

For birds the default AF is 10 and for fish, crustaceans and insects the default AF is 100 (see Table 3). In case of random tested species, the percentages of the ratios not covered by the AF are less than 5% for fish and insects and 6.6% for crustaceans when using an AF of 100. For birds this percentage is 7.3% when using the official AF of 10. These percentages of species not covered are slightly lower when corrections are implied for inter-test variation (see Appendix B).

In case of standard tested species, the percentages are less than 5% for fish and crustaceans and 6.4% for insects when using an AF of 100. If the risk assessment for birds would be based on the Bobwhite quail or Japanese quail as the standard test species, the percentages for an AF of 10 would be lower than 5%, but for the mallard duck these percentages would be greater than 5% (i.e. 17.8%).

Note: this exercise only covers the inter-species-sensitivity and does not include any other types of uncertainty.

Table 3: Percentages of ratios not covered by the specified assessment factor. Values in bold are the values that belong to the official AF to be used in risk assessment.

Random species tested: percentage of ratios not covered by the specific assessment factor

Assessment factor	Insects	Crustaceans	Fish	Bird
100	**3.8%**	**6.6%**	**3.0%**	0.8%
10	16.9%	19.5%	12.1%	**7.3%**

Standard species tested: percentage of ratios not covered by the specific assessment factor

Assessment factor	Insects *Chironomus spec.*	Crustaceans *Daphnia magna*	Fish *Oncorhynchus mykiss*	Bird Bobwhite quail	Bird Mallard duck	Bird Japanese quail
100	**6.4%**	**4.5%**	**0.7%**	0.0%	1.3%	0.0%
10	21.4%	16.0%	5.0%	**4.4%**	**17.8%**	**3.9%**

Discussion

In PPPs ERA, current practice in Europe is to use the result of the most sensitive tested species and to divide this value by a factor of 5-100, depending on the species tested and the endpoint considered (e.g. acute versus chronic effects). It has been suggested that parts of these overall assessment factors of 5-100 cover interspecies differences in toxicity, though it is unknown whether the actual numbers used are appropriate for this purpose (EC, 2002). The results presented above indicate that the assessment factors currently used in acute risk assessment of PPPs cover the toxicological sensitivity of 82.2 - 100% of the species, depending on the species considered. However, this does not include potential variation in the other dimensions of ecological sensitivity, i.e. exposure, recovery and indirect effects (Section 4.2). As further extrapolation steps are necessary to cover the remaining uncertainties when using laboratory data to estimate effects in the field, it would be necessary to characterise all components of the overall AF as well as possible. In the following, this has been done only for the AF interspecies differences.

The lower Tier of the ERA is usually driven by the most sensitive test species or species group. For herbicides, the algae and macrophytes typically are the most sensitive group, while the crustaceans and insects typically are most sensitive for insecticides. In the research presented here, all available PPPs were used for the calculations. When the assessment would have been based on insecticides only, the outcome would have been slightly different and the percentages not covered by the AFs somewhat smaller.

The calculations presented above were based on acute toxicity data only. One could ask whether the results are also applicable to chronic toxicity data. However, no comparable large databases are available for chronic toxicity, and subsequently, similar calculations as presented above can currently not be performed for chronic data. However, Luttik et al. (2005) applied another approach to assess whether there is a difference in interspecies variation between acute and chronic toxicity data for one particular compound in a paper that was produced in response to a charge from the British Department of Environment, Food and Rural Affairs (DEFRA) to provide guidance to British and other EU regulators on the assessment of long-term risks to wild birds and mammals from their exposure to PPPs. They suggested that, in the absence of a strong rationale to the contrary, it should be assumed that reproductive data are at least as variable as acute data and that strategies developed for acute data could be applied to long term toxicity data as well. Considering only the two main bird test species for which reproduction data are available (Mallard and Northern Bobwhite), a comparison of the interspecies standard deviation for both acute and reproduction data suggested that the two are equally variable. In the same paper, an analysis of a very limited data set also suggested that this conclusion holds regardless of which endpoint is triggered in the reproduction study (Luttik et al., 2005).

In conclusion, risk assessment based on the standard aquatic test species and an AF of 100 appears to provide varying levels of protection: fish appear to be the best protected group (only 0.7% of the ratios are not covered by the AF), followed by crustaceans (4.5%) and insects (6.4%). Risk assessment based on the standard bird species, i.e. Bobwhite quail and Japanese quail, and an AF of 10 appears to provide almost the same level of protection, respectively 4.4% and 3.9 % of the ratios are not covered by the AF. These percentages would be 17.8% for the Mallard duck. The level of protection for fish seems to be higher than for birds. Choosing a random insect species for each test rather than the standard test species of *Chironomus* might provide a better level of protection. The percentages not covered might decrease from 6.4% to 3.8%.

If the aim of the risk assessment is for example to protect at least 95% of the species in any taxonomic group, it appears that the AF is consumed by the uncertainty from the between species variability where a standard species is tested: for bird assessment based on testing one of the two quail species and for the insect and crustacean species (percentages not covered are close to 5%). This means that there is no room for other sources of uncertainty in these AFs. For fish, there is still some room left for other uncertainties. It is evident that in case an AF is not covering the uncertainty for a general risk assessment it is also not covering the uncertainty in a risk assessment for endangered species. The reader is referred to Appendix B for more detailed information and results of additional calculations.

5.1.2.2 Coverage based on toxicological sensitivity: The surrogate species approach

Many species are not tested for legislative purposes and testing of other species is undesirable, either because (1) of animal welfare reasons or, (2) they cannot be tested because they are not surviving in the laboratory or, (3) they are so rare in the environment that testing should not be done. While it is currently not common practice in PPP ERA schemes, it has been suggested that in these cases a more common and closely related species can be tested to predict the toxicity of the species of concern (Fairchild et al., 2008; Sappington et al., 2001; Raimondo et al., 2008; Dwyer, 2005). That closely related species is sometimes called the surrogate species. In a general way, it was assessed below whether the surrogate species approach is a valid approach.

Comparison of toxicity data of the same genus

Using the same database as outlined in Section 5.1.2.1, the toxicological sensitivity of closely related species was compared to explore whether closely related species can serve as a surrogate for endangered species. It was assumed for these calculations that a species in the same genus can be considered a closely related species. The ratios in toxicity values between different species within one genus were calculated as a proxy for the variation in toxicological sensitivity between closely related species. Appendix B details the number of compounds and species available for this exercise.

In case of using an AF of 100 for the 3 groups of aquatic species the percentages of the ratios not covered by the specified AF are 1.6% for insects, 3.2% for crustaceans and 1.9% for fish, (see Table 4). If the standard test species are being used (instead of species in the same genus), these ratios are respectively 6.4%, 4.5% and 0.7% (see Table 3 in assessment factors Section 5.1.2.1). This suggests that testing a closely related fish species will generally not be more conservative than testing the Rainbow trout. For crustaceans and insects, the results suggest that testing a species from the same genus may result in a more conservative assessment. When applying an assessment factor of 10 to closely related test species, between 6.4 and 11.3% of the ratios would not be covered (see Table 4).

Table 4: Species tested from same genus: percentage of ratios not covered by the specified assessment factor

Assessment factor	***Insects***	***Crustaceans***	***Fish***
100	1.6%	3.2%	1.9%
10	9.5%	11.3%	6.4%

In conclusion, testing of surrogate species, which is not current practice in PPPs, could slightly improve the outcome of the risk assessment but the gain in knowledge is only marginal. Even when using a surrogate species (a closely related species from the same genus) for testing, to reach a protection level of 95%, one would have to use a safety factor of 100 (in the case of crustaceans, insects and fish). This means that, when using a surrogate species, the AFs can not be substantially lowered; they have to be in the same range as for the standard test species.

5.1.2.3 Examples from literature

The following Sections give examples, from the scientific literature, of comparison between standard test species and endangered species in relation to their toxicological sensitivity. This comparison aims to provide an indication whether the systematic use of tests species (as surrogates) for the risk assessment of endangered species would be a scientifically sound methodology or if unexpected toxicological responses in endangered species compared with the test species have been demonstrated.

For fish, static acute toxicity tests from test species (rainbow trout, *Oncorhynchus mykiss*; fathead minnows, *Pimephales promelas* and sheepshead minnows, *Cyprinodon variegatus*) were compared with data for several US listed endangered species (Apache trout, *Oncorhynchus apache*; Lahontan cutthroat trout, *Oncorhynchus clarki henshawi*; greenback cutthroat trout, *Oncorhynchus clarki stomias*; bonytail chub, *Gila elegans*; Colorado pikeminnow, *Ptychochelius lucius*; razorback sucker, *Xyrauchen texanus*; Leon Springs pupfish, *Cyprindodon bovinus* and desert pupfish, *Cyprindodon macularius*) for carbaryl, copper, 4-nonylphenol, pentachlorophenol, and permethrin. The results indicated that the surrogate and listed species were of similar sensitivity with differences less than two-fold (except in two cases with above two fold). The authors proposed that a safety factor of two would provide a conservative estimate for listed cold-water, warm-water, and euryhaline fish species (Sappington et al., 2001). However, in that study only toxicity tests from one laboratory were compared, hence omitting a lot of uncertainty. Dwyer et al. (2005) compared common test species (fathead minnow, sheepshead minnow and rainbow trout) and 17 listed or closely related species in acute 96-hour water exposures with five chemicals (carbaryl, copper, 4-nonylphenol, pentachlorophenol, and permethrin). There wasn't a single species that was the most sensitive for each of the chemicals. For the three standard test species evaluated, rainbow trout was the most sensitive and was equal to or more sensitive than listed and related species 81% of the time. The authors proposed to estimate an LC_{50} for a listed species using a factor of 0.63 applied to the geometric mean LC_{50} of rainbow trout toxicity data, and a low- or no-acute effect concentration could be estimated by dividing the LC_{50} by a factor of approximately 2, which supported the US-EPA approach. From a chronic perspective, early life-stage toxicity tests with copper and pentachlorophenol (were conducted with two species listed under the US Endangered Species Act (the endangered fountain darter, *Etheostoma fonticola*, and the threatened spotfin chub, *Cyprinella monacha*) and two commonly tested species (fathead minnow and rainbow trout). Results were compared using LOECs based on statistical hypothesis tests and by point estimates derived by linear interpolation and logistic regression. In this context, sub-lethal end points, growth (mean individual dry weight) and biomass (total dry weight per replicate) were usually more sensitive than survival. Overall, fountain darters were the most sensitive species for both chemicals tested, with effect concentrations lower than current chronic water quality criteria for biomass LOEC whereas spotfin chubs were no more sensitive than test species. The authors recommended that protectiveness of chronic water-quality criteria for threatened and endangered species could be improved through (1) the use of safety factors or (2) conducting additional chronic toxicity tests with species and chemicals of concern (Besser et al., 2005).

Reptiles have no standard test guidelines and the outcome of avian toxicity studies were proposed to be used as a surrogate. The results of the literature survey (Weir et al., 2010) showed that reptiles were more sensitive than birds in more than 3/4 of the chemicals investigated and that dietary and dermal exposure modelling indicated a relatively high exposure in reptiles, particularly for the dermal route. The authors concluded that caution was warranted to use birds as surrogates for reptiles and emphasised the need to better understand both hazard and exposure assessment in reptiles.

For amphibians, a comparison of the relative sensitivity between amphibians and fish for chemicals was performed using acute and chronic toxicity data from the US-EPA ECOTOX database and were supplemented with data from the scientific and regulatory literature (Weltje et al., 2013). Overall, toxicity data for fish and aquatic stages of amphibians were highly correlated and fish were generally more sensitive (for both acute and chronic) than amphibians, with a few exceptions. For acute toxicity data, amphibians were between 10- and 100-fold and 100-fold more sensitive than fish for only four and two of 55 chemicals respectively. For chronic toxicity data, amphibians were 10- and 100-fold more sensitive than fish for only two substances (carbaryl and dexamethasone) and greater than 100-fold more sensitive for only a single chemical (sodium perchlorate). The comparison for carbaryl was subsequently determined to be unreliable and that for sodium perchlorate is a potential artefact of the exposure medium. Only a substance such as dexamethasone, which interferes with a specific aspect of amphibian metamorphosis, might not be detected using fish tests. From these datasets, the authors concluded that that additional amphibian testing on top of the fish tests would not be necessary. The dexamethasone example furthermore illustrates that additional testing of amphibians would be required if the substance of interest interferes with a physiological feature which is characteristic for amphibians, such as metamorphosis. The challenge will be to predict such effects based on the structure of the chemical of interest and knowledge about the molecular receptors that play a key role in the physiological processes that are characteristic for amphibians.

In 2013, the EFSA PPR panel investigated in its Guidance on tiered risk assessment for plant protection products for aquatic organisms in edge-of-field surface waters, how well aquatic life stages of amphibian are covered by fish as test species. The panel reported that "*an analysis of acute toxicity data for a large number of amphibian species (Fryday and Thompson, 2012) and a comparison with fish acute toxicity data (see Appendix C) shows that the rainbow trout is a good surrogate test species for predicting the acute toxicity of PPPs for larval stages of amphibian species living in the aquatic compartment of the environment. Similar results were found by Aldrich (2009). By using the same AFs as have been applied for fish, the achieved level of protection will be the same for both groups of organisms*. [It is assumed that this covers the aquatic stages of vulnerable (endangered) amphibians]. *The assessment is only valid for acute toxicity (mortality) and will not necessarily be predictive of chronic toxicity. However, a recent study indicates that the same is also applicable for chronic toxicity (Weltje et al., 2013)*[12]". Terrestrial life stages of amphibians are to be addressed in a future GD on PPP RA for amphibians and reptiles (EFSA-Q-2011–00987) under the mandate of the revision of the GD on terrestrial ecotoxicology. However, there are some indications that terrestrial life stages of the European common frog *(Rana temporaria)* are more sensitive than anticipated in the current ERA as exposure to several PPPs at the recommended application rate resulted in up to 100% acute mortality (Brühl et al., 2013).

An external report delivered to EFSA in September 2012 (Fryday and Thompson, 2012) also concludes that a large number of toxicity values were found for aquatic stages of amphibians suitable for comparison with fish data. A far smaller body of data was found for toxicity of PPP to terrestrial phases of amphibians both in numbers of values and range of compounds making comparisons with bird and mammal data more difficult. The authors recommend that for terrestrial amphibians, methods must be developed that will estimate the extent of dermal exposure under field conditions. However, estimating dermal exposure and exposure through food will not allow full risk assessment in the absence of reliable amphibian or suitable surrogate toxicity data.

In conclusion, for some cases it has been shown that the rainbow trout is a good predictor of the sensitivity of other species, in particular for other fish species and for the aquatic life stages of

[12] Chronic amphibian toxicity data were retained for analysis if they were from studies of at least a 10-d duration, employed either static-renewal or flow-through aqueous exposure study designs, and reported apical endpoints of potential population relevance (i.e. they were related to survival, growth, development [including metamorphosis], or reproduction). The lowest long-term population-relevant NOEC, was identified for each chemical for subsequent comparison with fish data. Chronic fish toxicity data were retained if they were from static-renewal or flow-through laboratory aqueous exposures of at least 21 d and reported apical endpoints.

amphibians. When the same assessment factors are used, the level of protection will be the same as achieved for fish species. In other cases like for instance predicting the toxicity for reptiles from bird toxicity values and predicting the toxicity for terrestrial life stages of amphibians showed to be difficult and it was advised by the authors to develop better test for those groups.

5.1.2.4 Coverage based on exposure

PPP assessments use standard exposure scenarios for in-field and for edge of field (for soil as well as for receiving surface water) locations. In the exposure assessment, it is assumed that these standard exposure scenarios are sufficient and should cover the exposure of tested and non-tested taxa[13]. The 90th percentile concentration in space and time has been selected and fixed as a worst case realistic exposure scenario (FOCUS, 2001). This means that there is a 10% chance (in space and in time) that the actual exposure is higher than what is assumed in the ERA. However, it is noted that this 90th percentile is selected from the peak concentration and not from the average concentration, which is considered a conservative choice.

The scientific basis for the choice of the 90th percentile has not been described in detail and the level of uncertainty neither. For example, a spatial and temporal specification of this 90th percentile is lacking. An interesting question in this context is what is a “worst case” from an ecological point of view: being highly exposed over many years in one place or being highly exposed in one year over many different places? This question is especially important for endangered species as it would be an undesirable choice if the endangered species lives in the one area where higher exposures are being expected over many years. It is therefore recommended that risk managers motivate their choice on the basis of a risk/benefit analysis and for risk assessors to document the analysis of the environmental consequences of the choice of the 90th percentile.

In case that RA cannot exclude risk, landscape modelling as well as monitoring of field observation data as basic input for the modelling becomes necessary, depending on the mobility of the endangered species. For this, it will be necessary to know how much time an endangered species spends in the different landscape types (including other fields with either the same PPP regime or other regimes) and to estimate the potential exposure concentrations in those other landscape types. Landscape modelling for the PPP ERA scheme is in development (EFSA PPR Panel, 2015).

Skin and inhalation as exposure routes are generally not considered in the current PPP ERA schemes. For these and other exposure routes, co-occurrence needs to be established (e.g. Driver et al., 1991).

For the aquatic compartment, direct exposure through the water compartment seems to be a worst-case scenario for direct exposure, because it includes a multitude of different exposure routes (oral, dermal, gills) and a long exposure duration (continuous). Exceptions are limited to exposure to specific food products (e.g. secondary poisoning) and/or transition layers (e.g. sediment dwelling organisms) containing high levels of toxic stressors. A similar reasoning applies to terrestrial and sediment compartments, i.e. organisms living in the soil and/or sediment. Most exceptions can be expected for organisms that are being exposed through a combination of different exposure routes, e.g. water organisms dwelling in sediment and terrestrial organisms dwelling over land or in the air. Even more, in some of these cases there are different life stages that can have a different sensitivity as well.

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[13] This is similar to the effect assessment, where not all taxa are/can be assessed (e.g. fungi and reptiles) and where it is assumed that the assessment factors on the test species data also cover for the non-tested taxa.

Assessment of biomagnification is standard in risk assessment of PPPs. In general, two routes are taken into account:

1) water – fish – fish-eating bird and fish-eating mammal,
2) soil – earthworm – earthworm-eating bird and earthworm-eating mammal.

The fish route is normally based on a biomagnification study with fish (standard OECD test 305) but can also be calculated by using a Quantitative Structure Activity Relationships or QSAR (EFSA PPR Panel, 2009). The biomagnification for earthworms is normally based on a QSAR (EFSA PPR Panel, 2009). It is generally assumed that these two routes cover the biomagnification potential of compounds, although not many studies are available to underpin this conclusion. One of the reasons for this is the lack of bioconcentration factors and biomagnification factors for many different types of food.

Jongbloed et al. (1996) and Traas et al. (1996) presented a more detailed description of a three trophic level approach based on data for DDT and Cd: plants and invertebrates at the first, small birds and mammals at the second, and birds of prey and mammal predators at the third trophic level. Exposure of top predators via separate food chains is analysed. However, most top predator species are exposed via more than one food chain (food web). Therefore, a species-specific approach was followed too, for which four bird of prey species and two beasts of prey species with different food choices were selected: sparrow hawk, kestrel, barn owl, little owl, badger, and weasel. The most critical food chains for secondary poisoning of top predators were soil → worm/insect → bird → bird of prey for dichlorodiphenyltrichloroethane (DDT), and soil → worm → bird/mammal → bird of prey for cadmium (Cd). The overall risk for the selected top predator species was much lower than the risk based on these critical food chains, because the critical food chains constituted only a minor part of their food webs. Species feeding on birds (sparrow hawk) and on small carnivorous mammals (barn owl) were exposed to DDT and Cd to a much higher extent than species mainly feeding on small herbivorous mammals (kestrel and weasel). The authors proposed to include exposure via the pathways soil → worm/insect → bird/mammal → top predator in procedures for derivation of environmental quality objectives for persistent and highly lipophilic compounds. In the bird and mammal guidance document of EFSA PPR Panel (2009), a method is presented that allows calculation of the biomagnification potential for any carnivorous or insectivorous species of concern.

There are indications that the assessment of biomagnification in current ERA schemes is not always sufficiently protective for endangered species. The example presented here does not relate to PPPs, but to another group of persistent chemicals, i.e. PCBs, which are assessed using similar biomagnification models. A study by Peters et al. (2014) explored the sudden death of reintroduced Eagle Owls living in the southern part of the Netherlands. Chemical analysis showed that these dead owls contained very high PCB levels; even higher than any other values reported in literature. Analysis of the foraging behaviour of the owls indicated that the PCBs are likely stemming from a diffuse source, most likely the widespread historical soil contamination due to industrial emission of incineration products. The tentative conclusion of this study was that biomagnification of PCBs in the food web of the Eagle Owl is higher than the models and the biomagnification factors currently applied in environmental risk assessments of PCBs. Subsequently, it is likely that current PCB soil standards provide insufficient protection for the Eagle Owl.

In conclusion, the exposure scenarios currently applied in ERA schemes for PPPs in general seem to be sufficiently conservative to cover endangered species. However, it would be good if the scientific basis of the 90th percentile that has been chosen as a generic benchmark for PPP exposure was strengthened, particularly in relation to its spatial and temporal dimensions. Furthermore, there are some specific exposure conditions that are relevant for endangered species and which are insufficiently covered in current PPP ERA schemes, notably PPP exposure through multiple routes, including inhalation and dermal exposure, and biomagnification of persistent PPPs.

5.1.2.5 Coverage based on indirect effects

It is scientifically challenging to document in how far indirect effects are covered in ERA. Direct and indirect effects are considered equally important and this is taken into account in field studies as requested by the legal frameworks for PPPs (although not always in a quantifying manner). It is underlined that an indirect effect can only occur if a direct effect is being allowed when using or releasing the potential stressor.

The PPR panel is continuing the development of explicit guidance to deal with indirect effects related to herbicide use and the killing of “weeds” in the field. This practice obviously takes away food/hosts of insects, which raises concern of indirect effects. Some countries already take that into account at the management level. For instance in the UK, various stewardship schemes are put in place that farmers can sign up to in order to provide food and habitat for farmland birds.

5.1.3. Conclusion on the coverage of endangered species in the PPP ERA scheme

With the few exceptions of rare plants and amphibian larval stages, endangered species are not explicitly covered in the guidance documents on the RA for PPPs. However, endangered species might be covered when addressing vulnerable species during the assessment and defining specific protection goals in the future based on species vulnerability aspects (toxicological sensitivity, probability of exposure, recovery potential and responsiveness to indirect effects).

The analyses presented in Sections 5.1.2.1 and 5.1.2.2 show that the assessment factors currently used in hazard/effect assessment of PPPs provide varying levels of protection for different species groups (0.7%-17.8% unprotected, depending on which group is considered and which species is being tested). As a consequence, it seems likely that the toxicological sensitivity of a limited number of endangered species is not covered by the current assessment factors. Other aspects of ecological vulnerability (i.e. exposure, recovery and indirect effects) are not included in this assessment and may modulate the ultimate protection level, either resulting in a higher or lower protection level.

5.2. Coverage in ERA of genetically modified organisms

5.2.1. Overview ERA of GMOs with a focus on genetically modified plants (GMPs)[14]

Within the European Union (EU), the application of genetic engineering is regulated for domestic and imported goods. The EU legal frame for GMOs is set by Directive 2001/18/EC (EC, 2001) for their release into the environment, and Regulation (EC) No 1829/2003 (EC, 2003b) for the marketing of derived food and feed products. According to GMO legislation, GMOs and derived food and feed products are subject to a risk analysis and regulatory approval before entering the market in the EU. In this risk analysis process, the role of EFSA is to independently assess and provide scientific advice to risk managers on any possible risks that the use of GMOs may pose to human and animal health and the environment. The main focus of EFSA in the field of GMOs lies in the evaluation of GMO market registration applications (referred to hereafter as GMO applications) and in the development of risk assessment and monitoring guidelines. The decision on whether a certain risk is acceptable and whether a

[14] Few applications for GM micro-organisms and none for deliberate release into the environment of GM animals have been received. Guidance for risk assessments of both GM micro-organisms and GM animals groups have been published by the EFSA GMO Panel (2011a, 2013).

GMO or a derived product can be placed on the EU market is not part of the risk assessment itself, but part of the wider risk analysis.

As part of the risk assessment, potential adverse effects that GMOs may pose to the environment, including biodiversity, are evaluated. The EFSA GMO Panel has developed guidelines for the ERA of GM plants and living GM animals (namely fish, insects, and mammals and birds) (EFSA GMO Panel, 2010, 2013, respectively). Although some of the intended uses of GM animals could fall in the scope of this Scientific Opinion (e.g. GM fish in aquaculture), the focus is exclusively on GM plants, as to date no applications for commercial release of GM animals have been submitted to EFSA.

ERA evaluates potential adverse effects on the environment arising from a certain course of action such as cultivation of a GM plant, and is one of the important safeguards to ensure the protection of the environment and biodiversity. Potential direct and indirect, as well as immediate, delayed and cumulative long-term adverse effects are considered on a case-by-case basis taking into account the plant species, traits, receiving environments and intended uses, and the combination of these characteristics.

Seven areas of environmental concern are considered during the ERA of GM plants. These involve: (1) potential effects on plant fitness due to the genetic modification, including vertical (plant-to-plant) gene flow; (2) horizontal (plant-to-bacteria) gene flow; (3) interactions between the GM plant and target organisms; (4) interactions between the GM plant and non-target organisms; (5) plant effects on human and animal health; (6) effects on biogeochemical processes; and (7) impacts of the specific cultivation, management, and cropping practices associated with the use of the GM plant.

In line with a number of internationally agreed risk assessment principles, the guidelines require ERAs: (1) to use quantitative information where available; (2) to use a comparative approach whereby the level of risk is estimated through comparison with an appropriately selected comparator and its associated farm management and cropping practices; (3) to be case-specific; and (4) to be iterative by examining previous conclusions in the light of new information (EFSA GMO Panel, 2010).

As outlined in Directive 2001/18/EC, ERA follows six steps (Figure 3), consisting of: (1) problem formulation as a critical first step; (2) hazard characterisation that examines potential hazards and the seriousness of potential harm; (3) exposure characterisation that considers levels and the likelihood of exposure and thus how likely it is that harm occurs; (4) integrative risk characterisation in which the magnitude and likelihood of harm are integrated to estimate the level of risk; (5) mitigation of the identified risks to reduce an identified risk to a level of no concern; and (6) evaluation of the overall risk based on proposed risk mitigation measures (EFSA GMO Panel, 2010).

Problem formulation is given a central role in ERAs, as it enables a structured, logical approach to detecting potential risks and scientific uncertainties by summarising existing scientific knowledge and explicitly stating the assumptions and principles underlying the risk assessment. Problem formulation involves: the identification of characteristics of the GM plant capable of causing potential adverse effects (hazards) and pathways of exposure through which the GM plant may adversely affect human and animal health or the environment; the definition of assessment endpoints, which are explicit and unambiguous targets for protection extracted from legislation and public policy goals; and outlining specific hypotheses to guide the generation and evaluation of data in the subsequent successive risk assessment steps. This process also requires the development of a methodology – through a conceptual model and analysis plan – that will help to direct the risk characterisation and to produce information that will be relevant for regulatory decision-making (Raybould, 2006; Wolt et al., 2010; Gray, 2012). Information considered in problem formulation includes published scientific literature, expert opinions, research data and relevant data derived from molecular, compositional and agronomic/phenotypic analyses performed during GM plant development.

The overall conclusions of the ERA provide the basis for the Post Market Environmental Monitoring (PMEM), which focuses on risks to human health and the environment identified in the ERA and remaining scientific uncertainties (EFSA GMO Panel, 2011b). In the EU, the objectives of PMEM according to Annex VII of Directive 2001/18/EC and the Council Decision 2002/811/EC are: to confirm that any assumptions regarding the occurrence and impact of potential adverse effects of the GMO, or its use, in the ERA are correct; to identify possible unanticipated adverse effects on human health or the environment which could arise directly or indirectly from GM plants and which were not anticipated in the ERA; and to further inform the ERA. The scientific knowledge obtained during monitoring of GMOs, along with the experience gained from their marketing/cultivation as well as any other new knowledge generated through research, can provide valuable information to risk assessors to update ERAs and to resolve any remaining scientific uncertainty.

PMEM is composed of case-specific monitoring (CSM) and general surveillance (GS). CSM is not obligatory, but may be required to verify risk assessment assumptions and conclusions, whereas GS is mandatory in all cases. Due to different objectives between CSM and GS, their underlying concepts differ (Sanvido et al., 2012). CSM enables the determination of whether, and to what extent, anticipated adverse effects occur during GM plant deployment, and thus to relate observed changes to specific causes. It is mainly triggered by scientific uncertainties that were identified in the ERA. Therefore, a hypothesis is established that can be tested on the basis of newly-collected monitoring data ("bottom-up approach"). In GS, in contrast, the general status of the environment that is associated with the GM plant deployment is monitored without any preconceived risk hypothesis in order to detect any possible effects that were not anticipated in the ERA, or that are long-term and cumulative. Should any such effects be observed, they are studied in more detail to determine whether the effect is adverse and whether it is associated with the use of a GM plant. GS data can originate from various sources: (1) farm questionnaires; (2) existing surveillance networks (such as plant health surveys, soil surveys, ecological and environmental observations); (3) scientific literature; (4) industry stewardship programs; and (5) alert issues.

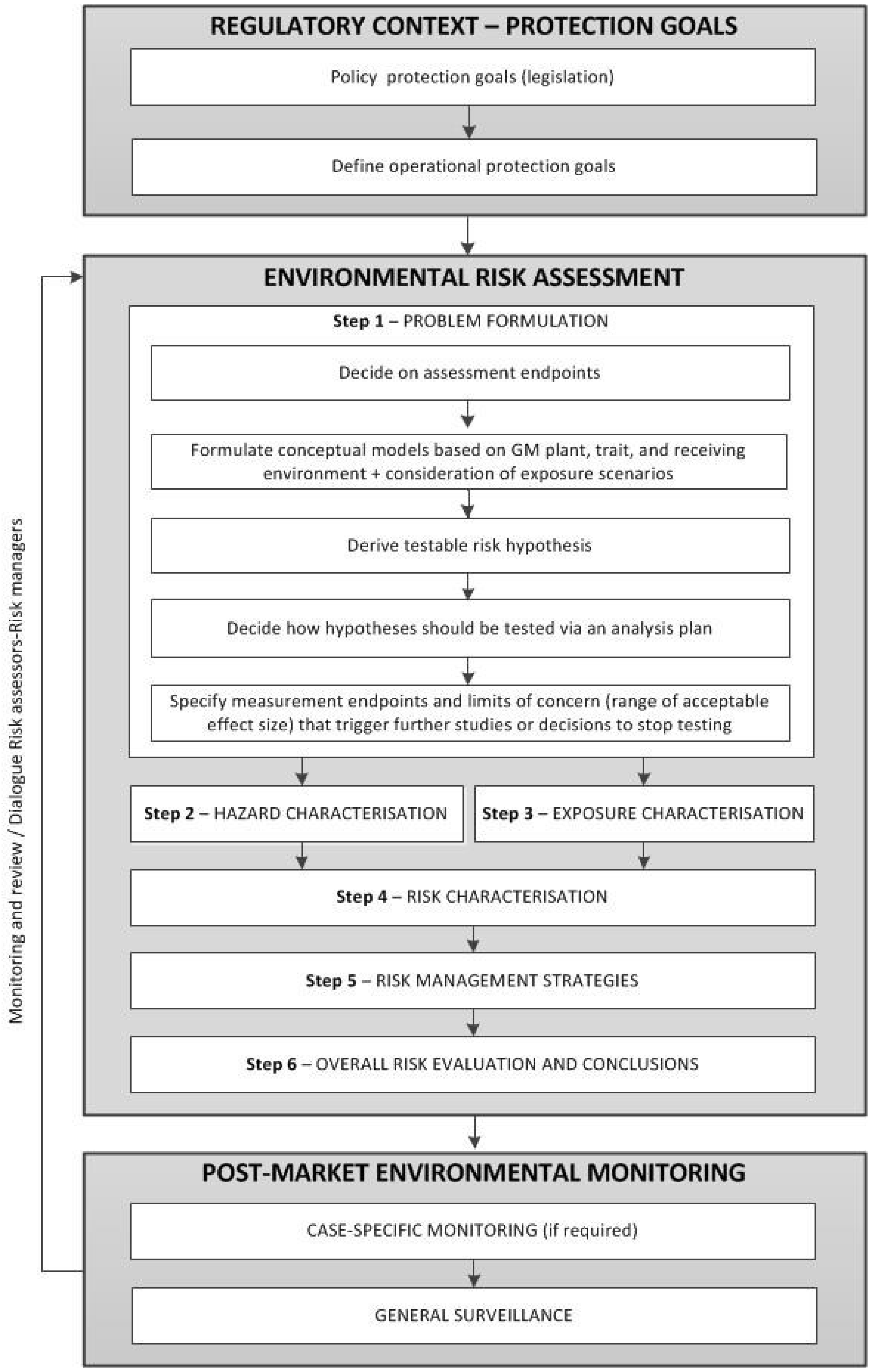


Figure 3: Schematic diagram representing the key steps of the environmental risk assessment (ERA) of GMOs, and the interplay between protection goals outlined in the legislation, ERA and post-market environmental monitoring. Adapted from EFSA GMO Panel (2010) and Sanvido et al. (2012)

5.2.2. Coverage of endangered species in ERA of GM Plants

Species of conservation concern, which include endangered species (both plants and animals), are explicitly addressed in the ERA of GM plants, especially in the problem formulation phase. During the problem formulation, conceptual models are developed that define how the GM plant could cause harm to valued species. ERA of GM plants should cover potential adverse effects arising from the intended[15] and unintended[16] changes in the GM plant.

Four plausible scenarios are considered to describe how GM plants may cause harm to endangered species:

- *Scenario 1* focuses on the exposure of valued species to the transgene products when feeding on living or dead plant material, and on potentially altered interactions between the GM plant and associated fauna;
- *Scenario 2* covers potential adverse effects on valued species caused by the altered invasiveness and persistence potential of the GM plant;
- *Scenario 3* considers the consequences of introgressive hybridisation of the transgene(s) to cross-compatible wild relatives through vertical gene flow;
- *Scenario 4* focuses on the impact of altered farm management practices (e.g. effects on weeds and their associated fauna).

The plausibility of the above mentioned scenarios is to be explored on a case-by-case basis. For each plausible scenario, a conceptual model describing how GM plants could harm an endangered species is to be formulated. Each model consists of a series of events or discrete steps that must occur for harm to the assessment endpoints to be realised (pathway to harm). For each step, a conservative risk hypothesis is formulated that needs to be tested (Raybould, 2011). If evidence suggests that one step in the pathway cannot take place, then the formulated risk hypothesis can be invalidated and one can conclude that the likelihood that any hazard will be realised via that pathway is negligible.

5.2.2.1. Scenario 1a: Exposure of endangered species to transgene products

Several non-target organisms (NTOs) are likely to be exposed to the newly expressed protein by the transgene (e.g. insecticidal protein) in GM plants when cultivated. These NTOs can be exposed to the transgene products when feeding on plant material (including pollen) or honeydew excreted from sap-sucking species, and/or when feeding on prey/host organisms which have previously been feeding on the GM plant (Andow et al., 2006; Romeis et al., 2006, 2008). NTOs occurring in the soil ecosystem can be exposed to the transgene products introduced into the soil via physical damage to plant tissues, via decomposition of shed root cells during plant growth, via decomposing plant residues remaining in fields after harvest, which might be incorporated into the soil during tillage operations, and possibly via root exudates (reviewed by Icoz and Stotzky, 2008). By-products from GM plants (e.g. pollen, detritus) can be transported in water courses to downstream water bodies where aquatic NTOs can be exposed to transgene products through consumption (Rosi-Marshall et al., 2007; Axelsson et al., 2010, 2011; Chambers et al., 2010; Tank et al., 2010). These species however are only at risk if the newly expressed proteins from the transgene show toxicity at a realistic level of exposure.

The ERA of GM plants to endangered species under scenario 1 should consider (1) the activity spectrum of the newly expressed protein by the transgene to define which taxa are susceptible; and (2) the level of exposure of potential sensitive species to the transgene product.

Based on the familiarity with the transgene product (e.g. knowledge on its mode of action and spectrum of activity) effects on NTOs can be predicted during the problem formulation phase. Potential adverse

[15] Intended changes are those that fulfil the original objectives of the genetic modification

[16] Unintended changes are those which go beyond the primary intended changes of introducing the transgene(s)

effects caused by the intended genetic modification [e.g. the expression of a protein from *Bacillus thuringiensis* (Bt)] are typically evaluated within different Tiers that progress from highly controlled laboratory studies representing worst-case exposure conditions [e.g. 10-fold estimated environmental concentration (EEC) of the newly expressed protein] to more realistic but less controlled field studies (Romeis et al., 2008). Moving to a higher Tier is only considered relevant if adverse effects are detected at a lower Tier, or if unacceptable scientific uncertainty remains. The use of worst-case exposure conditions (e.g. 10-fold EEC) in early-Tier studies adds certainty to the conclusions drawn from the test and enables to account for possible intra- and interspecies variability in sensitivity (Romeis et al., 2011). No specific margin of exposure for early-Tier studies is recommended in the EFSA guidance for the ERA of GM plants (EFSA GMO Panel, 2010).

Because not all of the exposed species can be tested from a practical point of view, the toxicity of the transgene products is tested generally on a representative subset of species (Romeis et al., 2006, 2008, 2013; Rose, 2007). Usually, a representative subset of NTOs are selected for testing purposes based on the ecological relevance of the species, the likely exposure of the species to the GM plant under field conditions, species susceptibility to the transgene product, and testability. The selected species serve as surrogate species for the endangered species, as those cannot be tested for legal reasons. Ideally, surrogate species should have equal or greater sensitivity to the potential stressor than the species they represent in the ERA and thus knowledge of the effects on these species provides reliable predictions about effects on many other species (Raybould et al., 2011).

If the lower-Tier studies indicate that the activity of the transgene products is not limited to the target species, but also affects other valued taxa, then the temporal and spatial exposure to the potential stressor under field conditions is characterised for the sensitive taxa. Depending on the level of exposure of the endangered species and worst-case assumptions on the sensitivity level of the endangered species to the transgene products, the risk is determined and, if needed, particular mitigation measures are proposed.

5.2.2.2. Scenario 1b: Potentially altered interactions between the GM plant and associated fauna

NTOs might also be adversely affected by unintended changes in the GM plant (Arpaia, 2010). Unintended effects of the genetic modification are considered to be consistent differences between the GM plants and its appropriate comparator, which go beyond the primary intended effect(s) of introducing the transgene(s) (EFSA GMO Panel, 2010). The presence of unintended effects in GM plants can be due to different reasons (e.g. insertional, pleiotropic effects). Unintended adverse effects of GM plants on endangered species are only restricted to endangered herbivores feeding directly on the plants and their associated natural enemies.

For the assessment of unintended effects, EFSA proposes a weight-of-evidence approach based on (1) data generated during the comparative analysis of the GM plant with its appropriately selected comparator at molecular, compositional, and agronomic/phenotypic levels; and (2) on data from *in planta* (field and laboratory) studies with NTOs. In these studies, one focal species of each relevant functional group needs to be tested (EFSA GMO Panel, 2010).

5.2.2.3. Scenario 2: Altered persistence and invasiveness of GM plant

The possibility that GM plants might invade non-agricultural habitats has been acknowledged (Keeler, 1989; Crawley et al., 1993). More recently the prospect of GM plants with abiotic stress tolerance has reignited interest in the potential for such plants to become persistent or invasive (e.g. Nickson 2008;

Wilkinson and Tepfer 2009). Enhanced fitness[17] of GM feral plants in semi-natural or natural habitats may reduce the diversity or abundance of endangered fauna and flora. For example, native plant species may be displaced, which in turn may affect species that use those plants as food or shelter (EFSA GMO Panel, 2010).

Problem formulation focuses on the potential of a GM plant to be more persistent or invasive than its conventional counterpart. To assess potential for persistence (weediness) and invasiveness, general agronomic and phenotypic characteristics are measured in multi-location agronomic field trials representative of the different environments where the GM plant may be grown, and compared with those of its comparator and non-GM reference varieties.

5.2.2.4. Scenario 3: Introgressive hybridisation potential

Vertical gene flow and introgressive hybridisation between the GM plant and cross-compatible wild relatives may adversely affect endangered species. Depending on which plant and which transgenes are involved, vertical gene flow to wild relatives may decrease the fitness of hybrid offspring. If rates of gene flow are high, this may cause wild relatives to decline locally or to become extinct (e.g. swarm effect, outbreeding depression) (Ellstrand, 2003). An increased fitness in vertical gene flow recipients might enable them to become more invasive in semi-natural and natural areas, as a result of which endangered plants may be displaced and associated fauna may be adversely affected.

Owing to ecological and genetic barriers, not all relatives of GM plants share the same potential for hybridisation and transgene introgression (Jenczewski et al., 2003; Chèvre et al., 2004; FitzJohn et al., 2007; Wilkinson and Ford, 2007; Devos et al., 2009; Jørgensen et al., 2009). For transgene introgression to occur, both species must occur in their respective distribution range of viable pollen. This requires at least partial overlap in flowering in time and space, and sharing of common pollinators (if insect-pollinated). Sufficient level of genetic and structural relatedness between the genomes of both species also is needed to produce viable and fertile oilseed rape × wild relative hybrids that stably express the transgene. Genes, subsequently, must be transmitted through successive backcross generations or selfing, so that the transgene becomes stabilised into the genome of the recipient.

The ERA of gene flow for endangered species should consider the likelihood of hybridisation and subsequent introgression with wild relatives and the occurrence of endangered wild relatives or endangered plant or animal species associated with the wild relative in areas where harm could occur.

Of all crops for which GM plant applications have been submitted for cultivation and/or import and processing in EU [mostly maize, followed by cotton and soybean, and, to a lesser extent, oilseed rape, potato, sugar beet, and rice], only oilseed rape and sugar beet have wild relatives occurring in Europe. Oilseed rape hybridises with several wild relatives in the *Brassica* family (FitzJohn et al., 2007) but none of the cross-compatible species are listed in the European Red List of Vascular Plants (Bilz et al., 2011). Sugar beet hybridises with several wild relatives in the *Beta* family (Bartsch, 2010). Some of these wild relative species (such as *B. nana*, *B. patula* and *B. webbiana*) are rare, endangered and red listed as they only survive in a few restricted areas. These wild relatives mostly occur in upland or coastal areas, which are usually remote from sugar beet cultivation and therefore they are unlikely to be exposed to pollen from cultivated beet.

[17] Enhanced fitness can be defined as a characteristic of an individual or subpopulation of individuals that consistently contribute more offspring to the subsequent generation (Wilkinson and Tepfer, 2009).

5.2.2.5. Scenario 4: Altered farm management practices

Endangered species might be indirectly harmed when the introduction of a GM plant requires specific management practices and cultivation techniques that lead to changes in farm management and production systems. These changes could alter food resources, foraging, and/or nesting habitats (e.g. host plants) of endangered species. Examples of GM plants than can cause significant changes in production systems are genetically modified herbicide-tolerant (GM HT) plants, which change herbicide regimes and facilitate the adoption of minimum tillage or no-till cultivation techniques, genetically modified insect-resistant (GM IR) plants, which reduce the use of some insecticides and require establishment of non-IR refuges with specific cultivation techniques, and GM drought-tolerant plants, which change irrigation regimes (EFSA GMO Panel, 2010).

5.2.3. Conclusion on the coverage of endangered species in the GM plant ERA scheme

ERA of endangered species follows the principles outlined in the EFSA guidelines on the ERA for GM plants and GM animals (EFSA GMO Panel, 2010, 2013, respectively). Endangered species are regarded as entities of concern that need to be protected, and are explicitly considered in the problem formulation phase of ERA. As a first step, plausible pathways to harm of endangered species need to be identified. Risk hypotheses derived from those pathways are then tested with existing data or with new studies that aim to characterise the risk as a function of hazard of and temporal and spatial exposure to the potential stressor under field conditions. Given the protected status of endangered species, conservative assumptions representing worst-case conditions, including local extinction risks, might be required. Specific recommendations are given in Section 8.7.2.

5.3. Coverage in ERA of invasive alien species

5.3.1. Overview ERA of IAS

The assessment of the risk that invasive alien species (IAS) pose to the environment is part of the Pest Risk Assessment (PRA).The PRA includes the pest identification or characterisation, the analysis of the entry, establishment and spread, the assessment of the impact on cultivated and managed plants and on the environment, and finally, the evaluation of the risk reduction options.

Assessment can be done for areas already invaded and where the species is established (retrospective ERA). The main interest of the PRA, however, is to perform prospective assessment of the risk posed by an IAS, for both the cultivated plants and the wider environment. The objective of prospective assessment is to define the magnitude of the impact and its probability of occurrence (risk assessment). This information can be considered by risk managers for possible implementation of risk reduction options.

As there is no "owner" of an invasive species that can be held responsible to produce the necessary data, the data on which the assessment is based comes from public sources or modelling. For the ERA of IAS the change in the ecological traits, in the ecosystem services provision level and in the state of biodiversity is assessed considering information available in the literature on the biology and ecology of the pest, the characteristics of the receiving environment and information of the environmental impact of the species, if available. The availability of studies on the impact in previously invaded areas can be important (but extrapolation requires some caution). Expert judgment is in many cases fundamental due to the lack of data and the complexity of the ecosystem responses to the perturbation caused by the invasive species. The collection of experts' judgment in Expert Knowledge Elicitation (EKE) procedure (EFSA, 2014b) provides probability distributions of the impact, which allows the joint estimation of the mean impact and the evaluation of the uncertainty of the estimation.

The assessment of uncertainty is an integral part of the risk assessment, and the evaluation of uncertainty is done for every stage of the PRA (see ISPM No. 11, 2004). For the ERA of IAS, two main sources of uncertainty are considered: one related to the data and modelling (including parameter estimation) projecting the potential distribution and abundance/prevalence of the pest, and one to the probability distributions provided by the expert involved in the EKE procedure for the assessment of the impact on ecosystem traits, ecosystem services and biodiversity components.

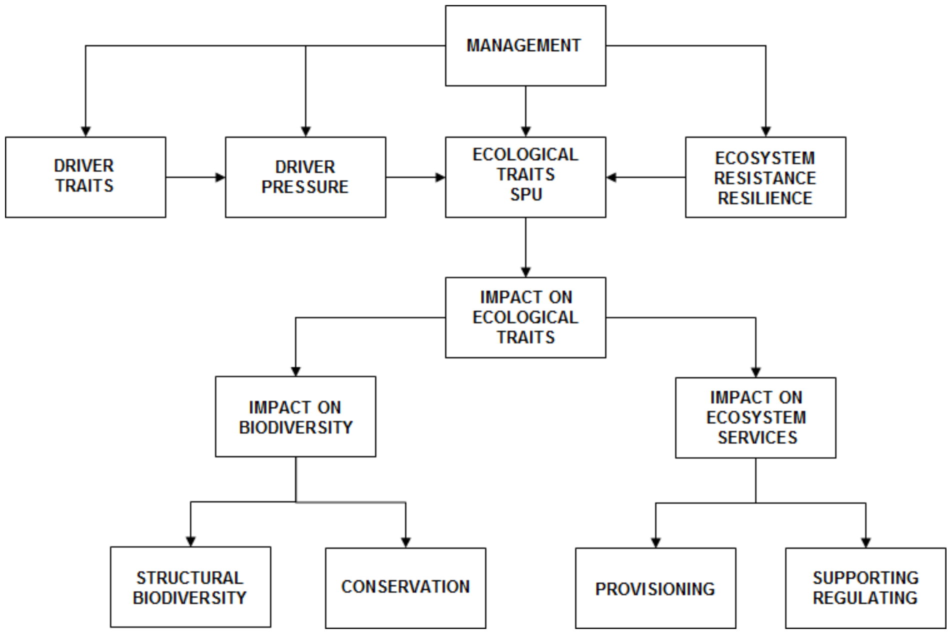
In modelling the potential distribution and abundance/prevalence of the IAS, uncertainty is taken into account by means of:

- Stochastic population dynamics models considering variability in biodemographic functions (e.g. the Kolmogorov model) or deterministic models able to simulate the effect of variability in biodemographic functions (e.g. von Foerster model);
- The use of confidence bands of the biodemographic functions to estimate the consequences of biological variability and uncertainty on the pest spatio-temporal population dynamics;
- The use of perturbation methods to explore the consequences of variability and uncertainty in environmental driving forcing variables on the pest spatio-temporal population dynamics.

In the EKE procedure, different experts' evaluations are combined to obtain a single probability distribution (mixture distribution). The probability distribution allows deriving a measure of the risk by assigning to each class of rating a numerical value. Then, a measure of the uncertainty in the estimated risk can be obtained using the Shannon entropy (Shannon, 1948).

The methodological approach developed by the Plant Health Panel for IAS is aimed at the standardisation of the assessment of impacts of these species on the environment and thus assists in making the assessment transparent and consistent (EFSA PLH Panel, 2011 and 2014; Gilioli et al., 2014). The impact can be assessed at different levels: at the level of ecological traits or at the more integrative levels of biodiversity and ecosystem services. While the consideration of biodiversity is to account for a nonutilitarian perspective (Callicott et al., 1999), which gives importance to the value of nature and to the conservation-related issues, the consideration of the ecosystem services gives major importance to a functionalist perspective (Callicott et al., 1999) that focuses on the contribution of functional biodiversity in defining how systems cope with IAS as drivers of ecosystem change, and how IAS can drive ecosystem functions (and services) to a less desirable state (Gilioli et al., 2014).

The risk that IAS pose to the environment is assessed at the end of a process (Figure 4) which includes a scenario analysis and the consideration of the impact on ecological traits at individual, population, community and ecosystem levels. Once the relevant traits are acknowledged for the environmental components directly or indirectly affected by the IAS, their connections with biodiversity and ecosystem services are identified, via the selection of the main traits–services clusters (De Bello et al., 2010) and traits–biodiversity clusters (EFSA PLH Panel, 2014).



Legend: SPU= service-providing unit

Figure 4: The PLH approach to assessing the effect of IAS on ecosystem services (From: EFSA PLH Panel, 2014).

In more detail, the approach is based on a framework that considers the population abundance of a pest or the prevalence of a disease as the driver of ecosystem change. The assessment is based on a scenario analysis organised as follows:
Identification of the service-providing unit. The impact of the pest is related to the environmental components or units responsible for the genesis and regulation of the ecosystem services, the so-called service-providing units (SPU), being specific for every organism (Luck et al., 2003). The structural and functional characteristics of the SPU represent the state of the system before the perturbation and allow defining the constraints and possibilities of ecosystem change under a perturbed regime (the presence of the driver);
Scale of the analysis. The scenario requires the definition of the spatial and the temporal scale (in its components of extent and resolution or grain) at which the assessment is performed. Also the pest abundance/prevalence scale has to be set. Spatial, temporal and abundance/prevalence scales are linked and their definition depends on the objectives of the assessment and the information on the state of the receiving ecosystem as well as assumptions on future trends;
Population pressure. The potential distribution of the pest abundance/prevalence in relation to the distribution of the pest's potential hosts and habitats represents the pressure of the driving force. The population pressure modifies ecosystem structure and ecological traits with effect on the ecosystem services level of provision and the state of biodiversity components;
State and reactions of the receiving ecosystem. Assumptions being made include the features that can modify or mitigate the degree of change in individual, population, community and ecosystem functional traits due to the pest population pressure. The change of the population pressure over time depends on the resistance and resilience of the receiving ecosystem, as well as of the effect of the management on the pest populations.
In the final step, the impact of the IAS is assessed in relation to the driver pressure and the state and the reactions (dynamics) of the receiving ecosystem and performed for the selected spatial, temporal and abundance/prevalence scales. Risk assessment can be performed at different levels.

The basic level is the assessment of the impact on the ecological traits. In the case of the environmental risk assessment of *Pomacea* spp. performed by the EFSA Plant Health Panel, the impacts of apple snails on the traits related to macrophytes, water quality and biodiversity were considered (EFSA PLH Panel, 2014). The ecological traits considered in the assessment are listed in Figure 5.

Traits assessed for impact relationship with snail biomass		
Traits related to the macrophytes	**Traits related to water quality**	**Traits related to biodiversity**
Edible macrophyte biomass	Oxygen concentration	Aquatic invertebrates biodiversity
Biomass of non-edible macrophytes	Phosphorous concentration	Amphibian biodiversity
Dominance (macrophytes/ phytoplankton	Sedimentation rate	Fish biodiversity
Macrophyte species diversity	pH (percentage of variation)	Bird biodiversity
Structural complexity of the habitat	Denitrification	Zooplankton biodiversity
		Zooplankton biomass
		Periphyton biomass

Figure 5: The ecological traits considered in environmental risk assessment of apple snail for the EU (from EFSA PLH Panel, 2014)

The more integrative levels of assessment consider the impact on the provisioning and regulating-supporting ecosystem services, and/or biodiversity components. In the environmental risk assessment of *Pomacea* spp. performed by the EFSA Plant Health Panel, the impacts on the ecosystem services and biodiversity components listed in Figure 6 were considered (EFSA PLH Panel, 2014).

Ecosystem services assessed for impact of snail invasion		Biodiversity components assessed for impact of snail invasion
Provisioning services	**Regulating and supporting services**	
Food	Climate regulation	Genetic diversity
Genetic resources	Water regulation/cycling /purification	Native species diversity
Fresh water	Erosion regulation	Native habitats, communities and/or ecosystems diversity
	Nutrient cycling	Threatened species
	Photosynthesis and primary production	Habitats or other ecological entities of high conservation value
	Pest and disease regulation	
	Pollination	

Figure 6: The ecological provisioning and regulating ecosystem services and biodiversity component assessed for the impact of apple snail invasion for the EU (from EFSA PLH Panel, 2014)

5.3.2. Coverage of endangered species in ERA of IAS

For the PLH ERA approach, the impact of IAS on endangered species is considered in the phase of the assessment of impacts on the biodiversity components. In the list of questions proposed in the Guidance on the environmental risk assessment of IAS (EFSA PLH Panel, 2011 p. 53), this corresponds to question 2.2.: “To what extent are there any rare or vulnerable species among the native species expected to be affected as a result of invasion?” In the Guidance, there is a generic reference to a prepared list of conservation values (protected individuals, groups of individuals, landscapes, habitats, ecosystems) in the risk assessment area. The rating is based on three qualitative levels (minor, moderate, major) associated with explanations and examples guiding the assessment.

In the application of the scheme proposed in the ERA for the apple snail, *Pomacea* spp. (EFSA PLH Panel, 2014) endangered species are included in the “Conservation component of biodiversity” under the definition of “Threatened species”. This snail was accidentally introduced in the Ebro Delta in Spain and is now invading the rice fields and the adjacent wetlands, threatening different plant and animal species. The scheme proposed in the *Pomacea* opinion is more flexible than the one in the Guidance and includes an assessment procedure allowing more specific investigations on the consequences of the establishment and spread of invasive alien species on endangered species. This is likely to be used in the future. The ERA is triggered as a task from the EC or a self-task.

In the case-study of the apple snail, interpretations are provided of how the snail biomass can affect the components of biodiversity. Reductions in macrophyte species richness and macrophyte abundance due to the apple snail feeding activity negatively affect all resident and transient organisms that depend on macrophytes at any life stage The macrophytes maintain biodiversity by providing varied and structurally complex habitats for macroinvertebrates, zooplankton and juvenile fish (Diehl, 1988; 1992; Persson and Crowder, 1998) and serve as food or the substrate for food (periphyton) consumed by macroinvertebrates (James et al., 2000), fish and waterfowl (Lodge et al., 1998).

The EFSA-PLH ERA scheme proposed in the opinion on the apple snail (EFSA PLH Panel, 2014) included the possibility to focus on specific taxa or groups of organisms. In the opinion, the impacts on three non-systematic categories of organisms, aquatic invertebrates, zooplancton periphyton, and three classes of vertebrates, amphibians, fish, birds are considered. Even if not specifically addressed in this case-study, the assessment can also be conducted at the level of specific endangered species. This possibility opens the question which endangered species have to be considered in ERA of PLH. Given that the IAS act primarily through indirect effects it is believed that the understanding of the relationships established between IAS and the whole recipient community is considered key. So it is important to see how the perturbation propagates in the ecosystem, and how endangered species or other taxa are threatened due to their functional relationship with the driver (IAS).

5.3.3. Conclusion on the coverage of endangered species in the IAS ERA scheme

In the risk assessment of IAS (EFSA PLH Panel, 2011), effects on endangered species are an essential part of the PRA procedure. In the proposed risk assessment approach, one central question to be answered is to what extent rare or vulnerable species (defined as all species classified as rare, vulnerable or endangered in official national or regional lists within the risk assessment area) are expected to be affected as a result of invasion. Once the relevant traits are acknowledged for the environmental components directly or indirectly affected by the IAS, their connections with biodiversity and ecosystem services are identified, via the selection of the main traits–services clusters (De Bello et al., 2010) and traits–biodiversity clusters (EFSA PLH Panel, 2014). One peculiarity of the ERA for plant pests is that the PLH Panel does not use thresholds for impact assessment. The PLH Panel aims at defining the impact on ecosystem services and biodiversity component, including endangered species. In most cases a prospective assessment is performed and the objective is to define the magnitude of the impact and its probability of occurrence (risk assessment). This information can be considered by risk managers for possible implementation of risk reduction options.

5.4. Coverage in ERA of feed additives

The five categories of Feed additives are defined in Article 6 of Commission Regulation (EC) No 1831/2003 as follows: (i) Technological (preservatives, antioxidants, emulsifier, thickners, stabiliser, gelling agents, binders, radionuclide control, anticacking agents, acidity regulators, silage additives, denaturants), (ii) Sensory (colourants and flavourings), (iii) Nutritional (vitamin, trace elements, aminoacids, urea), (IV) Zootechnical (digestibility enhancers, gut flora stabilisers, favourably affecting the environment, other zootechnical additives), (v) Coccidiostats and Histomonostats.

5.4.1. Overview ERA of FAs

The procedure used for ERA of FAs is analogous to those deployed by ECHA (EUR 20418 EN/2) and EMA (Eudralex 7AR1a) for ERA of industrial chemicals and veterinary medicines, respectively. In accordance with Commission Directive 2001/79/EC and Commission Regulation (EC) 429/2008, the FEEDAP Panel primarily looks at effects in three compartments: soil, freshwater, and sediment under sea cages of fish farms. So, the organisms of focus will in theory be the most sensitive species in either of these compartments, depending on application of the feed additive, although surrogate species in standardised tests are used to establish No Observed Effect Concentrations (NOEC). A problem with assessing safety of feed additives to the environment is that often the substances are not perceived as particularly toxic, at least not to mammals, as they are used in animal feeds and sometimes also in human food. However, use levels can be very high and in some cases other groups of animals can be expected to be much more sensitive than mammals and, therefore, the risk to the environment cannot be ignored. In some cases, such as flavourings, the Panel is asked to assess the safety to the environment for entire groups of chemically defined compounds and these groups sometimes contain well in excess of 100 chemicals. In short, the ERA of feed additives can be a lot cruder than that used by for example for PPPs and focusses more on Predicted Environmental Concentration (PEC) than on the effect side.

Consideration of the environmental impact of additives is however important since administration of additives typically occurs over long periods, often involves large groups of animals and the constitutive active substance(s) may be excreted to a considerable extent either as the parent compound or its metabolites. The approach taken by the FEEDAP Panel reflects in particular: (i) the common practice in which manure is stored and spread in Europe and the way feed additives leach to groundwater and drain or runoff from grassland arable land to surface water; (ii) the different European fish production systems including ponds, tanks and sea cages.

To determine the environmental impact of additives, a stepwise approach is followed. All additives have to be assessed through Phase I (see Figure 7) to determine if an environmental effect of the additive is plausible and whether a more detailed Phase II (see Figure 8) assessment is necessary. Exemption from Phase II assessment may be made on one of two criteria, unless there is scientifically-based evidence for concern (EC, 2008). The two criteria for exemption relate to the chemical nature, biological effect and conditions of use of the additive. Exemptions apply where the impact is assumed to be negligible, i.e. where the additive is:

- a physiological or natural substance (e.g. vitamins, amino acids, carotenoids) that will not result in a substantial increase of the concentration in the environment, considering the intrinsic toxicity of the excreted substance(s). It is important to note that even if a chemical is naturally occurring in the European environment an increase in its concentration may cause toxicity to biota (such as seen for Zn and Cu).

- intended for non food-producing animals only (e.g. pets[18]), because manure originating from these animals are typically not gathered in a systematic way to be spread on fields by farmers.

Phase I

For the ERA related to the terrestrial target animals, the FEEDAP Panel recommends a tiered approach (Figure 7) starting with screening models as a worst case (assuming that 100% of the dose ingested is excreted as the parent compound) to derive the Predicted Environmental Concentrations (PECs) in soil, groundwater and surface water, and (aquatic) sediment. If the worst-case PEC falls below pre-set trigger values (soil < 10 µg/kg; groundwater < 0.1 µg/L; surface water < 0.1 µg/kg; sediment 10 µg/kg) the additive is considered to be of no risk to the environment and no further assessment is required. These trigger values were selected based on known ecotoxicity of chemicals used as feed additives at the time of publication of the FEEDAP Opinion (EFSA, 2007). The trigger values for PEC in soil and groundwater are also the same as those used by EMA for veterinary medicines.

When a risk cannot be excluded based on the exposure screening models (e.g. concentration of the substance in the environment might be increased above background or a threshold of concern), PECs can be refined either on the basis of degradation, metabolism data, dilution during the withdrawal period and/or by using more sophisticated models. For the PEC refinement of groundwater and surface water, the FEEDAP Panel proposes the use of the FOCUS models developed initially for the exposure assessment of PPP, but have been tailored for feed additives guaranteeing that the exposure assessments are standardised.

For the ERA related to the aquatic target animals, the guidance (EFSA FEEDAP Panel, 2008) describes two different exposure models to take account of the difference between aquaculture operations in open sea (cages) and inland facilities (ponds, tanks or raceway systems). For sea cages, it is assumed that organisms living on or in the sediment in the deposition zone underneath an aquaculture operation are at greatest risk. The reason for this assumption is that fish cages are always located in water with sufficient current for provision of oxygen to the fish and removal of ammonia, resulting in rapid dilution of any dissolved substance in feed and excreta. For this reason, it is proposed to focus the risk assessment for such systems on the sediment compartment. For raceway, pond, tanks and recirculation systems, it is proposed to focus the environmental risk assessment on the water phase only because the effluent from land based aquaculture systems is running through a sedimentation tank before release into the environment. Refinement of the PECs can be based on information on metabolism and/or degradation.

[18] See the guidance document on the assessment of additives intended to be used in pets and other non food-producing animals (EFSA FEEAP Panel, 2011): Pets and other non food-producing animals are defined as 'animals belonging to species normally nourished, bred or kept, but not consumed by humans, except horses' (Article 1.1 or Regulation (EC) No 429/2008).

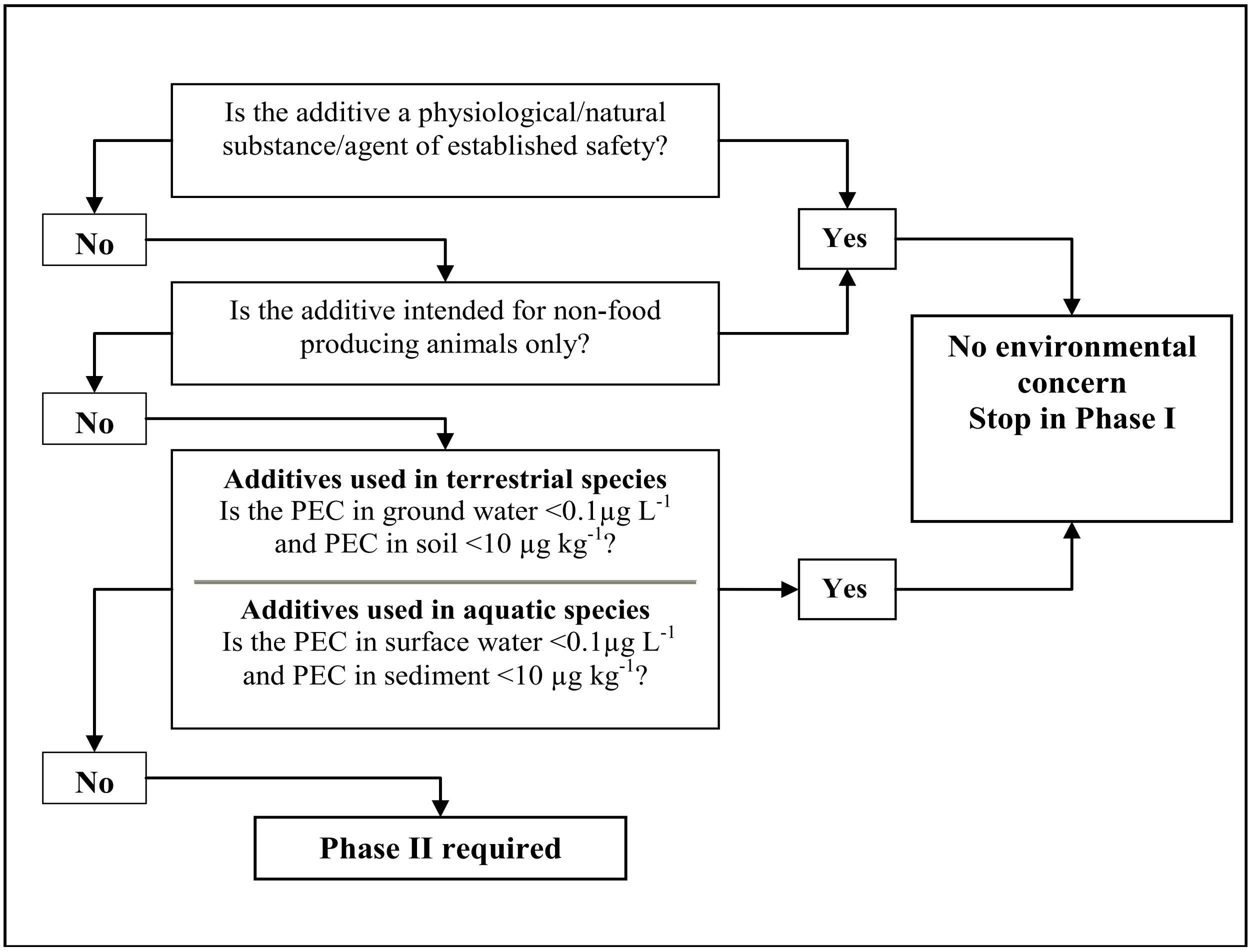


Figure 7: Phase I decision tree for the environmental risk assessment

Phase II

The aim of Phase II is to assess the potential for additives to affect non-target species in the environment, including both aquatic and terrestrial species or to reach groundwater at unacceptable levels. It is not practical to evaluate the effects of additives on every species in the environment that may be exposed to the additive following its administration to the target species. The taxonomic levels tested are intended to serve as surrogates or indicators for the range of species present in the environment. For example, earthworms could be used to represent soil invertebrates, chlorella for aquatic plants, and rainbow trout for aquatic vertebrates.

The Phase II assessment is based on a risk quotient approach, where the calculated PEC and Predicted No Effect Concentration (PNEC) values for each compartment is compared (Figure 8). The PNEC is determined from experimentally determined endpoints divided by an appropriate assessment (safety) factor. The more data are available, the lower is the assessment factor applied. The PNEC value is calculated for each compartment of concern.

The Phase II assessment is based on a tiered approach (Figure 8).

The first Tier, Phase IIA, makes use of a limited number of fate and effect studies to produce a conservative assessment of risk based on exposure and effects in the environmental compartment of concern.

To start, a comparison should be made between the initial PEC and the PNEC (the initial PEC should also consider the potential accumulation in soil):

- If the ratio of the PEC to the PNEC is lower than 1 no further assessment is required, unless bioaccumulation is expected;

- If the PEC/PNEC is > 1, a more refined PEC can be calculated based on data not considered in Phase I.

If the refined PEC/PNEC ratio predicts an unacceptable risk (ratio > 1), a Phase IIB to refine the environmental risk assessment is necessary.

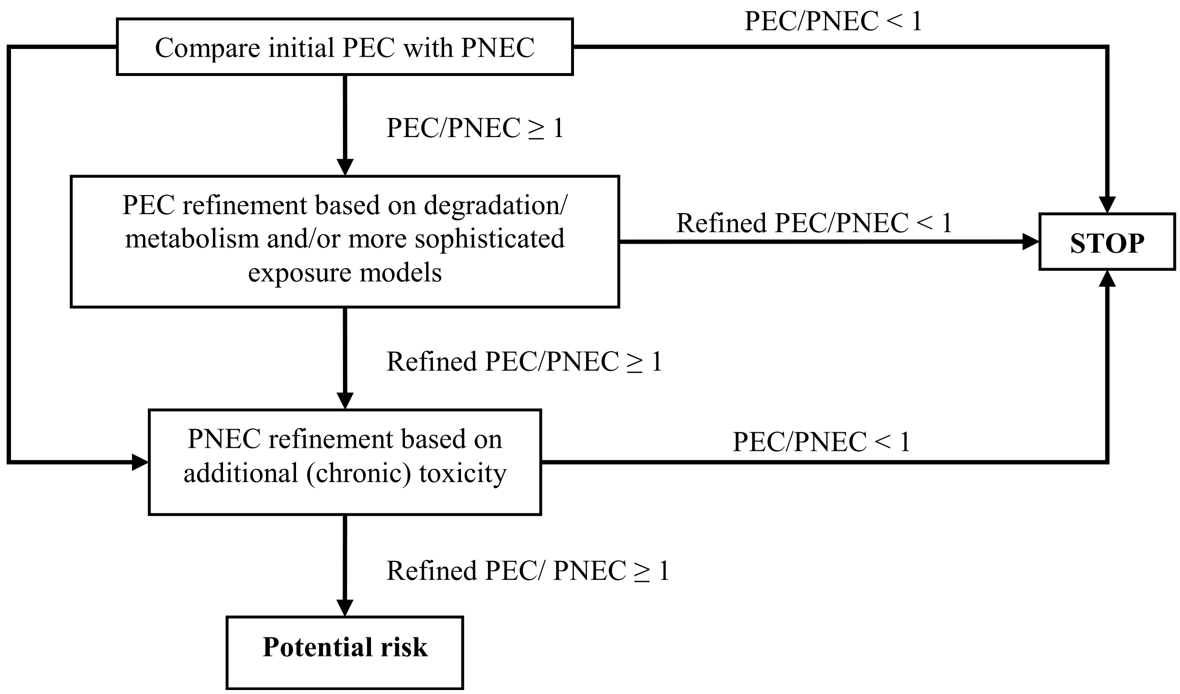


Figure 8: Phase II decision tree for the environmental risk assessment of soil and aquatic compartment for terrestrial animals

5.4.2. Coverage of endangered species in ERA of FAs

The ERA approach used by the FEEDAP Panel aims to protect all species, including those that are endangered. In many cases this is achieved by establishing that the feed additive under assessment is either a naturally occurring product and that its use in animal feeds will not raise its concentration in the environment, or by calculated prediction that its concentration will not exceed set thresholds of concern (Soil and Sediments < 10 µg/kg; Water Compartments < 0.1 µg/L). It is the assumption that residues of feed additives used in agri- or aquaculture will have no harmful effects on the environment at concentrations below these thresholds. These threshold concentrations were set to exceed those shown to have adverse effects in studies with substances used as feed additives. In cases where these criteria are not satisfied the risk assessment is based on the ratio between PEC and PNEC being < 1. Here, the PEC is calculated with a conservatism dictated by available data, always assuming worst-case scenario. The PNEC is derived from toxicity data using relevant species with appropriate assessment factors depending on data richness (L(E)C50 from each of three trophic levels: 1,000; Species sensitivity distribution data: 1 – 5). As this assessment approach is designed to protect all species, endangered species are implicitly included and are assumed to be protected. A major uncertainty in the FEEDAP ERA is the appropriateness of the assessment factors. Although the ERA is assumed to be based on conservative extrapolations, there is a lack of knowledge that would allow to conclude if endangered species as a group are more sensitive than other species to the environmental effects of feed additives.

It may be argued that endangered species are mainly animals and plants which need specific ecological conditions to survive. For example plants adapted to low nutrient conditions and animals that require very clean water, a large habitat or specific food. Whilst the maximum legal amount of nitrogen that can be applied to a field is outside of EFSA's remit, it is used by FEEDAP to calculate the amount of a feed additive that may be spread on a field through manure. FEEDAP does not cover the eutrophication of sensitive aquatic and terrestrial ecosystems since it is assumed that manure is applied on agricultural fields only. The eutrophication caused by sea cages is also not taken into account although this is likely to have a greater impact on flora and fauna in the sediment underneath a mariculture installation than that caused by feed additives.

Coccidiostats and histomonostats are active against protozoa and this is the only category of feed additives used that are designed to control eukaryotic organisms. There are currently no feed additives on the European market that are used to control taxa, which include endangered species.

5.4.3. *Conclusion on the coverage of endangered species in the FAs ERA scheme*

The technical Guidance for assessing the safety of feed additives for the environment (EFSA FEEDAP Panel, 2008) does not mention endangered species in the protection goals or elsewhere. However, the FEEDAP ERA does not tolerate effects on any species in the environment and, thus, endangered species are implicitly included and are assumed to be protected[19]. The threshold values used in Phase I should be validated against currently available ecotoxicity datasets for chemicals used as feed additives. Considering the nature of feed additives and the very large number of products in need of assessment, it is difficult to envision an alternative approach that would further ensure the safety for endangered species from additives in animal feeds.

[19] The information available does not allow the EFSA Scientific Committee to provide an evidence based assessment of the level of protection actually provided by the default factors recommended for the PNEC derivation. In addition, on a case-by-case basis, the recovery option, e.g. as described in the EFSA PPR guidance on aquatic organisms (EFSA PPR Panel, 2013), might be explored and consider applicable. In those cases, the protection of endangered species might require special attention

5.5. Conclusion for ToR2

There are four types of potential stressors undergoing ERA within EFSA's remit and (mainly) in an agricultural context: PPPs, GMOs, IAS and FAs.

For GMOs and IAS, the protection of endangered species is explicitly dealt with during the problem formulation phase in the respective ERA schemes. These ERA schemes allow a tailor made-assessment and the selection of one or more relevant endangered species.

For PPPs, the PPR Panel adopted an approach to species selection for prospective risk assessment of an individually assessed potential stressor using (or leaving the option for) the concept of vulnerable species. Only in a few cases, specific groups of endangered species are explicitly mentioned in the guidance documents on the ERA for PPPs, such as rare plants and amphibian larval stages. Endangered species are assumed to be partly covered by the vulnerable species approach. While the vulnerable species concept takes account of exposure, sensitivity and/or recoverability, then it does not usually consider that the conservational state of a species can already be unfavourable. Furthermore, examples in this opinion demonstrate that while part of the endangered species are covered by this approach (for instance fish and aquatic amphibians), others may not be (see the reasons set out in Section 4).

For FAs, the ERA does not tolerate population effects on any species in the environment and, thus, endangered species are implicitly included by the assumption that no FA is allowed on the market should a species be at risk.

Thus, it currently varies among EFSA ERA schemes to which degree (implicit or explicit) endangered species are covered and how they are covered.

The level of protection afforded by these four ERA schemes for endangered species seems to vary. However, the limited data availability does not allow to draw a firm conclusion and also does not allow an assessment of the level of protection achieved (regardless whether endangered species are implicitly or explicitly covered).

Hence, risk assessment is conducted via selected (test) species, with assessment factors and extrapolations to endangered species (bottom-up approach). There is however a growing need for a landscape assessment (per potential stressor) plus population modelling (top down approach), which is discussed herein in Section 8.5.

6. PREVENTIVE RISK MITIGATION MEASURES PROPOSED AT THE FINAL STEP OF ERA

The third question of the ToR asks, in case the current ERA schemes would not adequately cover endangered species, "*what risk mitigation measures can be envisaged to prevent endangered species being put at risk from stressors resulting from the application of a regulated product?*"

As described in Section 5, the possibility exists that endangered species may not always be adequately covered in prospective ERA schemes, mainly due to many unknowns about endangered species and the absence of data[20].

"Risk mitigation measures" mean technical or practical provisions that can help to reduce risk from a (regulated, potential) stressor, its intended use or its estimated spread in the environment. Risk mitigation measures can be proposed by risk assessors at the end of the ERA process to inform the risk managers. The following Sections describe improving conditions for endangered species as well as stressor-related measures that are considered of importance to help protect endangered species.

6.1. Improving conditions generally supporting endangered species in an agricultural context

A recent study by Schneider et al. (2014), demonstrates that landscape properties are very decisive for farmland biodiversity. Diversity in the type of habitats and their structure at the farm scale are more decisive for biodiversity than the distinction between organic and conventional farming (Dauber et al., 2003; Woodcock et al., 2005; Diekötter et al., 2010).

Habitat creation, aiming at increasing local and landscape scale biodiversity and service provision[21] in a typical Dutch agricultural landscape, was envisaged through increasing the network of semi-natural elements in the landscape (ECORYS for The Netherlands Ministry of VROM, 2007). "Green-blue veins" is a network of vegetation (green) and water (blue) elements in the landscape. Two types of non-crop elements were considered: the robust elements (25m wide like dikes, creek banks small wood slots) and the fine elements (minimum of 3m wide like field margins, road verges and ditch banks). Robust elements compose the core of the network, and were supported by fine elements. One of the factors for successful maintaining biodiversity of different groups of invertebrates is the density of semi-natural elements in the landscape (e.g. Billeter et al., 2008). However not only the area occupied is important, but also the spatial arrangement and management techniques adopted for these landscape elements. One important contribution of this "Green-blue veins" project is that it developed both quantitative (density of elements in the landscape) and qualitative (e.g. plant species composition and management techniques) standards for both types of elements to optimise their role as habitat providers for biodiversity. Indication of the type of species to plant, but also the frequency and area of mowing is given. This originates higher habitat diversity not only in space but also in time, which is crucial to maintain viable populations of many species, including rare arthropod species. For finer elements, the question of spatial arrangement plays an important role. For them to be most effective the distance between them should not be large (high proximity). The reason has to do with the dispersal ability of many groups of insects (10s of meters) into crop areas. In this context the project gave also indication on the optimal size of a crop depending on the density and proximity of surrounding semi-natural elements. On larger fields (large crop areas) this means

[20] Section 8 herein provides suggestions for improvement of the current ERA schemes and as such constitutes a preventive measure prior to ERA.

[21] This project was primarily concerned with the regulatory services of pest control and pollination. The aim was that the entire network acts as habitat from which insects could disperse into in-field areas with the robust elements also acting as sources for passive dispersal over large distances. The working group considers this initiative also important to support endangered species, as one of the cultural ecosystem services.

that these finer elements may exist not only on the margins of the fields, but also inside them (in-crop vegetated zone). Proximity between semi-natural habitats was also mentioned as a key factor explaining diversity of many arthropod groups in agricultural landscapes (e.g. Hendrix et al., 2007).

The above type of mitigation will work only if the potential stressor does not impair the viability of the endangered species. These mitigation measures do not mitigate the adverse effects of potential stressors. They merely optimise the living conditions for endangered species, provided that they “survive” the potential stressors.

As said above, EFSA risk assessments may conclude that vulnerable species (possibly including endangered species) are at risk from the potential stressor under assessment and that risk mitigation measures related to this potential stressor are needed. However, risk management measures only related to one single stressor might not be enough to offer the required level of protection. This is because vulnerable species (possibly including endangered species) are likely to be influenced by various variables other than the potential stressor, such as availability of food and habitats. It is however not in the remit of EFSA to advise on issues beyond its mandate on potential stressors. Actions (with respect to habitats, may be possible to Member States under different EU legislations (e.g. the Habitat Directive), under which guidance is available, for instance regarding how to support farming in Natura 2000 areas (EC, 2014).

6.2. Measures to avoid co-occurrence of endangered species with the potential stressor

A first strategy for risk mitigation to prevent endangered species being put at risk from the use or spread of an assessed potential stressor, is to reduce the spatial and temporal exposure of the endangered species to the potential stressor. This can be achieved through the use of spatial isolation distances or special in-field measures.

> For example, it is standard practice under GM Plant ERA to advise on refuge areas for mitigating potential risk by ensuring sufficient untreated zones. However, if species of conservation concern are identified in neighbouring areas, isolation distances or even restriction of cultivation might be recommended. Also in-field mitigation measures aim to limit temporal exposure to the potential stressor. To reduce exposure to NTOs, possibly including endangered species, risk mitigation might include the planting of non-GM plants as border rows (EFSA GMO Panel, 2009) or, where feasible, detasseling of GM maize plants in border rows in order to limit Bt maize pollen dispersal outside of the field (EFSA GMO Panel, 2010).
>
> For PPP, the provision of buffer strips is a common in-field mitigation measure. Buffer strips may have the crop plant or not, but it is not sprayed (although it might be influenced by the farmer’s activity, e.g. by spray drift). EFSA is advising in its prospective ERA on the width of the buffer strip necessary to reduce the risk at the edge of the field to an acceptable level. In addition, regardless whether a risk was determined or not in ERA, crop-free buffer strips (e.g. connected to borders of any water courses in the Netherlands) may be required. Off-field risk mitigation measures include nature reserve management. A further example would be to avoid co-occurrence of amphibians on fields during movement between water bodies and summer habitat and the application of PPP with high amphibian toxicity (see Brühl et al., 2011; Lenhardt et al., 2014). This could be part of management plans in conservation areas, those which were set up for protection of amphibians in particular.

The prevention of co-occurrence between the potential stressor and the endangered species, however, does not necessarily prevent the occurrence of adverse effects, i.e. indirect effects cannot be excluded. The EFSA ERAs help to develop appropriate mitigation through the collection of information on hazard, exposure, spatial and temporal overlap between the *sphere of influence* of the potential stressor under assessment and the endangered species; and on the habitats hosting the endangered species.

The implementation of suitable risk mitigation measures by risk managers may be challenging when the occurrence of the endangered species is scattered across the landscape (Liess et al., 2010 for aquatic species). From a scientific point of view, the availability of refuge areas and off-field habitats is linked to *(and should be taken into account when)* deciding on the allowable use/spread of the potential stressor. For example, *encouraging (farmers) to support* a landscape structure having more traits that allow the required habitats to support a sustainable population influences positively the (*allowance of*) safe use/spread of the potential stressor or the flexibility of certain use restrictions. Population models may be used as a tool to evaluate the importance of landscape structure and the availability of refuge areas on population dynamics.
For the above two reasons, the SC underlines the need for both local and landscape scale risk assessments.

6.3. Measures linked specifically to the type of potential stressor

Below Sections describe mitigation measures as currently provided in the sectorial legal frameworks per potential stressor and/or in the corresponding ERA schemes. Their adequacy to also cover for endangered species is discussed. In General, to be effective to mitigate risk also for endangered species, the generic mitigation measures now used in the current authorisation schemes for potential stressors may be developed in a more site-specific manner, should be context-specific, should account for the biology of the endangered species and the reasons for its endangerment (EFSA Colloquium, 2014a).

6.3.1. For PPPs

Regulation (EC) No 1107/2009 (concerning the placing of PPPs on the market), while not mentioning endangered species explicitly, states that PPPs shall have no unacceptable effects on the environment with particular regard on non-target species, biodiversity and ecosystems. Likewise, the Directive 2009/128/EC (EC, 2009) (on establishing a framework for Community action to achieve the sustainable use of PPPs) does not specifically mention endangered species. However, Art. 12 (b) of this directive implies the minimisation of PPP use in protective areas, which may have derived their status from the occurrence of endangered species. This Directive also sets practical goals for sustainable use of PPPs (and in the future also biocides) from which also endangered species could profit. It stipulates the implementation of “National Action Plans aimed at setting quantitative objectives, targets, measures, timetables and indicators to reduce risks and impacts of PPP use on human health and the environment and at encouraging the development and introduction of integrated pest management (IPM) and of alternative approaches or techniques”. Main areas of action include the training of farmers and advisers, inspection of spraying devices (e.g. narrow nozzles to reduce spray drift), water protection by buffer zones, and promotion of and development of guidance on IPM.

Mitigating the Risk of Plant Protection Products in the Environment (MAgPIE) was discussed in a SETAC Workshop in 2013 (MAgPIE 2013, 2014), and communicated to the Standing Committee of the Food Chain and Animal Health (Section PPP) at the EC. The objective of this workshop was to help achieve a better harmonisation within Europe in the area of risk mitigation measures to the environment and related risk assessment and management. The expected outcome is to provide European regulatory authorities with a toolbox of risk mitigation measures designed for uses of PPP for agricultural purposes.

A variety of risk mitigation options for the in- and off-field risk from PPP use are described by the EFSA PPR Panel (2014a). For specific PPP with a likely use pattern that may threaten endangered species, it might be possible to specify “mitigation labelling” that encourages farmers or environmental managers to undertake more local ERAs to inform acceptable use in the local context. For the remit of EFSA, this suggests the development of rules for prospective risk assessments leading to recommendations on risk mitigation by PPP labelling saying “Recommendations for risk mitigation. If the intended use of this product is in the vicinity of an endangered species habitat, the following must be considered prior to use:

[insert specific issues]". In practice, this would require adoption of the idea by risk managers and guidelines, and a (local) map where the label requirements apply.

In addition, it is noted that country specific "restrictions" on the use of products are already in place. For instance, in the UK, there are statutory designations and associated management guidelines/prescriptions on local/regional scale provided for Sites/Areas of Special Scientific Interest (SSSIs/ASSIs), National Nature Reserves (NNRs), Local Nature Reserves (NRs), Areas of Outstanding Natural Beauty (AoNB), Environmentally Sensitive Areas (ESA), Special Areas of Conservation (SACs), and Special Protection Areas (SPAs). Here overarching guidance should not really be developed or set out to go against what is trying to be achieved in those sites and to biodiversity (Species and Habitat) Action Plans where certain management actions are favoured or not. Given that in such site specific and mainly conservation-targeted areas the adequate requirements for assessments and mitigation are likely to be entirely case (species or site) specific, it is possibly more efficient to encourage change through financial compensation based initiatives that focus on farmers' participation and information, on user training and integrated or voluntary reporting, than by imposing fixed regulatory risk assessments or risk management requirements. One example of such voluntary programme is in the UK (http://www.voluntaryinitiative.org.uk/en/home) to promote the responsible use of PPP. In its first year the Voluntary Initiative (VI)'s Integrated Pest Management Plan (IPMP) is set to cover more than two million hectares. The initiative's Chairman Richard Butler said: "In under a year, this is a tremendous performance, and shows yet again UK farmers preference for the voluntary approach and their willingness to follow best practice." (http://www.voluntaryinitiative.org.uk/ipmp). Thus, mitigation measures proposed upon ERA, for appropriate use of the potential stressor, whether proposed under EU-wide authorisation of regulated products or in response to local site specific concerns, should ideally be able to tie in with the site/species specific management plans.

Another example is the approach reported for the "Protection of biodiversity of free living birds and mammals in respect of the effects of PPP" on behalf of the Federal Environment Agency (Jahn et al., 2014). The authors made Proposals and Recommendations for the PPP risk management in respect of particularly endangered species, which constituted most of further use restrictions. The authors report that the current "*concepts try to exclude mortality due to PPP applications among identified key species. The current concepts have proved to be successful when three preconditions were fulfilled: 1. All relevant species have been considered during the tests, 2. There is no misapplication of PPPs, 3. There are still enough resources (habitat, food and shelter) for the species in the agricultural landscape*." The authors remind however that "*Regarding 1. and 2., some examples demonstrated that the risks have not been excluded in a sufficient extent. For instance, rodenticides or molluscicides (e.g. Methiocarb, Metaldehyd,) are still applied in sites where European Hamsters (Cricetus cricetus) occur. Another example is the poisoning of Hoopoes (Upupa epops) after the application of the molluscicid Methiocarb. The implementation of restrictions on the local level after the occurrence of the poisoning could not "repair" the severe damage to the particularly endangered species. Moreover, the current concepts are static because they do not consider changes in habitat choice of species."* The proposals made by the authors per endangered species mostly constitute a combination of a reduction of PPP applications, restrictions in the application of fertilisers, the establishment of less densely growing cultures and the increase in crop diversity.

6.3.2. For GMOs

It is standard practice for GMOs that if the ERA identifies risks, strategies to manage these risks may be required and should be defined by applicants. Applicants should then evaluate the efficacy and reliability of any risk mitigation measures and conclude on the final level of risk resulting from their application. Remaining identified risks and risk management measures should be considered when formulating PMEM plans. These provisions are described by the EFSA GMO Panel (2011b). Other than those mentioned above in 7.2, no further specific risk mitigation measures for species of conservation are envisaged. PMEM reports are requested annually from the applicant and action would be taken if there were concerns raised regarding the protection goals. A renewal of the authorisation is required after a period of 10 years of the GMO being approved.

6.3.3. For IAS

It is standard practice in the ERA of invasive alien species to include a list of risk reduction options with the aim to reduce/mitigate the damage caused by the pest in natural environments. An overview of the available risk reduction options for pests in natural environments is presented in the ERA guidance document of the EFSA PLH Panel (EFSA PLH Panel, 2011) and includes general practices such as for example more local spraying of chemicals (e.g. herbicides), physical methods to remove the invasive alien species, biological control methods and other methods to prevent the movement and spread of pest species (e.g. restrictions on trade). In this context, the aim of the risk reduction options is to contain existing outbreaks and ensure that no further pest movements are made into such environments. By these actions, eventually the endangered species may benefit from the reduction of the pressure of the driving force (population abundance/prevalence) on components of the ecological niche or on traits of the endangered species. Endangered species may also benefit from risk reduction options preventing or limiting the entry, establishment and spread of IAS. However, the implementation of risk reduction options may cause negative environmental effects. This is particularly relevant for IAS that affect natural or semi-natural environments like many forest insects or pests that can have a wide ecological niche and attack not only crops but also non-cultivated plants. In these cases control methods may have a direct or indirect negative impact on endangered species.

> An example is the set of methods used to control apple snails in rice fields that may cause negative environmental effects on the wetlands like (1) keeping rice paddies dry for a long period that might negatively influence rice paddy biodiversity, (2) burning vegetation and removal of plants along river banks to prevent egg laying and survival of snails that might have a negative effect on the flora and fauna of river ecosystems in wetlands and (3) treating rice paddies and/or irrigation canals with (a) lime, (b) saline water, (c) snail attractants containing methaldehyde or (d) saponins that may result in negative effects on both the rice and the natural wetland ecosystem (EFSA PLH Panel, 2014). Another interesting example is the ongoing assessment on risk associated to the planned release in Portugal of the bud-galling wasp *Trichilogaster acaciaelongifoliae* for the biological control of the invasive plant *Acacia longifolia*. Invasive *Acacia longifolia* in Portugal has formed extensive thickets in coastal sand dunes, along rivers, road edges and on mountain slopes and is becoming a dominant species out-competing many native species and plants associations. The PLH Panel performed an ERA to evaluate both the likelihood of negative impacts of the release of the wasp on cultivated Acaciae species, and the positive impacts on biodiversity and ecosystem services provisioning. The positive effect on biodiversity is a consequence of the negative effect on *A. longifolia*, and hence an example of an indirect impact of the wasp (EFSA PLH Panel, 2015).

6.3.4. For FAs

Mitigation or monitoring, both risk management responsibilities, are currently not foreseen in the FEEDAP authorisation process in Europe. From a scientific point of view, the aim of the risk assessment prior to authorisation is to reduce the use level of the feed additive below its effect level. Hence, no effects should be generated in the environment, therefore no risks and no risk mitigation measures are proposed at the end of the ERA. Should this objective not be met, a renewal of authorisation is foreseen after a period of 10 years of the feed additive being on the market.

6.4. Conclusion for ToR3

The main measures to generally support endangered species in an agricultural context, are the availability and quality of diverse farm habitats. It is however not in the remit of EFSA to advise on issues beyond its mandate on ERA of potential stressors.

EFSA risk assessments may conclude that vulnerable species (possibly including endangered species) are at risk from the potential stressor under assessment and that risk mitigation measures related to this potential stressor are needed. These may include measures to avoid co-occurrence of endangered species with the stressor and measures linked specifically to the type of potential stressor. Currently recommended risk mitigation measures have been detailed by the PPP, GMO and PLH Panels. For these areas, risk mitigation is considered as essential to complement ERA, since ERA can provide the information for the scale and the safe use/spread of the potential stressor. To be effective to mitigate risk also for endangered species, the generic mitigation measures now used in the current authorisation schemes for potential stressors may be developed in a more site-specific manner, should be context-specific, should account for the biology of the endangered species and the reasons for its endangerment. The FEEDAP ERA legal framework does not foresee to formulate risk mitigation measures at the end of ERA.

Additional to the currently applied mitigation measures, the following are considered of importance when protecting endangered species in an agricultural context.

For PPP, one can consider the development of rules for prospective risk assessments leading to recommendations on risk mitigation by PPP labelling saying "Recommendations for risk mitigation. If the intended use of this product is in the vicinity of an endangered species habitat, the following must be considered prior to use: [insert specific issues]". The consequences of such labelling might require some kind of compensation for farmers with ecologically more valuable land.

There is a suite of possible risk mitigation measures available ranging from small scale to well organised and remunerated incentives. Some of the risk mitigation measures are in the hands of local authorities rather than being implemented on the EU level, but consistency in such local implementation is considered important. Furthermore, mitigation measures proposed upon ERA, linked to the scale/use/spread of the potential stressor, should ideally tie in with the site/species specific management plans and those decisions are probably best made at a local level rather than at the EU-wide level of the e.g. the authorisation of regulated products.

As the farmers will be in most cases the in-field risk managers, their education and training should be supported. The importance of risk mitigation measures should be well communicated and emphasised in the farmer communities. In addition, advisory services and forecasting tools should be made available to farmers so that specific risks can be quantified by them at the local level.

7. IS MONITORING NEEDED TO CHECK THE EFFICACY OF RISK MITIGATION MEASURES FOR THE OCCURRENCE OF ENDANGERED SPECIES?

The fourth question of the terms of reference asks "*Is monitoring needed to check the efficacy of risk mitigation measures for the occurrence of endangered species?*"

Managing the various stressors threatening biodiversity is of critical importance for adequate protection of biodiversity. Potential stressors that undergo ERA can be managed for instance by the risk mitigation measures as discussed in Section 6. *A priori*, it is considered important to monitor the level of protection achieved by all management measures taken. Two stressor-oriented monitoring schemes can be envisaged: first, compliance monitoring and second supplementary monitoring (EFSA Colloquium, 2014). These are discussed below in view of protecting endangered species in an agricultural context. Also monitoring of endangered species *per se*, as well as monitoring the potential for unanticipated adverse effects to occur, are discussed below.

7.1. Compliance monitoring

Compliance monitoring is mainly stressor-oriented. The aim would be to check whether farmers implemented the recommended risk mitigation measures. Both regulatory authorities and the product owner (where applicable) would be responsible for compliance monitoring. The existing monitoring systems based on farmer questionnaires could be one of the tools to collect the necessary information. the design of framers questionnaires has been discussed in EFSA GMO Panel, 2011b.

For PPP: EFSA is not involved in PPP environmental monitoring. Information on if and how this is done for PPP and a feedback mechanism to the ERA with respect to co-occurrence would be welcomed. For endangered species, it may be recommendable to make extra efforts locally where the endangered species occur.

> Possible sources of useful information are (1) The Water Framework Directive (providing information on PPP concentrations in surface waters, but not on how PPPs are being used by farmers and the Sustainable Use Directives, which are tools to monitor compounds, their concentrations and their impacts over longer distances; (2) The EU Habitat and Birds Directives, Red Lists and monitoring regimes for invasiveness and weediness; and (3) national initiatives, such as imidacloprid monitoring in France (ongoing, no reference yet).

For GMOs: A plan for post-market environmental monitoring (PMEM) of GM plants is mandatory in all applications for deliberate release of GMOs submitted under Directive 2001/18/EC and Regulation (EC) No 1829/2003. The overall PMEM has two objectives: (1) to confirm or reject potential adverse effects that the GM plant or its use may have on human and animal health or the environment as identified during the ERA; and (2) to identify potential unanticipated adverse effects on human health or the environment which could arise directly or indirectly from GM plants and that the pre-marketing ERA would not have detected. Therefore, according to its two distinct objectives, PMEM is respectively composed of (1) case-specific monitoring (CSM) and (2) general surveillance (GS). On the one hand, the hypothesis-driven CSM is not compulsory but may be included in a GMO application in order to confirm the outcomes of the ERA. In the case where risk management strategies (mitigation measures such as buffer zones, isolation distances, to limit exposure of endangered species) have been put in place due to identified risks or critical uncertainty (e.g. gaps in the scientific information), their efficacy could be monitored under CSM in order to determine the reduction in exposure. In such cases, the monitoring results can be used to modify the risk management strategies, so that they are proportional to the remaining levels of risk. On the other hand, GS, which is not hypothesis driven, is required in all cases for each GMO application even if no adverse effects have been identified in the ERA. In the context of GS, the applicant should therefore monitor all aspects of the environment that need to be protected from harm (i.e. protection goals as set by the risk managers, and that could be specified to include endangered species). In its 2011 guidance document on PMEM, the EFSA GMO Panel detailed the tools to monitor unanticipated adverse effects under GS: (1) the monitoring of the GMP and its cultivation site(s) mainly through farmer questionnaires, (2) the monitoring at larger scale by utilising the data collected by existing environmental monitoring networks active in biodiversity surveys at local/regional/national scale, and (3) the review of the scientific literature. The EFSA GMO Panel assesses the scientific quality (e.g. methodology) of the PMEM plans.

For invasive species, surveillance and monitoring is advisable in any case, even if no management measures are undertaken to combat the pest. This is for example the case when action would result in a more negative effect on the environment than without action, when no effective risk reduction options are available, when the pest is already too widespread for cost-effective action or when the pest is not likely to cause damage or will die out without intervention, e.g. because it cannot reproduce.

For feed additives, no monitoring is currently envisaged, see above.

7.2. Current status monitoring at the European level

In the general scheme of species conservation in general and endangered species specifically it is important to realise that “potential stressors” as defined in this opinion in most cases contribute only a minor proportion of the integrated pressures biota experience. The WWF Living Planet Report (WWF, 2014) has the general attribution between the threats listed as contributing to the declines in animal populations were 37% from exploitation (Fishing, hunting etc.), 31% habitat degradation and change, 13% from habitat loss and only 5% from invasive species, 4% from pollution and 25% from disease. However, for endangered species within the “sphere of influence” of the European agriculture it may in many cases be these “potential stressors” that may tip the balance and possibly also are the technically easiest (although not financially cheapest) to mitigate. While general European level monitoring is not targeted at endangered species it may however provide evidence of areas, species groups or habitats that are under most pressure and thus where the protection of endangered species warrant extra attention including ERA considerations. As Monastersky (2014) lists 26% of mammals, plus 13% of birds and 41% of amphibians (based on combining IUNC, 2014c; Pimm et al., 2014; Scheffers et al., 2012; Mora et al., (2013)) as “threatened species” it makes sense to focus attention first on ensuring best possible ERAs for endangered species where the pressures observed on species in general are highest.

The current monitoring data received and collated by EEA and JRC is based on national reporting of monitoring data originating from the member states and is in the main reported either under Article 17 of the Habitats Directive (HD) or the Water Framework Directive (WFD). In both cases, the monitoring is aimed at status monitoring for either the conservation status of “species of Community interest” (HD) or “Good Ecological Status” of waterbodies (a measure under WFD that integrates biology, hydrology, physical and chemical and pollution criteria). While both HD and WFD monitoring include agricultural threats (e.g. use of biocides, hormones and chemicals for HD and some pesticides under WFD), quantitative analysis of the separate “stressor factors” and apportionment will most often not be possible. This monitoring can thus contribute to holistic risk assessment, but not directly to the assessment of the degree of risk incurred or present from specific potential stressors. None of the monitoring under HD or WFD specifically includes “endangered species”. In fact while the baseline reference site selection for the WFD sometimes include rare species, it must not include an endangered species as expected in the baseline population.

In relation to endangered species this general status monitoring still provides input to where the overall pressures on habitats are highest or increasing at the European scale, and so direct where special attention might need to be focused on possible ERA efforts and local “supplementary monitoring” (see below) specifically related to endangered species. E.g. the data under the HD are integrated at the level of bio-geographical regions by EEA, which, when combined with e.g. bird data from the Pan-European Common Bird Monitoring Scheme (PECBMS), could be used to highlight general areas (regions or habitats) especially under high overall pressure. Similarly, analysis under the WFD can provide a general European level picture of regions or habitats under high pressure. Additionally, at least for chemical based stressors there is some monitoring of the original 33 priority substances (or substance groups) set out in 2008 and a further 15 added in the 2012 review. Again, these data can be used to ensure that the PEC used in the ERAs remains relevant under current usage patterns. Likewise, this data should also be considered when discussing any extra assessment factors potentially deemed relevant for endangered species.

Where special "location specific" ERA consideration is relevant for an endangered species, either at the scale of a member state or a habitat, the national HD and WFD monitoring data held by the geographically relevant member states are likely to be very useful in gaining a picture of the overall pressures experiences by the biota in the location of concern.

7.3. Supplementary monitoring

Specific experimental monitoring studies could be designed to investigate a particular research question or bio-geographical area of concern. Those can be linked to the efficacy of the implemented risk mitigation measure(s) for an potential stressor. The spatio-temporal scale for such experimental monitoring, the choice of monitoring endpoints and the number of spatio-temporal measurements, must be considered with care to yield meaningful answers. Instead of asking specific research questions only, this type of monitoring could also be directed at generating relevant baseline data needed to confirm ERA assumptions. As supplementary monitoring is considered stressor-oriented, it should ideally be conducted by research funders and the product owner if applicable (EFSA Colloquium, 2014).

7.4. Monitoring of (endangered) species

As society considers that specific measures should be undertaken to protect endangered species from harmful effects, regulatory authorities should bear the responsibility to ensure this type of monitoring. The data generated through the monitoring of endangered species should enable the assessment of their status and trends over time. Although relevant data may be collected through existing surveillance and nature conservation networks, it is recognised that the collected data may not necessarily be fit for purpose (i.e. enable the assessment of their status and trends over time). In addition, it may be challenging to determine the cause of an observed effect.

Monitoring of endangered species is therefore an activity that is not (always) linked to a particular stressor. For potential stressors that undergo ERA, examples have been published and the below examples helped to explore how existing species monitoring activities can inform the ERA of potential stressors (plus efficacy of mitigation or other management measures).

A first set of examples demonstrate that chemical usage patterns in time and geography are reflected in monitoring, that safety failures can be overcome by revising mitigation measures, and that mitigation efficacy and the process of recovery can be monitored.

- *Polybrominated flame retardants (PBDEs) in gannet eggs: responding to increased use and then restrictions of use.* PBDEs have been found to have toxic effects in vertebrates. The most toxic (the penta- and octa-PBDE mixtures) are now banned, but it was unknown how quickly environmental levels would subsequently fall. Crosse et al. (2012) used gannet eggs as a sentinel to provide the first detailed long-term temporal trend of PBDE contamination in UK waters. The authors found penta BDE was the main contaminant, and that levels in eggs closely tracked modelled usage, with concentrations falling from peak to pre-usage levels within 10 years of the ban. This is much more rapid than the decline of other persistent pollutants in the environment.

- *Decline in organochlorine (OC) residues following restrictions and then bans in their use*. In the 1950s, the use of OC insecticide seed-dressings, such as aldrin and dieldrin, led to thousands of birds of various species being found dead and dying each spring in recently-sown cereal fields across Britain and to declines in predatory bird populations. It was established that population declines of birds-of-prey in the U.K. were due to a combination of reduced reproduction (caused by DDT) and reduced survival (caused by aldrin, dieldrin and others). Pressure to phase out the organochlorines steadily mounted, and in Britain their use gradually declined through progressive restrictions over the next 25 years. Over the same period, egg-shell thickness and population levels of birds-of-prey gradually recovered, residues declined in their body tissues, and by the 1990s the numbers and distributions of Peregrines, Sparrow hawks and other raptors in Britain had recovered (Newton et al., 1993). Later, regional differences in observed OC residue levels in both kestrels and sparrow hawks were related to levels of usage of OCs in those regions (Walker and Newton, 1999).

A second type of example shows that important species differences (e.g. in TD/TK, see Appendix C) can be found during monitoring.

- *Species differences in residue magnitude between sparrow hawks and kestrels.* When comparing data among the raptors monitored by the PBMS, Newton et al.(1993) found that the sparrow hawk had higher levels of most pollutants than the kestrel and showed less decline in levels during the study period. There were probably three reasons for this difference. The sparrow hawk eats other bird-species (herbivores and insectivores), and hence feeds higher in the food chain than does the kestrel, which eats mainly herbivorous voles. Secondly, birds in general are less able to metabolise organochlorines and some other pollutants than are mammals (Walker, 1983), so for this reason too the bird-eating sparrowhawk would tend to accumulate higher levels than the mammal-eating kestrel. Thirdly, sparrowhawks are less able than kestrels to metabolise (by oxidation) organochlorines within their own bodies (Walker et al., 1987). It was not surprising, therefore, that sparrow hawks suffered a more marked and widespread population decline than kestrels.

The above examples are typical food web accumulation examples that could be clearly linked to direct effects of a particular chemical. For indirect effects, the cause of harm is more difficult to know from general monitoring, i.e. observed trends can hardly be linked with a causing effect. This is because monitoring (of birds for example) is mainly based on counting the number of individuals and when the observation is equal to disappearance, one cannot investigate the cause of disappearance. Therefore, indications for the cause of harm for indirect effects are more likely to come from observational reports rather than from monitoring. Additionally, the hypothesis for a cause can be tested through modelling or by weight of evidence. The question can still remain open if the trend (observed in monitoring) is related to the chemical alone and what is the relevant contribution of other stressors. The determination of the chemical having a mechanistic cause remains necessary in this respect.

If the prospective ERA concludes positively and strongly on the likelihood of indirect effects, then ERA can advise monitoring techniques that are built on the case-specific context and the formulated risk hypothesis.

7.5. Conclusion for ToR4

A priori, it is considered important to monitor the level of protection achieved by all management measures taken to protect endangered species, including risk mitigation measures as discussed in Section 6.

Two stressor-oriented monitoring schemes can be envisaged: first, compliance monitoring and second supplementary monitoring. At present only the GMO Panel is actively involved in regulated monitoring of GMOs, from which also endangered species could benefit. For invasive species, surveillance and monitoring is advisable in any case. For PPP and feed additives, EFSA is currently not involved in monitoring. At the member state level information on chemical and biological monitoring, for instance conducted within the context of the Water Framework Directive (WFD), may be used in the re-registration of the PPP.

Additionally, one can envisage monitoring of the (endangered) species *per se*, not linked to a particular stressor. For direct effects, typical food web accumulation examples have shown that effects on the endangered species could be clearly linked to direct effects of a particular chemical. For indirect effects, the question is likely to remain open if the trend (observed in monitoring) is related to the chemical alone and what is the relevant contribution of other stressors. If the prospective ERA concludes positively and strongly on the likelihood of indirect effects, then ERA can advise monitoring techniques that are built on the case-specific context and the formulated risk hypothesis.

Ideally for risk assessors of potential stressors, knowledge from diverse monitoring schemes should be fed back to them so that their assessments can be refined where needed. Currently however, information about the level of protection achieved, and whether or not this is adequate, is not always available for risk assessors (except to a certain extent for GMOs, for which yearly monitoring reports are submitted to EFSA).

8. Options for extended coverage of endangered species in ERA schemes

Based on the analysis presented in Section 4, it was concluded that no general answers can be given to the question "*Are endangered species more vulnerable to potential stressors than other species*?", but that anecdotal examples illustrate why, where and when endangered species can be more vulnerable. Subsequently, it was shown in Section 5 that it currently varies among ERA schemes to which degree endangered species are covered and how they are covered.

During the collection of relevant information for Sections 3, 4 and 5, additional approaches for covering endangered species were compiled and their usefulness for ERA was discussed. While this is not a guidance document, the aim of the present Section is to provide some general suggestions on how to extend the coverage of endangered species in the ERA schemes falling under EFSA's remit. These suggestions are presented as generic approaches which should be elaborated further when considering their implementation in practice. The possible implementation is left at the discretion of the individual panels and responsible risk managers. Increasing the complexity of the ERA should be balanced against the win of knowledge and the environmental safety. It is discussed in Section 8.6 at which different spatial scales endangered species can be addressed in ERA schemes.

It should be noted that the details of the legal protection status of endangered species as described in the Habitat Directive (or the respective national legislations) with respect to potential stressors under ERA could not be concluded in this opinion (see Section 1). In particular when defining protection goals for endangered species it needs to be clarified if single individuals need to be taken into account or if (sub-)populations can be the target. Furthermore, the probably often impaired population viability of endangered species (i.e. unfavourable conservation status as defined in the Habitat Directive) needs to be taken into account in the practical implementation of the suggested options.

8.1. Setting specific protection goals for endangered species

In order to account explicitly for the protection of endangered species in ERA schemes, the first step is to define specific protection goals. This involves a detailed specification of what needs to be protected where and when, to what level and with what level of certainty, given our current state of knowledge.

After the identification and selection of *endangered species or species groups that are in the sphere of influence of the potential stressor that* should be protected, an explicit protection goal should contain the following dimensions:

- The *ecological entity of the selected endangered species* to be protected. Should all individuals be protected or is the aim to protect the population, i.e. some individuals may suffer adverse effects (including welfare), as long as the viability of the population is not impaired? The definition of an adequate protection level requires careful consideration of the societal values attached to endangered species and the ecosystems they are part of.
- The *attribute*: What is the type of the effect, e.g. lethal, sub-lethal?
- A *temporal* specification of the protection goal. Should endangered species always be fully protected or are temporary reversible effects allowed?
- A *spatial specification* of the protection goal. Should endangered species be protected everywhere or only in certain locations?
- The *magnitude*: What is the level of effect expressed in x% (e.g. x = 1, 5 or 10) of the population?
- The *level of (un)certainty* of the assessment. How certain must the assessment be, given the current state of knowledge?

When defining specific protection goals for endangered species, it is important that all Panels involved apply similar and consistent approaches, so that inconsistencies in protection goals are avoided. It should furthermore be noted that full protection of endangered species cannot be realised by EFSA's ERA schemes alone, and cannot be considered in isolation from species-specific and/or localised efforts to protect endangered species, e.g. within the context of the Habitat Directive. Ideally, EFSA's ERA schemes should help to safeguard that the potential stressors do not impede the realisation of the protection goals as defined for endangered species within other regulatory contexts.

Starting point for defining specific protection goals for endangered species is the recognition that biodiversity is a common and prominent legal protection goal for all environmental risk assessments performed by EFSA. However, as noted in the parallel opinion on protection goals (EFSA SC, 2016a), it has not yet been clarified by the legislator how to measure and evaluate biodiversity (and to what extent it should be protected) in the prospective ERA schemes of potential stressors that fall under EFSA's remit, and the coverage of endangered species in particular. One line of thought is that the ERA schemes for which EFSA is responsible should provide "sufficient" protection for endangered species against direct and indirect adverse effects of potential stressors that fall under EFSA's remit.

However, it is considered unfeasible to provide full protection to all endangered species, everywhere and all the time – and to some extent also not required in the circumstances where endangered species are outside the "sphere of influence" of the potential stressor. The protection goals for endangered species in EFSA's ERA schemes should thus be further specified in terms of what is being protected where and when, to what level and with what level of certainty. Even when protection goals are fully defined based on the available knowledge, there is a higher likelihood that the ecotoxicological knowledge for endangered species is incomplete and the uncertainty around the provided protection consequently larger.

One option to deal with the practical unfeasibility of protecting all endangered species, everywhere and all the time, is to distinguish between ecologically critical subpopulations, which are essential for a species' survival in a particular region, and subpopulations which are not (e.g. the sink and source population). The underlying rationale is that critical subpopulations should be provided with a higher level of protection than non-critical subpopulations. The exact definition of critical and non-critical subpopulations requires careful consideration as the distinction may have serious implications for managing potential stressors. For the purpose of this opinion, critical subpopulations are loosely defined as subpopulations which are essential for the survival of the endangered species in a particular area. When this concept is further operationalised, ecological criteria are needed to distinguish between ecologically critical and non-critical subpopulations, as well as (monitoring) data to assess the status of a subpopulation in a particular area, e.g., at a local or regional scale.

A further option when defining protection levels, is to distinguish a number of species groups for which common protection levels or endpoints are defined, e.g. individual versus population level protection. Examples include vertebrates, invertebrates, plants and microbes. Regarding the spatial dimension, distinction could be made between (1) the application area of the potential stressor, (2) agricultural area, i.e. the area that surrounds the application area and which is also reserved for agricultural purposes, and (3) non-agricultural area (action at a distance). The rationale behind this spatial differentiation is that the level of protection provided to endangered species may differ between these areas. Temporary adverse effects could for example be allowed in the application area of the potential stressor, but not in the non-agricultural area.

Regarding the (un)certainty of the assessment, distinction can be made between uncertainties that can be assessed (the known unknowns), for example using probabilistic techniques, and uncertainties that cannot, i.e. ignorance (the unknown unknowns). Details on how to deal with uncertainty in scientific assessments is the subject of the Guidance Document of the EFSA Scientific Committee under development (EFSA SC, 2016c).

Tables 5 and 6 provide some tentative examples of how the different dimensions of protection goals for endangered species could be specified. The values listed should not be taken as proposed values, but are meant to illustrate the principles and to stimulate a discussion. The choice of the entities related to the specific protection goals for endangered species must be considered in concert with the available methods and tools, e.g. using field data or mathematical population models. It is furthermore important that the different dimensions of the protection goals are consistent, i.e. the spatial and temporal dimensions of the exposure and the effect assessment must be compatible. Ultimately, the establishment of specific protection goals for endangered species is a task for risk managers, preferably in a dialogue with different stakeholders. EFSA recommends to hold a stakeholder workshop for setting specific protection goals for endangered species. It would be necessary to develop a limited number of options for specific protection goals of endangered species, including an indication of their socio-economic consequences, that could serve as case-studies at the workshop.

Table 5: Tentative examples of specific protection goals for *critical subpopulations* of endangered species.

Species Group	Vertebrates	Invertebrates	Plants & Algae	Microbes
Entity	Individual	Population	Population	Population
Protection Level				
Application area[1]	*x*% (e.g. *x* = 1, 5 or 10) of the individuals in the population may temporarily suffer from sub-lethal adverse effects, but must recover within *y* weeks (e.g. *y* = 1, 2 or 4 weeks)	The population may temporarily suffer from adverse effects (i.e. drop in numbers), but must recover within *y* weeks (e.g. *y* = 1, 2 or 4 weeks)	The population may temporarily suffer from adverse effects (i.e. drop in numbers), but must recover within *y* weeks (e.g. *y* = 1, 2 or 4 weeks)	The population may temporarily suffer from adverse effects (i.e. drop in numbers), but must recover within *y* weeks (e.g. *y* = 1, 2 or 4 weeks)
Agricultural area[1]	*x*% (e.g. *x* = 1, 5 or 10) of the individuals in the population may temporarily suffer from sub-lethal adverse effects, but must recover within *y* weeks (e.g. *y* = 1, 2 or 4 weeks)	The population may temporarily suffer from adverse effects (i.e. drop in numbers), but must recover within *y* weeks (e.g. *y* = 1, 2 or 4 weeks)	The population may temporarily suffer from adverse effects (i.e. drop in numbers), but must recover within *y* weeks (e.g. *y* = 1, 2 or 4 weeks)	The population may temporarily suffer from adverse effects (i.e. drop in numbers), but must recover within *y* weeks (e.g. *y* = 1, 2 or 4 weeks)
Non-agricultural area[1]	Full protection for all individuals (no temporal adverse effects)	Full protection for the population (no temporal adverse effects)	Full protection for the population (no temporal adverse effects)	Full protection for the population (no temporal adverse effects)
Uncertainty				
Probability	If probabilistic assessment is possible, *x*% certainty (e.g. *x* = 50, 90 or 95) of realizing the protection goal	If probabilistic assessment is possible, *x*% certainty (e.g. *x* = 50, 90 or 95) of realizing the protection goal	If probabilistic assessment is possible, *x*% certainty (e.g. *x* = 50, 90 or 95) of realizing the protection goal	If probabilistic assessment is possible, *x*% certainty (e.g. *x* = 50, 90 or 95) of realizing the protection goal
Ignorance	- Prescribed RA methods - Conservative assumptions if probabilistic assessment is impossible - Assessment factor for unknown unknowns: 1-10 (case-specific)	- Prescribed RA methods - Conservative assumptions if probabilistic assessment is impossible - Assessment factor for unknown unknowns: 1-10 (case-specific)	- Prescribed RA methods - Conservative assumptions if probabilistic assessment is impossible - Assessment factor for unknown unknowns: 1-10 (case-specific)	- Prescribed RA methods - Conservative assumptions if probabilistic assessment is impossible - Assessment factor for unknown unknowns: 1-10 (case-specific)

[1] The specific protection goal of species moving between different types of areas is determined by the dominant area type in their foraging range.

Table 6: Tentative examples of specific protection goals for *non-critical subpopulations* of endangered species.

Species Group	**Vertebrates**	**Invertebrates**	**Plants & Algae**	**Microbes**
Entity	Individual	Population	Population	Population
Protection Level				
Application area[1]	*x*% (e.g. *x* = 5, 10 or 25) of the individuals in the population may temporarily suffer from sub-lethal adverse effects, but must recover within *y* weeks (e.g. *y* = 2, 4 or 8 weeks)	generic ERA applies	generic ERA applies	generic ERA applies
Agricultural area[1]	*x*% (e.g. *x* = 5, 10 or 25) of the individuals in the population may temporarily suffer from sub-lethal adverse effects, but must recover within *y* weeks (e.g. *y* = 2, 4 or 8 weeks)	The population may temporarily suffer from adverse effects (i.e. drop in numbers), but must recover within *y* weeks (e.g. *y* = 2, 4 or 8 weeks)	The population may temporarily suffer from adverse effects (i.e. drop in numbers), but must recover within *y* weeks (e.g. *y* = 2, 4 or 8 weeks)	The population may temporarily suffer from adverse effects (i.e. drop in numbers), but must recover within *y* weeks (e.g. *y* = 2, 4 or 8 weeks)
Non-agricultural area[1]	Full protection for all individuals (no temporal adverse effects)	Full protection for the population (no temporal adverse effects)	Full protection for the population (no temporal adverse effects)	Full protection for the population (no temporal adverse effects)
Uncertainty				
Probability	If probabilistic assessment is possible, *x*% certainty (e.g. *x* = 50, 90 or 95) of realizing the protection goal	If probabilistic assessment is possible, *x*% certainty (e.g. *x* = 50, 90 or 95) of realizing the protection goal	If probabilistic assessment is possible, *x*% certainty (e.g. *x* = 50, 90 or 95) of realizing the protection goal	If probabilistic assessment is possible, *x*% certainty (e.g. *x* = 50, 90 or 95) of realizing the protection goal
Ignorance	- Prescribed RA methods - Conservative assumptions if probabilistic assessment is impossible - Assessment Factor for unknown unknowns: 1-5 (case-specific)	- Prescribed RA methods - Conservative assumptions if probabilistic assessment is impossible - Assessment Factor for unknown unknowns: 1-5 (case-specific)	- Prescribed RA methods - Conservative assumptions if probabilistic assessment is impossible - Assessment Factor for unknown unknowns: 1-5 (case-specific)	- Prescribed RA methods - Conservative assumptions if probabilistic assessment is impossible - Assessment Factor for unknown unknowns: 1-5 (case-specific)

[1] The specific protection goal of species moving between different types of areas is determined by the dominant area type in their foraging range.

8.2. Options for extending the coverage of endangered species into generic assessments

As outlined in Section 5, different risk assessment strategies have been developed for the different potential stressors falling under EFSA's remit. In generic assessments[22], such as commonly applied for PPP and FAs, no explicit distinction is made between endangered and non-endangered non-target species. The protection level provided in these assessments is typically implicitly defined in procedures and criteria such as the application of a tenfold safety factor to the lowest available NOEC or the determination of the 5^{th} percentile in a species sensitivity distribution of NOECs, to which an appropriate extra assessment factor is being applied to account for all remaining uncertainties. These procedures and criteria provide a kind of baseline protection for all species in the ecosystem.

It is important to note however, that prospective risk assessment of potential stressors at a European scale cannot always guarantee the realisation of the specific protection goals at lower scales. Local conditions may give rise to risks unanticipated in generic ERA schemes, e.g. due to co-exposure to other stressors or modulating factors. The principle is for example illustrated by the increased exposure of rusty blackbirds in the North American Acadian ecoregion attributed to a combination of higher bioavailability of MeHg here than in other wetlands and waterbodies, and the high trophic position of the birds (Edmonds et al., 2010, 2012) (See Section 4.2.1 for details). The example shows that location-specific conditions may give rise to a species-specific assessment, i.e. if these location-specific conditions influence the impact of the stressor on the endangered species in such a way that the targeted protection level is impaired.

One option to extend the protection of endangered species in generic assessments is by increasing the generic protection level currently applied in ERA schemes to a level where all endangered species are considered to be protected. Examples of this approach include the application of a higher assessment factor, (e.g. increasing the standard AF of 10 used in chronic risk assessments used in birds and mammals to 50) or lowering the fraction of species affected (e.g. to use HC_1 instead of the HC_5). A disadvantage of this approach is that this increases the generic protection level for all species to address a specific problem (i.e. protecting endangered species). This can be avoided by using the results of the ERA with higher AF only in a region where certain endangered species are expected. A further disadvantage is that our current state of knowledge does not allow reliable statements about what protection level is sufficient for endangered species. With other words, we currently do not know how high that increased AF should be. A further option could be to generally base the ERA for endangered species on the ecological threshold option, thus not allowing for recovery, and applying the outcome of such ERA only in areas where the endangered species may occur. The protection of endangered species can alternatively also be achieved by implementing additional mitigation measures (see Section 6). Therefore, when it comes to the specific protection of endangered species, a more feasible approach is to supplement generic assessments with the option to perform species-specific and/or site-specific assessments.

[22] The term "generic assessment" is used in this opinion to refer to assessments which are strongly protocolled, such as the assessments of PPPs and FAs. These generic assessments typically use detailed and standardised protocols to assess exposure, effects and risks, and do generally not focus on one particular geographical area. Generic assessments are distinguished from "case-by-case assessments", such as the assessment of GMOs and IAS, which are less protocolled. These assessments are typically tailored on the characteristics of the stressor and the exposure situation that are being assessed. Case-by-case assessments often do focus on a particular geographical area, although this is not a necessity.

The (potential) presence of an endangered species in the sphere of influence of the potential stressor is a prerequisite for triggering a species-specific and/or site-specific assessment. As such, the protection of a specific endangered species will often be an issue at a local, regional or national scale. However, the protection of a specific endangered species may also require regulation at European or even higher spatial scales, e.g. if the endangered species has a large foraging range (e.g. the mutton bird, alias Short-tailed shearwater, alias *Ardenna tenuirostris*) or a widespread geographical distribution. This implies that the spatial scale of the assessment cannot be fixed *a priori*, but depends on the sphere of influence of the potential stressor, the habitat of the endangered species and their mutual overlap. It might be useful to compile a list of endangered species that are likely to be affected on large scales where EU assessments are appropriate.

Before performing a species-specific assessment, it should be assessed whether the potential stressor at hand may contribute significantly to the overall stress for the endangered species. After all, it is not very efficient and effective for the endangered species to only perform a detailed prospective assessment for the potential stressor at hand and propose subsequent mitigation measures if more than e.g. 95% of the stress is caused by other stressors.

Al the above options can only be considered for implementation after consultation with the responsible risk managers.

8.3. Addressing endangered species in ERA using surrogate species

Assessing the impact of potential stressors on endangered species is complicated by the fact that experimental studies in which these species are subjected to potential harm are ethically unacceptable and practically often unfeasible. This means that a workaround is needed. One option is to select and assess a non-endangered surrogate species which is closely related to the endangered species. This approach is currently applied for GMOs (under the focal species concept Section 5.2.1.) and biological invasions (Section 5.4.1 and more details provided in Carstens et al., 2014; Delano et al., 2011), and it has also been proposed for chemical substances such as PPPs and FAs (Fairchild et al., 2008; Sappington et al., 2001; Raimondo et al., 2008; Dwyer, 2005). It is based on the assumption that closely related species are likely to have similar traits as the endangered species, and therefore also a comparable ecological vulnerability. The uncertainties involved in extrapolating the assessment results for a surrogate species to the endangered species at hand, particularly when considering its recovery potential and the viability of the population, can be covered by the application of one or more assessment factors. The values of these factors depend on the level of similarity between surrogate and endangered species.

The use of surrogate species in risk assessment as a stand-in for endangered species is not without debate (Banks et al., 2014; Munns 2006; Banks et al. 2010; Ebner et al., 2009). The overarching question is whether – and under which conditions – selected surrogate species adequately reflect the ecological vulnerability of the endangered species. While the use of surrogate species offers a practical tool to get an impression of the ecological vulnerability of an endangered species, the selection of the surrogate species often "*is indefensibly simplistic and vague, driven by apparent similarities in physiology, phylogeny, or life history*" (Banks et al., 2014, p. 770).

Several studies have evaluated the similarity in toxicological sensitivity between taxonomically related species, with varying results. Some studies conclude that the variation in toxicological sensitivity between closely related species is limited and can be covered by an adequate safety factor (Fairchild et al., 2008; Sappington et al., 2001). However, the analysis presented in Section 5.1.2.2 of this opinion shows that the gain in knowledge from testing surrogate species of the same genus is only marginal.

There are also studies that focus on the role of life history traits in the selection of surrogate species. These traits determine the similarity in terms of population outcomes rather than individual responses to stressors. Banks et al. (2010) explored how life history traits relate to how a surrogate species and the species it represents respond to the effects of toxicants. In their discussion they warn that "*For most endangered/threatened wildlife, extrapolations from surrogate species' responses to disturbance seem woefully simplistic in the context of more complex ecological factors that influence their population dynamics*" (p. 180). In a later study, Banks et al. (2014) applied a trait-based model to assess population responses to toxic insult in a suite of parasitoid wasps that provide biological control of agricultural pests. Based on this modelling study, they conclude that "*Taken together, our results suggest that the ability to predict the fate of a suite of species using the response of just one species (the surrogate species concept) is widely variable and potentially misleading.*"

It can be concluded that the selection of appropriate surrogate species is a critical step in the use of surrogate species approach in risk assessment. The level of similarity in terms of ecological vulnerability should be a key factor in the selection process. Scientifically based and transparent criteria are needed to assess the level of similarity between surrogate and endangered species covering all areas of ecological vulnerability, i.e. exposure, (toxicological) sensitivity, recovery potential and sensitivity to indirect effects. It might be wise to choose a surrogate species from the same traits-group (see next section 8.4).

8.4. Addressing endangered species in ERA using trait-based approaches

The following Sections will explore how taxa-specific traits can help (1) to identify if an endangered species may not be covered by generic ERA and (2) with the identification of the traits or the surrogate species that will help to cover such endangered species in the ERA scheme. The impact of a stressor on a particular endangered species depends on the stressor characteristics, species characteristics and environmental characteristics (e.g. the landscape configuration and other species and stressors). The interactions between these factors determine whether or not an adverse effect eventually arises. In most cases, it is possible to either measure or model the nature of these interactions. For example, a fate model is often used to describe the interactions between a chemical substance and the environment. Likewise, a laboratory toxicity test can be considered a technique to determine (part of) the interactions between a chemical substance and a particular species. A population or ecosystem model constitutes a technique to describe the interactions between the species of interest, the landscape and/or other species and potential stressors.

If sufficient knowledge is available about the species characteristics that drive these interactions between stressor, species of interest, landscape and other species and potential stressors, this information may be used to make projections about the likelihood of adverse impacts. This idea lies at the heart of trait-based approaches, which are essentially modelling approaches in which the species-of-interest is represented by a limited number of species characteristics or traits (e.g. home range, age, feeding habits). Based on these traits, the endpoint of interest is assessed, e.g. external exposure or toxicity. Depending on the level of knowledge, trait-based approaches can be used to make qualitative as well as quantitative projections about the impacts of potential stressors.

Recently, several researchers have proposed to incorporate species traits in risk assessment of chemicals to better understand and predict the potential for toxic effects of chemicals to a broader range of taxa than the traditional laboratory species. De Lange et al. (2009) developed a method to predict ecological vulnerability in wildlife using autecological information related to potential exposure, sensitivity and recovery capacity. The analysis resulted in an ordinal ranking of vulnerable species. Rubach et al. (2011) developed a framework for the application of trait-based assessment based on the population vulnerability conceptual model of Van Straalen by grouping vulnerability traits into three major categories: (1) external exposure, (2) intrinsic sensitivity, and (3) population sustainability. The authors provided a preliminary inventory of traits within each major category including data sources to quantify those traits and assess their relative contribution to potential toxic effects on particular taxa (Rubach et al., 2011). They argue that trait-based approaches allow to investigate species-substance interactions and to predict toxic effects at different levels of biological organisations, i.e. individual, population, community and ecosystem level. The authors discuss key aspects including the development of ecological models describing toxicokinetic (TK) and toxicodynamic (TD) processes of a given chemical in individual organisms, species life history and the connectivity of populations to determine their recovery, and the food web relations at community and ecosystem level that determine indirect effects of chemicals. Van den Brink et al. (2013) proposed a similar framework aiming to integrate more ecological data using trait-based approaches and ecological modelling.

Trait-based approaches are promising techniques to address two main challenges in risk assessment of endangered species:

- Increase the ecological relevance of the assessment. Processes such as exposure, recovery and indirect effect strongly depend on the complex interactions between potential stressor, landscape, species-of-interest and other species. Although these processes are relevant for all species, they may be especially relevant for endangered species because of their high degree of food and habitat specialisation, their low population numbers and/or the high level of background stress.

- To cover the fact that the sensitivity of an endangered species to an potential stressor cannot be tested experimentally. Considering that it cannot be deduced from the existing literature that endangered species have a priori different sensitivity, and considering the scarcity of testing possibilities with endangered species, the SC sees great potential for the application of molecular and sequencing techniques to detect the presence or absence of receptor targets and to predict the toxicological sensitivity of (endangered) species. The SC strongly encourages further exploration of these techniques and their potential for use in prospective risk assessments. This aspect is elaborated in detail in Appendix C.

8.4.1. Comparative trait-based assessment for endangered species

The prediction of the ecological vulnerability of a species to an potential stressor in the absence of empirical data requires detailed knowledge about the potential stressor, the endangered species, (including the viability status of the respective populations), other relevant species and potential stressors, the landscape, and their mutual interactions. Such detailed knowledge is currently lacking, impeding the development of a reliable and quantitative prediction model for ecological vulnerability. Nonetheless, the insight that species traits are important determinants of ecological vulnerability can be put into practice for assessing the risks of potential stressors to endangered species by making an inventory of relevant traits of the endangered species and comparing those with the traits of the species included in the standard ERA schemes. The question to be answered is then whether the endangered species possesses specific traits which are likely to result in a (1) higher exposure, (2) higher toxicological sensitivity, (3) lower recovery potential, and/or (4) a higher sensitivity to indirect effects, than the species generally considered in ERA. This question can be answered by listing the species traits that determine the processes in each of these four subdomains and comparing these traits between the standard (test) species and the endangered species. For example, an endangered species may have a higher exposure to a potential stressor if a relevant exposure route is not included in standard tests and/or exposure assessments. Likewise, an endangered species may be more sensitive to the potential stressor if it lacks certain enzymes responsible for metabolism or elimination of the potential stressor (See Appendix C for more details). A comparative trait-based assessment would provide a qualitative indication of whether an endangered species is sufficiently protected under current ERA schemes. If the outcome indicates that the endangered species is more vulnerable, this could be used as an argument to apply an additional safety factor to the results of the generic assessment, or to motivate the application of extra local mitigation measures (including use restriction) to the potential stressor in the vicinity of the endangered species of concern. As such, it is a supplement to the generic assessment. It should be noted that a comparative trait-based assessment does not take site-specific conditions into account and as such cannot replace a site-specific assessment. Nonetheless, the identification and determination of relevant species traits can be of great potential value where prospective risk assessment is aimed at the protection of endangered species, e.g. in the form of a checklist. The current Section provides a provisional outline for such a checklist, with the main aim to stimulate the discussion about its feasibility and further development. It should be stressed that most questions presented below cannot be answered in general, but should be answered for a combination of a particular endangered species with a particular potential stressor. This is particularly true for the questions addressing external exposure and toxicological sensitivity. The questions on recovery and indirect effect can only be answered if the direct effects of the potential stressors are known. After each question that is presented below, an indication is given of the type of information that is needed to answer the question.

8.4.1.1. Traits related to external exposure

The main parameters driving the external exposure of a species have been discussed in Section 4.2.1: (1) the exposure routes (e.g. food, ventilation, dermal contact), (2) the concentration level in the exposure media, (3) the exposure duration, and (4) the bioavailability of the potential stressor. The following questions could help exploring whether a specific endangered species has a higher external exposure than the exposure scenario applied in current ERA schemes:

Message to the public: please add tools and tests that can aid to fulfill datagaps on any of the below points.

Increased external exposure

- Is the endangered species likely to be exposed to the potential stressor via routes not considered in current ERA schemes? To address this question, one could look into e.g. food choice, ventilation media, other contact media, seasonal variation (see Section 4.2.1 example on sparrow hawk).
- Is the concentration level of the potential stressor in the exposure media (e.g. selective food items) of the endangered species likely to be higher than assumed for species addressed in current ERA schemes, e.g. because it dwells selectively in certain locations where concentrations occur? To address this question, one could look into e.g. measurements or predictions from multimedia fate, bioaccumulation and biomagnification models, or seasonal variation (see Section 4.2.1 example on kestrel).

- Does the endangered species live in a habitat in which the availability of the potential stressor is higher than assumed in current ERA schemes? To address this question, one could look into field measurements of the potential stressor, habitat characteristics or seasonal variation of the potential stressor in the field.
- Is it likely that circumstances occur under which the contact duration between the exposure media and the endangered species is likely to be longer than assumed in current ERA schemes? To address this, one could pay attention to life span, test duration, field exposure scenario etc.

Message to the public: please add tools and tests that can aid to fulfill datagaps on any of the below points.

Reduced external exposure

- Does active avoidance of the potential stressor occur? Little data will be available on endangered species, but the potential for food avoidance or escape behaviour could be obtained from toxicity tests carried out with other species.
- Is exposure to the potential stressor in the field partial or complete? Partial exposure occurs if the endangered species is also exposed to uncontaminated area or food, e.g. if the area of exposure and the home range of the endangered species overlap only partly, or if seasonal migration to uncontaminated areas occurs. This will reduce average exposure. To address this, one could pay attention to the location-specific exposure situation.

8.4.1.2. Traits related to (toxicological) sensitivity

Traits related to toxicological sensitivity are split up in traits governing toxicokinetics (i.e. the internal exposure) and traits governing toxicodynamics (i.e. the sensitivity of the organism to the internal exposure). More background information and relevant scientific literature is reviewed in Appendix C.

Internal exposure (toxicokinetics)

Species traits governing the internal dose can be life history characteristics (e.g. hibernation and molting) as well as traits on the genomic and biomolecular level (e.g. metabolic activity). The following questions may help to assess if the internal exposure of endangered species is likely to be higher than anticipated in the current ERA scheme:

- Is the endangered species small compared to the species considered in current ERA schemes? Does the endangered species have a higher intake rate of relevant exposure media (e.g. food, water, air) than the standard species considered in current ERA schemes? To address this question, one could look into field metabolic rate, food choice and seasonal variation.
- Is it likely that, once inside the endangered species (e.g. through the digestive system or the gills) but still outside the membrane of the organism, the potential stressor will be released more easily from relevant exposure media (e.g. food) than in the standard species considered in current ERA schemes? To address this question, one could look into food choice, bioavailability food matrix, lipid content food etc.
- Is the endangered species likely to have an increased uptake rate of the potential stressor compared to the species considered in current ERA schemes? To address this question, one could look into uptake rate (absorption capacity, e.g. fat content for POPs / active uptake rate for polar substances).
- Is it plausible that high contaminant uptake rates are compensated for by the endangered species through high elimination rates (excretion, metabolism, dilution)?

- Is the endangered species likely to have a lowered rate of metabolising the potential stressor compared to the species considered in current ERA schemes?
- Is the endangered species likely to have a lowered excretion rate of the potential stressor compared to the species considered in current ERA schemes?
- Does the endangered species have specific organs in which the potential stressor can accumulate over time? To address this, one could look at internal storage organs or sequestration.
- Is it likely that circumstances occur under which the accumulated potential stressor can later be released or remobilised internally resulting in harmful concentration levels? To address this, one could look at hibernation, lactation or seasonal variation in dietary intake.
- Does the endangered species have specific organs through which the potential stressor can be eliminated? To address this, one could look at molting, integument, etc.
- Does the endangered species have a higher growth rate compared to the species considered in current ERA schemes, resulting in more dilution?
- Does the endangered species have particular life stages with traits that may result in a relatively high internal dose. To address this, one could check for e.g. increased absorption, reduced elimination or different metabolism compared to the standard test species in the current ERA.

(Toxicological) sensitivity on the organism level (toxicodynamics)

Species traits governing sensitivity are related to the presence or absence of substance-specific receptors and the toxicity pathway involved, i.e. the cascade of adverse effects triggered by the substance after the primary lesion.

- Is it likely that the endangered species has molecular receptors which have a higher affinity for the potential stressor than those in the species used in standard toxicity tests?
- Does the endangered species have a higher number of molecular receptors than the species used in standard toxicity tests? To address this, one could look at the receptor density.
- Is it likely that binding of the potential stressor to the molecular receptor triggers a toxic mechanism which is more adverse in the endangered species than in the species used in standard toxicity tests? To address this, one could look at the toxicity pathways and AOPs.
- Does the endangered species have a reduced capacity to repair the adverse effects of the potential stressor (e.g. antioxidant capabilities, heat-shock proteins, DNA repair mechanism, metallothioneins. etc.) compared to the species used in standard toxicity tests?.
- Does the species have particularly sensitive life stages (e.g. embryos, larvae or juveniles), e.g. life stages with high affinity receptors, more receptors, other toxic pathways or reduced repair mechanisms?

8.4.1.3. Traits related to recovery

The recovery potential of the endangered species only needs to be assessed in cases where adverse effects on the species are expected. The reader is referred to the recovery opinion for a more detailed discussion of the species traits that govern recovery (EFSA SC, 2016b).

- Does the endangered species have a relatively slow reproduction rate? To address this question, one could look into age at reproduction, number of offspring and survival of offspring.

- Can the affected area easily be recolonised by other source populations? To address this question, one could look into site-specific spatial configuration of (potential) habitat and source populations, and dispersal capacity.
- Is there a periodic co-occurrence of sensitive life stages and high exposure events? To address this question, one could look into site-specific information on exposure patterns and life stages.
- Does the occurrence of adverse effects co-occur with other critical stressor events (e.g. food shortage, flooding, draught, temperature stress)? To address this question, one could look into site-specific information on exposure patterns and other stressors.

8.4.1.4. Traits related to indirect effects

Sensitivity to indirect effects is relevant in cases where a direct effect is expected on a species that has a relationship with the endangered species. The affected species may be a target as well as a non-target species of the potential stressor. The relationship between endangered and affected species can be quite diverse, e.g. a food source, a competitor or symbiont. Distinction can be made between one-way indirect effects and more complex indirect effects. One-way indirect effects are transmitted directly from the affected species to the endangered species. More complex indirect effects involve the transfer of effects to other species which in turn may affect the endangered species. This may involve several feedback mechanisms which can be quite complex. For the latter, ecological networks are relevant; see also Section 4.2.4. For one-way indirect effects, one could more easily check which endangered species might be affected by the potential stressor. For instance, if the stressor affects a particular species in the system, it can be checked which endangered species depend on this species as a food source or are otherwise strongly interacting with the affected species (see Section 4.2.4 for different interaction types). Such a check is difficult for complex indirect effects. Here, it might be helpful to check the position of endangered species in the food web and other ecological networks. Species that are high up in the food chain and depend on a limited number of food items (i.e. food specialists) might be more strongly affected by indirect effects. In more general terms, a species that is highly connected in an ecological network may have a higher chance of being affected, but it may also be more resilient to these changes because they may be compensated by other connections. Similarly, a species that is poorly connected may have a lower chance of being affected, but its resilience may also be smaller. The latter has been confirmed for real food webs in a study by Montoya et al. (2009). The mapping of the ecological networks of the endangered species seems a good starting point for assessing the vulnerability to indirect effects of potential stressors.

- Does the endangered species have a high position in the food web that is affected by the potential stressor?
- Is the endangered species poorly connected in an ecological network which is affected by the potential stressor?
- Does the endangered species critically depend on a species that is directly or indirectly affected by the potential stressor (e.g. in a food web, host-parasite network or plant-pollinator network)?

8.4.2. A trait database for endangered species

Application of the checklist presented in the previous Section requires extensive data on specific species traits. The data needed cover a wide range of traits, varying from generic life history traits to genetic traits, biomolecular traits and site-specific traits such as actual population size and habitat preference. Many different databases on species traits exist which may be used to retrieve part of the required data. Some of these databases are well established, e.g. on life history traits, others are in development, e.g. on genetic and biomolecular traits. The available databases have been set up to serve a variety of different purposes, and, as a consequence, the coverage of these databases in terms of species groups and species traits varies hugely. Below follows a tentative list of potentially useful databases.

8.4.2.1. Life history traits and distribution data

Question to the public: please add during the public consultation more relevantdatabases

- *Fishbase* (www.fishbase.org): Database with species names, geographical distribution and life history data on more than 28,000 species.
- *FishTraits* (www.fishtraits.info): Database of >100 traits for 809 (731 native and 78 non-native) fish species found in freshwaters of the conterminous United States. The database contains information on four major categories of traits: (1) trophic ecology, (2) body size and reproductive ecology (including life history), (3) habitat preferences, and (4) salinity and temperature tolerances.
- *TRY* (www.try-db.org): Database containing more than 5 million trait records for 1,100 traits of 2.2 million individual plants, representing 100.000 plant species.
- *LEDA* (www.leda-traitbase.org): Database on the life history traits of the Northwest European flora.
- *Polytraits* (http://polytraits.lifewatchgreece.eu/): A database on biological traits of polychaetes (bristle worms, Polychaeta: Annelida). It covers information about morphological, behavioural, reproductive and larval characteristics of polychaete taxa which has been collected from the literature.
- *BWARS* (www.bwars.com): A database on bees, wasps and ants, including a detailed account for each species, with distribution maps, photos, life history, conservation status and identification tips.
- *Life-history trait database of European reptile species* (http://scales.ckff.si/scaletool/ and doi: 10.5061/dryad.hb4ht).
- *Life-history trait database of European amphibians* (http://bdj.pensoft.net/articles.php?id=4123).
- *AnAge* (http://genomics.senescence.info/species/): A database of animal ageing and longevity.
- *European Bird Traits Database* (http://scales.ckff.si/scaletool/index.php?menu=6): The database contains data about 90 functional traits for each of 495 European bird species. Traits cover different groups of traits as morphological traits (wing size, bill size, weight etc.), reproductive traits (clutch size, number of broods, egg mass, length of incubation etc.) or traits relating to food or habitat preferences. It can be used for various analyses which deal with avian traits. Data are available on request only.
- *Ecological Traits of New Zealand Flora* (http://ecotraits.landcareresearch.co.nz/).
- *Lotic Invertebrate Traits for North America* (http://pubs.usgs.gov/ds/ds187/)): A database of traits for North American invertebrates. A total of 14.127 records for over 2.200 species, 1.165 genera, and 249 families have been entered into the database from 967 publications, texts and reports
- *Freshwater Biological Traits Database* (http://www.epa.gov/ncea/global/traits/): A database containing traits data for 3.857 North American macroinvertebrate taxa, and includes habitat, life history, mobility, morphology and ecological trait data. These data were compiled for a project on climate change effects on river and stream ecosystems.

8.4.2.2. Genome and biomolecular traits

- *EnSembl* (www.ensembl.org): This is a collection of databases with genome data for vertebrates and other eukaryotic species.
- *KEGG* (http://www.genome.jp/kegg/): A collection of databases containing metabolic pathways (372 reference pathways) from a wide variety of organisms (>700). These pathways are hyperlinked to metabolite and protein/enzyme information.
- *Cytochrome P450 Homepage* (http://drnelson.uthsc.edu/CytochromeP450.html): A database with data on cytochrome P450 types in different species.
- *MetaCyc* (http://metacyc.org/): A database of non-redundant, experimentally elucidated metabolic pathways. MetaCyc contains more than 1.100 pathways from more than 1.500 different organisms.
- *Reactome* (http://www.reactome.org/): A curated, peer-reviewed knowledgebase of biological pathways, including metabolic pathways as well as protein trafficking and signalling pathways. Reactome includes several types of reactions in its pathway diagram collection including experimentally confirmed, manually inferred and electronically inferred reactions. Reactome has pathway data on more than 20 different organisms but the primary organism of interest is Homo sapiens. Reactome has data and pathway diagrams for >2.700 proteins, 2.800 reactions and 860 pathways for humans.

Besides the species-oriented databases listed above, there are several stressor-oriented databases that could be consulted to assess the vulnerability of endangered species, particularly in the field of toxicological sensitivity. Databases providing ecotoxicological data of chemicals for test species used in standard ERA provide background information but do not specifically address endangered species. These include the eChemPortal hosted by the OECD, which allows simultaneous searching of reports and datasets by chemical name and number and by chemical property. Direct links to collections of chemical hazard and risk information prepared for government chemical review programmes at national, regional and international levels are available (e.g. US-EPA, ECHA). In addition, the eChemPortal provides exposure and use information on chemicals. Other databases include Chembase (www.chembase.com/), ChemIDplus (http://chem.sis.nlm.nih.gov/chemidplus/), ChemSpider: (www.chemspider.com/), Pubchem (http://pubchem.ncbi.nlm.nih.gov/), DSSTox (www.epa.gov/comptox/dsstox/), European chemical Substances Information System.

Approaches and databases in the field of Quantitative Structure Activity Relationships (QSARs) may also provide a useful background to assess the (toxicological) sensitivity of endangered species. QSARs are mathematical models that relate the structure of chemicals to their biological activities. Key databases for QSAR and read-across include the OECD QSAR Toolbox (http://www.qsartoolbox.org/), a hazard identification tool, which contains QSAR relationship methodologies that can be used to group chemicals into categories sharing the same structural characteristics and/or MoA. EFSA (2014c) has discussed a typical workflow, which would first examine existing data and information for possible read-across and grouping using the OECD QSAR Toolbox and the databases discussed above. A second step would be to predict metabolism in the relevant species (e.g. human, rat) using metabolism prediction tools such as the expert systems METEOR (LHASA), OASIS-TIMES or the US EPA MetaPath pesticide database. Another option is to use molecular modelling tools to conduct 3-D docking studies in potential target receptors and enzymes and these studies can also be used to build QSAR models (EFSA, 2014c).

Although all the databases listed above provide useful information on species traits, there is not one central database that contains all information and which is organised in such a way that it can be used to assess the ecological vulnerability of endangered species to potential stressors under assessment. Such a central trait database on endangered species could greatly support the coverage of endangered species in ERA schemes. The development of such a database requires careful planning, e.g. concerning the type of data to be included, quality control procedures and database maintenance. It is envisaged that such a database could evolve over time as the ecological vulnerability of more endangered species is being assessed in ERA. The set-up of such a database should preferably be in a cooperative network between interested parties (See Recommendation Section 10).

8.5. Ecological modelling to assess species vulnerability

Linking individual traits and behaviours to population and community endpoints using ecological modelling is a promising tool that can aid the risk assessment of potential stressors, for both endangered and other species. Recently, the PPR panel issued an opinion on good modelling practice (GMP) in the context of mechanistic effect models for risk assessment of plant protection products (EFSA PPR Panel, 2014b). The general concepts described therein also apply for other potential stressors.

In general, and except in higher Tiers occasionally, effect models are currently not used in ERA mainly due to the lacking validation. Nevertheless, recently experience with the application of mechanistic effect models in the ERA for assessed potential increased and examples of this can be found in recent special issues of scientific journals on this topic (Grimm and Thorbeck, 2014; Galic and Forbes, 2014). Building population (and community) models that account for potential stressor sensitivity (ideally including dose-response data and not only threshold values) might be particularly difficult for endangered species because less is known on their sensitivity to potential stressors. Moreover, data for model validation might be more difficult to obtain than for other species. With respect to this lack of data, a way to build trust into models in risk assessment might be to develop a model for a common species similar to the endangered one under consideration, for which more data and knowledge is available; demonstrate the models credibility by comparing modelling results with measured data; and then apply the traits of the endangered species. An important feature of the implementation of such a model would be that it is flexible enough to apply it to different species. Another important scientific criterion that needs to be fulfilled for models used to assess risks of potential stressors for endangered species is that they have to follow the principles of Good Modelling Practice (e.g. EFSA PPR Panel, 2014b).

Ecological models can be applied on different spatial scales in ERA. Examples range from modelling population dynamics in small artificial systems to investigate how toxicants act on individuals (Gabsi et al., 2014) to landscape scale applications to assess recovery (Topping et al., 2014). The choice of model type and spatial scale in model development for risk assessment should be guided by the question that is to be answered, hence by the problem formulation of the specific task. For example, a small scale TK/TD model might be useful to gain insights into what processes determine toxicological sensitivity and help to translate toxicity data from tested to untested (endangered) species. On the other hand, if the question is how effects on the individual translate to population level effects, population (or even community) models are needed. Even if the protection goal is at the individual level (e.g. birds and mammals), population level modelling could still be necessary to account for other ecological processes (e.g. indirect effects or density dependent factors). Additionally, the spatial scale of protection needs thorough consideration when investigating the impact of novel technologies or invasions on endangered species.

There are various possible applications in ERA where ecological modelling might be helpful:

Models can be particularly useful for upscaling effects on individuals to the population and landscape level. Additionally, modelling can be used to investigate the effectiveness of different management strategies. Furthermore, it can help to identify the importance of the potential stressor under assessment compared to other threats that act on the endangered species. However, even if the contribution of other factors like for instance habitat destruction might be much larger, the potential stressor under assessment might be the one that can be more easily managed.

When applying models for risk assessment, a crucial part is the scenario development, hence in what environmental context the model is run, which is defined by abiotic parameters (e.g. climate, soil properties), agronomic parameters (e.g. landscape structure, crops), and biotic parameters (e.g. competition, food level and including the viability state of population). Consequently, to prospectively address the impact of potential stressors and the potential for ecological recovery of endangered species, it may be required to develop both mechanistic effect models and environmental scenarios. These scenarios and models should not only take into account the traits of the endangered species that determine their susceptibility and recoverability, but also the spatio-temporal context of relevant landscapes of the endangered species at risk with its land uses and non-crop habitats and other potential refuges that may act as sources for recovery (e.g. the ALMaSS approach developed by Topping et al., 2003). To date, standard environmental scenarios for prospective ERAs that allow integrated fate and effect modelling are in their infancy, for both endangered and non-endangered species. Nevertheless, the GMP opinion (EFSA PPR Panel, 2014b) gives some general insights into scenario development. As there are many different combinations of environmental conditions that could be applied in the risk assessment, it would be essential to develop a set of realistic worst-case scenarios. The use of existing databases and modelling approaches provides additional tools that can be used to create realistic scenarios for ERAs

The availability of databases as repositories for life history (individual) and population level information on a wide range of species provide an essential resource to develop models and validate model predictions about risks to endangered species (e.g. http://www.freshwaterecology.info/; http://www.demogr.mpg.de/en/laboratories/evolutionary_biodemography_1171/projects/compadre_plant_matrix_database_comadre_animal_matrix_database_1867.htm).

One example of a widely accessible approach to scale traits and/or behaviours to the population level is through the use of matrix models (Caswell, 2000). These models are based on life cycle graphs that depict the essential stages in a population, the transition rates (survival) between stages and the reproductive potential of each stage. Each of these rates could be the product of detailed functions on individual traits. For example, the survival of juveniles to adults might be a function of an environmental stressor and the local abundance of juveniles. Figure 9 depicts a simple life cycle graph based on three stages (eggs, juveniles and adults).

From this, a matrix of transition probabilities and reproductive rates can be created and used to scale up to the population dynamics through time. The use of these sorts of models provides a standard way to assess the sensitivity of the dynamics to particular traits such as survival and fecundity (Caswell, 2000). Recent advances (e.g. integral projection approaches (Easterling et al., 2000)) in the use of such approaches makes them useful for adapting them to flexible scenarios for understanding the risks associated with the implementation of potential stressors under assessment.

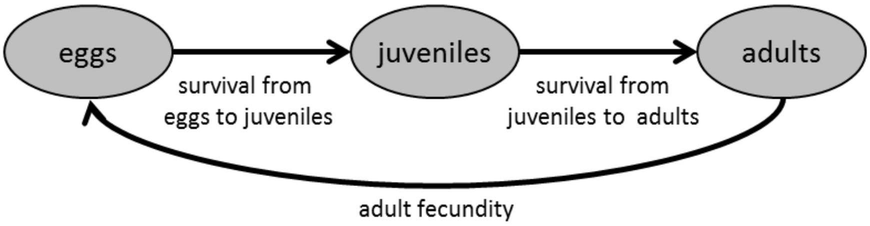


Figure 9: Hypothetical life cycle graph for three stages (eggs, juveniles and adults) with key trait variables (survival from eggs to juveniles, survival from juveniles to adults and adult fecundity)

Another example for techniques that has high relevance for the risk assessment of potential stressors to endangered species are agent-based (also called individual-based) approaches (DeAngelis & Gross, 1992; Grimm & Railsback, 2005). Such models take the individual organism as the unit of interaction. Entities in these models are ascribed traits or characteristics and interact with other individuals to give rise to collective behaviours and dynamics. Characterizing the individual traits is critical for these determine the higher level effects and hence predictions at the population, community and broader biodiversity scales. An example representation of this approach to ERA is illustrated in Figure 10. All relevant processes (e.g. including ageing, growth, movement, and predation) can be explicitly represented and the role of environmental stressors at the individual and population level can be evaluated under different scenarios (Loos et al., 2010).

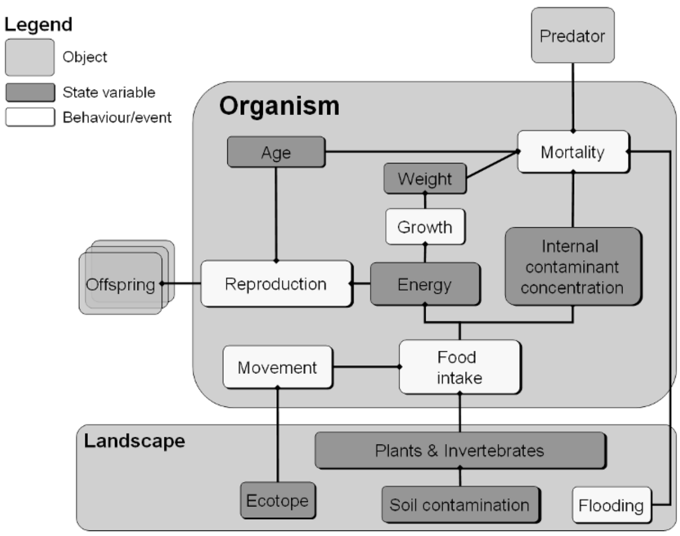
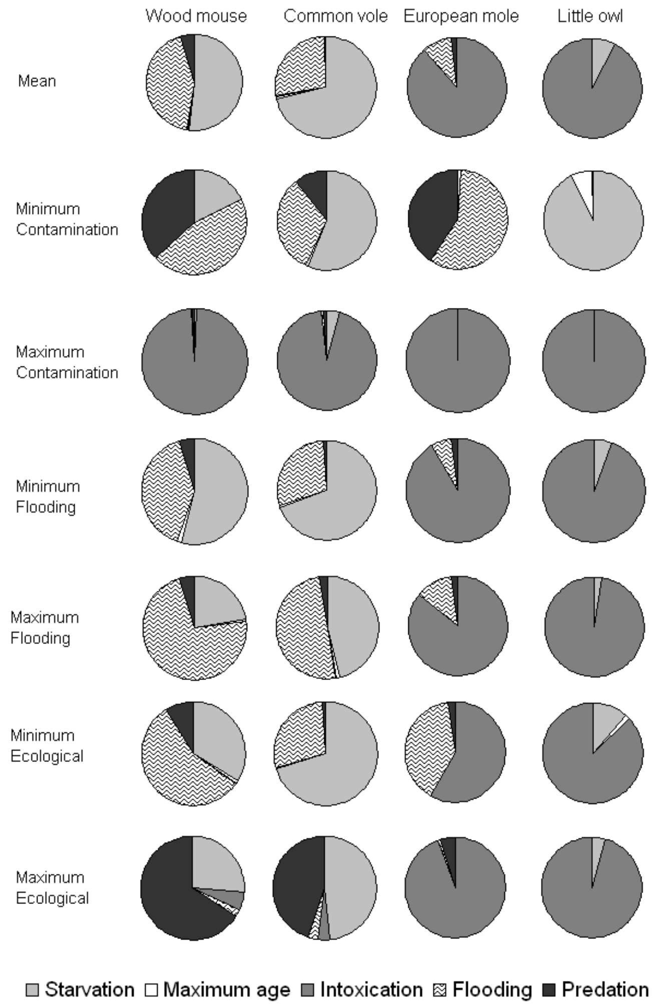


Figure 10: Schematic representation of an organism within a landscape with state variables (traits, other species, habitats) and behaviours[23].

[23] Reprinted from Loos et al, 2010, with permission from Elsevier

For example, Loos et al. 2010 examined the relative contribution of five ecological stressors (starvation, ageing, intoxication, flooding and predation) on mortality for the common vole, wood mouse, mole and little owl for different strengths of environmental stress. Results from individual-based modelling approaches provide visual impressions and quantitative support for the effect of different stressors (Figure 11).



Legend:

Figure 11: Relative contribution of starvation, ageing (maximum age), cadmium contamination (intoxication), flooding and predation to the mortality of 4 European mammals under seven different environmental stress scenarios (Mean: average stress scenario simulation. Minimum Contamination: minimum contamination stress simulation. Maximum Contamination: maximum contamination stress simulation. Minimum Flooding: minimum flooding stress simulation. Maximum Flooding: maximum flooding stress simulation. Minimum Ecological: minimum starvation and predation stress simulation. Maximum Ecological: maximum starvation and predation stress simulation)[23].

In conclusion, the type of model, the scenarios in which it is run and the spatial scale of the modelling depend on the precise question that is to be answered. Current effect models on both population and food-web level are generally not used in all ERA schemes. The SC advocates their adoption as one of the tools in developing a robust and quantitative approach to ERA for endangered species. The development of some standard models as well as the design of standard environmental scenarios might be helpful.

8.6. At what spatial scale can endangered species be addressed in ERA?

The following discussion explores the possible implementation of the scientific knowledge in this opinion. Without judging where this should be done, this discussion aims to give an insight in how ERAs at different spatial scales may help the protection of endangered species in the most efficient manner.

Endangered species not in the sphere of influence of the potential stressor

If there is no overlap between the sphere of influence of the potential stressor and the spatial distribution of endangered species, no further action is needed and there is obviously no need to consider the endangered species in an ERA. This concept is in parallel to the NAS scenario of no co-occurrence (NAS, 2013). This applies to any spatial scale, i.e. European, national or local. A lack of overlap between "the spatial distribution of the endangered species" and "the sphere of influence of the potential stressor" therefore seems a useful exclusion criterion for covering endangered species in ERA schemes at any spatial scale.

Endangered species in the sphere of influence of the potential stressor

When there is overlap, addressing endangered species should not *a priori* be limited to one particular spatial scale. This is because the appropriate scale depends on what is driving the ecological vulnerability of the endangered species, the sphere of influence of the potential stressor and the spatial distribution of the endangered species. These aspects are discussed in more detail here below.

Ecological vulnerability

The present opinion advocates the concept of ecological vulnerability to assess whether an endangered species is at risk of an potential stressor. However, ecological vulnerability is a multidimensional concept involving interactions between potential stressor, endangered species, landscape and other species, covering different levels of biological organisation, as well as different spatial and temporal scales. The appropriate level of assessment depends on what is triggering the ecological vulnerability. In first instance it is important to evaluate the relative importance of the potential stressor to the endangerment of a specific endangered species when compared to other stressors. If the potential stressor is considered important the causal relationship between the potential stressor and the vulnerability of the endangered species needs to be assessed, i.e. (1) a different/high exposure, (2) a high (toxicological) sensitivity, (3) a reduced recovery potential, and/or (4) a high sensitivity to indirect effects.

Exposure

There can be different reasons for an endangered species having a different/high exposure:
The endangered species is exposed through a route which is not covered in the standard ERA schemes. Identification of such highly specific exposure routes require: (1) the identification of media in which the potential stressor accumulates in relatively high concentrations, e.g. using fate and bioaccumulation models, and (2) the identification of endangered species that come into contact with

these exposure media, e.g. based on a database with feeding and behavioural characteristics of endangered species. Although the identification of such specific exposure routes can in theory be performed at any policy level, it seems most efficient to include this at a high level, such as the generic PPP assessments performed by EFSA or member states. However, more research and data is necessary to develop standard protocols for the identification of endangered species that may be at risk because of exposure through specific routes.
The specific local conditions (i.e. the combination of the use pattern of the potential stressor and the spatial distribution of the endangered species) result in a relatively high exposure. Such a situation should typically be addressed at the local level.

(Toxicological) sensitivity
There are several reasons why an endangered species can have a high (toxicological) sensitivity to an potential stressor, i.e.:
The species has TK/TD traits which make it particularly sensitive, e.g. the lack of detoxification pathways or the presence of highly sensitive molecular receptors. Identification of highly sensitive species based on TK/TD traits requires: (1) detailed knowledge about the (physicochemical) properties of the potential stressor, (2) detailed knowledge about the species traits that drive the interaction between potential stressor and organism. Although the identification of particularly sensitive endangered species can in theory be performed at any policy level, it seems most efficient to include this at a high level, such as the generic single-stressor assessments performed by EFSA or Member States. However, more research and data is necessary to develop standard protocols for the identification of endangered species that may have a high sensitivity to particular potential stressors because of particular TK/TD traits.
The specific local conditions result in an increased (toxicological) sensitivity of the endangered species, e.g. because of exposure to other physical and/or chemical stressors. Such a situation should typically be addressed at the local level.

Recovery
Assessment of recovery is relevant only if direct toxic effects on a particular endangered species are being expected. Recovery strongly depends on the configuration of the landscape, i.e. the application area and regime of the potential stressor and the spatial distribution of the endangered species. As such, it seems most logical to consider recovery of endangered species in regional or local assessments in which the spatial configuration of the landscape can be explicitly accounted for. Assessment of recovery at a national or EU scale seems relevant only for affected endangered species with a very large foraging range or species which are distributed over a large geographical area. However, both conditions seem unlikely since adverse effects on species that have a large foraging range would generally not be considered acceptable, and species which are distributed over a large geographical area are generally not endangered.

Indirect effects
Assessment of indirect effects is relevant if (i) a direct effect is expected on a species that is in turn directly or indirectly interacting with the endangered species or (ii) the stressor affects the state or functioning of the ecosystem which affects the endangered species. Knowledge about the ecological network of the affected and the endangered species is a prerequisite to assess indirect effects. Ecological networks typically have a strong local dimension (i.e. spatial configuration and presence of other species), although generic mechanisms may also be relevant for the identification of endangered species at risk of indirect effects. Since the knowledge base to assess indirect effects is still limited, it is proposed to assess potential indirect effects currently on a case-by-case basis.

It is concluded that the coverage of endangered species in ERA schemes cannot *a priori* be limited to one particular spatial scale. The higher spatial scale, such as considered in the substance-specific generic assessment of PPPs and FAs performed by EFSA and the Member States, seems the most appropriate level to identify endangered species which may be at risk because of highly specific exposure routes or a high toxicological sensitivity. Lower spatial scales, such as considered in site-specific and/or local assessments, seem most appropriate to cover the other dimensions of ecological vulnerability, i.e. (1) increased exposure due to location-specific conditions, (2) increased (toxicological) sensitivity due to location-specific conditions, and (3) recovery. Since the knowledge on the propagation of direct into indirect effects on endangered species is still limited, it seems most efficient to currently address these on a case-by-case basis on any spatial scale. For any assessment, a lack of overlap between “the sphere of influence of the potential stressor” and “the spatial distribution of the endangered species” is a useful exclusion criterion to cover endangered species.

8.7. Specific options

Given the ecological questions reviewed in Sections 4 and given the findings in Section 5, here below are possible options to extend specific ERA schemes for coverage of endangered species. These are theoretical options, presented as scientific information. Their feasibility or implementation is not assessed in this scientific opinion, since it is not a guidance document.

8.7.1. Options for ERA of PPPs

Following the general aspects mentioned in Section 5.1, coverage of endangered species on PPP risk assessment could further expand on the four aspects of species vulnerability (1) toxicological sensitivity, (2) probability of exposure, (3) recovery potential and, (4) toxicological sensitivity to indirect effects. The actual state of the respective population of an endangered species at time of exposure is also key information which should be considered, notably in a site-specific assessment. This could result in the refinement of existing assessment factors applied to ecotoxicological data obtained in the generic Tiered ERA schemes where needed. Alternatively, one can undertake a case-specific assessment. This may be especially important if the generic influences addressed in Section 4.2.6 (e.g. other stressors, population size, genetic diversity and habitat characteristics) are the driving forces behind the higher vulnerability.

On the sensitivity area, the exemplified approaches mentioned above, i.e. evaluating the efficiency of AFs in providing an adequate coverage for endangered species and literature reviews on comparing effects on standard species versus endangered species, could be extended. This can be done either by comparing sensitivity of species from the same taxonomic group or from different groups (e.g. such a check has been done for larval stages of amphibian species and, with the exception of a few compounds, their sensitivity could be tackled by the sensitivity shown by fish species (Fryday and Thompson, 2012; Weltje et al., 2013)). Despite the possible drawbacks of those approaches, namely the possible shortage of data, it could be useful to extend the knowledge to other groups of organisms to check if some sensitivity response patterns among or within organism groups can be seen, thus helping improving AF values.

However, a promising approach to tackle toxicological sensitivity is to use an approach based on analysing key traits influencing toxicological sensitivity to PPPs with specific MoA and comparing them between endangered and standard species, rather than an assessment based on surrogate species at taxonomic level. The advantage of this approach is that it can tackle not only sensitivity, but also the other 3 aspects of species vulnerability, since traits involved in exposure, recovery and indirect effects can also be listed. The examples and framework proposed for the aquatic compartment (Rubach et al., 2011; Van den Brink et al., 2013) and the few data existing for the in-soil compartment (e.g. Chelinho et al., 2011; 2014) provide promising expectations to this approach. Allied to the use of ecological models, this approach could better decipher the species-substance interactions and predict toxic effects at different levels of biological organisations i.e. individual, population, community and ecosystem level, from the receptor to the landscape level.

For this it is necessary to invest in data gathering, either via experimentation or literature reviews, to identify those traits involved in the processes of species vulnerability. Some trait databases do exist (or will become available soon), but they should be checked for data gaps and/or be extended to the groups of organisms of interest to PPP risk assessment.

Furthermore, the development of ecological modelling approaches at different levels of biological organisation (from individuals to populations and communities) and spatial scale (landscape level) could be done in order to include the key trait-based information defining vulnerability as input variables.

On the exposure side, a more in-depth knowledge of the different exposure routes linked to endangered species might be necessary (at least for endangered species with exposure routes that could be different from those usually considered in conventional risk assessment). Besides, a better assessment of the spatio-temporal co-occurrence of the species and the magnitude of exposure might also be necessary.

8.7.2. Options for ERA of GMOs

For the purpose of covering endangered species more explicitly, it can be suggested that during the problem formulation, risk assessors consider and report on all the scenarios of harm presented in Section 5.2.2. Depending on the level of available information/data on both hazard and exposure to support the ERA of endangered species, three ERA scenarios could be distinguished: (1) relevant hazard data are available but little or no exposure data; (2) an equal amount of hazard and exposure data exist, which may be sufficient or not to support the ERA of endangered species; and (3) available knowledge mainly cover exposure but hazard is not well characterised. Depending on the scenario, different ERA approaches could be followed with a different emphasis on either hazard or exposure.

In Section 5.2., it has been pointed out that during hazard characterisation, data generated with focal species might be extrapolated to endangered species. However, it would be recommended to make sufficiently conservative assumptions when using focal species. Theoretically, these conservative assumptions could involve the use of lower trigger values, worst-case exposure scenarios (e.g. in first-Tier laboratory studies with GM insect resistant (IR), use >> 10 × EEC), or specific measurement endpoints (e.g. sub-lethal endpoints, such as developmental time or reproduction).

Mathematical models are used to estimate environmental impact of GM IR plants on non-target Lepidoptera species (e.g. Perry et al., 2010; 2012). However, there is little data concerning sub-lethal effects on non-target species to parameterise such models (Perry et al., 2010). More complete datasets would be needed to refine the estimates of the models and thus to reduce uncertainties.

During the initial phase of the ERA, it is essential to identify endangered species potentially present in the receiving environment (if any). Databases of arthropod species can be used to identify abundance of endangered arthropods found in crops and field margins. A database of bio-ecological information on arthropod fauna in arable crops across Europe was established by Meissle et al. (2012). The database contains ecological information (on the taxonomy, geographical distribution, abundance, habitat, ecological function, and feeding guild of each species) for 3030 species representing 278 families and 30 orders, nine of which are species listed in the IUCN red list. However, the database presents some limitations and abundance records of arthropods occurring in field margins are scarce. Such databases could be expanded and updated to ensure its usefulness. Its potential use to support ERA and monitoring of transgenic plants is discussed in Romeis et al. (2014).

Species associations of wild relatives of crops are scarce and generally lack the less frequent and endangered species that would constitute targets for any hazard assessment. Thus it is required to identify associated non-target species that are most vulnerable to hazards arising from gene flow (Raybould and Wilkinson, 2005). A comprehensive knowledge and understanding of known associates of the crop wild relatives would also assist the process of prioritizing efforts to screen for likely consequences and where necessary, to trigger the instigation of corrective measures (Wilkinson and Tepfer, 2009).

When risks to endangered species are identified during the ERA, mitigation strategies should be clearly defined to reduce the spatial and temporal exposure of the endangered species to the potential stressor under assessment and to reduce the level of uncertainty. For instance, isolation distances from neighbouring areas where Lepidoptera of conservation concern have been identified, or sowing of strips of non-Bt maize around field edges. Remaining identified risks and risk management measures should be considered when formulating the mandatory PMEM plans. Ongoing specific monitoring of endangered species should serve to assess their status over time.

8.7.3. Options for ERA of IAS

The approach proposed in the Guidance on the ERA of IAS (EFSA PLH Panel, 2011) emphasises the need for an integrated assessment by focussing the analysis at the level of ecosystem services and biodiversity. For this purpose the analysis is conducted at large spatial scales with a low spatial and temporal resolution. The large scale at which the processes and the impacts are considered is a distinctive characteristic of the ERA-PLH approach compared to other ERA schemes in use in other EFSA Panels. Often the worst case scenario is the only considered scenario as the first indication of the possible risk posed by a new IAS, and no other details on the variability and heterogeneity of the impact are given.

However, there is scope for the PLH Panel to work more into the direction of developing assessments at higher spatial and temporal resolution. Convergence of the approaches of the PLH Panel and those followed by other Panels in EFSA working at higher spatial and temporal resolution should be encouraged. A first step in this direction was taken in the scheme proposed in the ERA for *Pomacea* spp. (EFSA PLH Panel, 2014) where the impact assessment can be conducted at the level of ecological traits. In principle, this also considers the distribution and the abundance of a species as well as the distribution of species/taxon traits in a community/ecosystem.

The assessment scheme proposed in the ERA for *Pomacea* spp. (EFSA PLH Panel, 2014) can then be further developed as a structured methodological framework in which the population pressure of the IAS is directly related to the impact on a single or a group of endangered species. The use of a scenario analysis may limit the complexity of ecological relationships to be considered. The consideration of resistance and resilience of the recipient ecosystem, as well as the role assigned to the pest management, contribute to the definition of the pressure of the driving force (pest population abundance/prevalence) on the endangered species. A suitable definition of the impact of the IAS in terms of spatio-temporal extent and resolution may support the convergence of the analysis conducted in the PLH Panel and that of other EFSA Panels.

In the ERA assessment scheme described in the apple snail opinion (EFSA PLH Panel, 2014), a methodology is proposed to represent the dependence of the ecological traits of interest for endangered species from the population pressure of the driving force (IAS). In the opinion the change in ecological traits is described by simple linear and non-linear functions depending on the variation of the density of the apple snail normalised to its maximum expected value. The availability of this functional relationships together with the potential distribution of the IAS population pressure obtained by population dynamics model, has been used to produce maps of the potential impact of IAS on ecological traits related to endangered species (EFSA PLH Panel, 2014). Such maps would have great potential in ERA schemes as they have high spatial resolution and cover the whole of Europe, and are linked to maps assessing ecosystems and their services.

8.7.4. Options for ERA of FAs

As indicated in Section 5.4.3, the ERA approach used by the FEEDAP Panel does not tolerate effects on any species in the environment, including those that are endangered. Despite the uncertainties indicated there, the consulted FEEDAP Panel could not foresee options that would further ensure the safety specifically for endangered species from additives in animal feeds. There are, however, options which could be considered to further ensure the safety of feed additions to the environment. These could include a modification in methods to test safety to soil organisms and introduction of safety testing of organisms living on dung. Such improvements should also benefit endangered species. The SC notes that no information is available to which extent endangered species are protected. Many of the issues/options noted for the assessment of PPPs are also applicable for the assessment of FAs. These will be considered when the FEEDAP guidance for testing of safety to the environment is reviewed.

8.8. Conclusions on options to extend coverage of endangered species in ERA

Potential approaches for current ERA schemes to extend the coverage endangered species in current ERA schemes include:

- Explicit inclusion of endangered species in ERA schemes would require a detailed specification of the protection goals for endangered species, particularly in terms of what species (groups) should be protected where and when, to what level and with what level of certainty. Establishment of these specific protection goals for endangered species requires a joint coordinated effort involving risk managers, risk assessors, scientists and other stakeholders.

- Different approaches can be followed to cover endangered species in ERA schemes. There is not one approach that suits all EFSA sectors (i.e. PPR, GMO, PLH and FEEDAP). For example, the use of surrogate species is frequently applied to assess GMOs, whereas a generic protection level in combination with a species-specific trait-based assessment (the vulnerable species concept) is more often used in the assessment of PPPs.

- Trait-based approaches, in which species traits are being used as indicators of potential (increased) risk, provide promising opportunities for including endangered species in ERA schemes. Further exploration and elaboration of the potential of this type of approaches is strongly recommended, i.e.:

    - Identification and validation of species traits that drive the ecological vulnerability of endangered species for different types of potential stressors, i.e. traits related to exposure, stressor sensitivity, recovery and sensitivity to indirect effects;

    - Development of a systematic procedure in which species traits are being used to obtain a qualitative and/or quantitative estimate of the environmental risk of potential stressors for endangered species;

    - Construction of a species trait database that can be used as a basis to assess the ecological vulnerability of endangered species for different types of potential stressors.

- The rapid advancements in "omics" and "in silico" techniques are resulting in large amounts of data which provide information about the molecular mechanisms and species traits that drive the sensitivity of organisms to potential stressors. Current practical and ethical limitations involved in testing endangered species in the field or the laboratory can be overcome if this type of information can be applied in a predictive way, i.e. to predict the sensitivity of species based on molecular traits regarding the phylogenetic relationships between endangered and non-endangered species of the same group.

- Mathematical models linking individual species traits and behaviours to populations, communities and landscapes provide a promising tool that can aid the risk assessment of potential stressors for endangered (if information on the actual impairment of the population is available at the ecologically relevant spatial scale) and other species.

- Because the coverage of endangered species in ERA schemes cannot *a priori* be limited to one particular spatial scale, risk assessment might need be conducted at different spatial scales. This also depends on the overlap between the sphere of influence of the potential stressor and the occurrence of the endangered species.

9. OVERALL CONCLUSIONS

What is an endangered species?

No generally accepted definition is available for endangered species since endangerment is related to spatio-temporal scales. In this opinion an endangered species is defined as a species that is either:

a) listed in one or more "red lists" as threatened (i.e. vulnerable, endangered, or critically endangered, or variants thereof), where the considered red lists are: (i) the European Red List, (ii) the global IUCN Red List of Threatened Species, and (iii) national and other regional red lists within Europe that follow the IUCN or another suitable classification scheme; or
b) rare based on the classification of Rabinowitz's seven classes of rarity (including "endemics", "classic rarity", "habitat specialists" and "truly sparse" species).

Are endangered species more vulnerable?

Examples show that endangered species can be more vulnerable than other species due to particular traits related to exposure, recovery and/or sensitivity to the potential stressor directly or to indirect effects. In general, endangered species are considered more vulnerable in view of general characteristics. Together with the general influences, related to habitat destruction and low genetic variation and low population sizes, there is theoretical evidence that some endangered species are likely to be more vulnerable than the standard species or the vulnerable taxa currently considered in ERAs. However, there is too little data to make empirically-based conclusions on whether endangered species are generally more vulnerable.

Endangered species often have a slow life history and show low intraspecific variability in their life-history traits as compared to other species. Often they are habitat specialists and have small geographic ranges or are diet specialists. In addition, top predators tend to be more often endangered than other species. A vulnerable species is a species with a relatively high sensitivity for the chemical at hand, high exposure and/or a poor potential for population recovery. Vulnerability can also be induced by indirect effects when the occurrence of a species depends on another species that is directly or indirectly influenced by the occurrence of that species. No convincing scientific evidence was found indicating that endangered species have in general a higher exposure than other species.

The SSD examples and the TK/TD considerations presented in this opinion do not provide conclusive evidence that endangered species are *per se* more sensitive towards potential stressors than other species. However, the anecdotal examples presented illustrate that species differences in toxicological sensitivity can, at least partly, be explained by differences in TK/TD mechanisms and traits, e.g. Since many of the endangered species are highly specialised, e.g. in their food or habitats, they may only have been exposed to a restricted range of natural chemicals and toxins, therefore resulting in the phylogenetic loss of certain detoxifying pathways relevant for potential stressors.

It is important to note that the statement above reflects only the toxicity part of vulnerability (short term exposure) and the other components of vulnerability remain at least equally important e.g. odonata are not very sensitive, but their long life cycle is not taken into account in laboratory studies.

It appears that not the potential stressor or the endangered species *per se* may be decisive for ecological recovery from impact, but their interaction with (the properties of) the environments impacted by stressors, in which endangered species (temporarily) dwell. However, it seems that endangered species more often exhibit traits that are related to a decreased ability for recovery (e.g. they often have a slow life history).

Most studies in invasion biology focused on the traits of IAS (specifically, traits related to their invasion success) or those of ecosystems (specifically, traits related to their vulnerability against IAS), whereas studies looking at traits of native species related to their vulnerability against IAS are rare. Regarding the latter, it is likely the type of interaction with IAS that makes them vulnerable and the lack of "eco-evolutionary experience" they have in interacting with such species.

Some endangered species, appear to suffer more from indirect effects than many non-endangered species, but due to their complex nature, indirect effects can be better evaluated from a case-by-case perspective.

Are endangered species appropriately covered in the current ERA schemes at EFSA.

There are four types of potential stressors undergoing ERA within EFSA's remit and (mainly) in an agricultural context: PPP, GMOs, IAS and FAs.

For GMO and IAS, the protection of endangered species is explicitly dealt with during the problem formulation phase in the respective ERA schemes. These ERA schemes allow a tailor made-assessment and the selection of one or more relevant endangered species.

For PPP, the PPR Panel adopted an approach to species selection for prospective risk assessment using (or leaving the option for) the concept of vulnerable species. Only in a few cases, specific groups of endangered species are explicitly mentioned in the guidance documents on the ERA for PPPs, such as rare plants and amphibian larval stages. Endangered species are assumed to be implicitly covered by the vulnerable species approach. Furthermore, examples in this opinion demonstrate that while part of the endangered species are covered by this approach (for instance fish and aquatic amphibians), others may not be (see the reasons set out in Section 4).

For FAs, the ERA does not tolerate population effects on any species in the environment and, thus, endangered species are implicitly included by the assumption that no FA is allowed on the market should a species be at risk.

Formulate as a recommendation,rather then in the conclusions. RS work with Jose. And maybe include after the PC.

Thus, it currently varies among EFSA ERA schemes to which degree (implicit or explicit) endangered species are covered and how they are covered.

The level of protection afforded by these four ERA schemes for endangered species seems to vary. However, the limited data availability does not allow to draw a firm conclusion and also does not allow an assessment of the level of protection achieved (regardless whether endangered species are implicitly or explicitly covered).

Hence, risk assessment is conducted via selected (test) species, with assessment factors and extrapolations to endangered species (bottom-up approach). There is however a growing need for a landscape assessment (per potential stressor) plus population modelling (top down approach).

Plant Protection Products

- With the few exceptions of rare plants and amphibian larval stages, endangered species are not explicitly covered in the guidance documents on the RA for PPPs. However, endangered species might be taken into account when addressing vulnerable species during the assessment and defining specific protection goals in the future based on species vulnerability aspects (toxicological sensitivity, probability of exposure, recovery potential and responsiveness to indirect effects).
- With respect to toxicological sensitivity, it was investigated if AFs cover for endangered species:
    - First Tier risk assessment for PPPs which is based on standard test species and standard assessment factors appears to provide varying levels of protection when comparing laboratory toxicity tests for different organism groups.
    - If the aim of the risk assessment for plant protection products is for example to protect at least 95% of the species in any taxonomic group, it appears that the assessment factor is consumed by the uncertainty from the between species variability. This means that there is no room for other sources of uncertainty in these AFs. For fish there is still some room left for other uncertainties.
    - It is evident that in case an AF is not covering the uncertainty for a general risk assessment it is also not covering the uncertainty in a risk assessment for endangered species.
    - Testing of surrogate species with the plant protection product could improve slightly the outcome of the risk assessment but the gain in knowledge is only marginal and still needs a comparable high AF as for non related species.

Genetic Modified Organisms

- Endangered species are regarded as entities of concern that need to be protected and are explicitly addressed in the problem formulation of GMO ERA. This cascades down to the scenarios used for exposure and hazard assessment and the selection of risk hypothesis to be tested during ERA.

Invasive alien species

- In the risk assessment of IAS effects on endangered species are an essential part of the ERA scheme. In the proposed RA approach, one central question to be answered is to what extent rare or vulnerable species (defined as all species classified as rare, vulnerable or endangered in official national or regional lists within the risk assessment area) are expected to be affected as a result of invasion.

Feed additives

- The technical Guidance for assessing the safety of feed additives for the environment does not mention endangered species in the protection goals or elsewhere. However, the FEEDAP ERA does not tolerate effects on any species in the environment and, thus, endangered species are implicitly included and are assumed to be protected.

Mitigation and monitoring

Protecting endangered species needs in some cases specialised mitigation and monitoring measurements and will be tackled in a site specific manner (e.g. specific conservation areas for weeds/hamsters in specific crops; financial compensation of farmers to implement specific land-use requirements that favour the red list species).

Two objectives of mitigation can be distinguished:

(1) to achieve a safe use of the product under assessment,

(2) specific risk mitigation measures that can be proposed as a result of observations from monitoring schemes.

There is a suite of possible risk mitigation or management measures available ranging from small scale to well organised and remunerated incentives. Some of the risk mitigation measures are in the hands of local authorities rather than being implemented on the EU level, but consistency in such local implementation is considered important.

For PPPs, the farmers will be in most cases the in-field risk managers; their education and training should be supported. The importance of risk mitigation measures should be well communicated and emphasised in the farmer communities.

A priori, it is considered important to monitor the level of protection achieved by all management measures or mitigation measurements taken to protect endangered species (either compliance or supplementary monitoring). At present only the GMO Panel is actively involved in regulated monitoring of the potential stressor. For invasive species, surveillance and monitoring is advisable in any case. For PPP and feed additives, EFSA is currently not involved in monitoring. At the member state level information on chemical and biological monitoring, for instance conducted within the context of the Water Framework Directive (WFD), may be used in the re-registration of the PPP.

Risk assessors would benefit from feedback of those monitoring schemes whether the proposed mitigation or management measures where adequate or not. This information is not always available (except to a certain extent for GMOs, for which yearly monitoring reports are submitted to EFSA).

Options to extend coverage of endangered species in ERA

It is the opinion of the SC that when endangered species are at stake, prospective ERA of potential stressors most probably cannot solve the situation alone, nonetheless, a better coverage of endangered species would be important and can be realised in the following ways:

- Explicit inclusion of endangered species in ERA schemes would require a detailed specification of the protection goals for endangered species, particularly in terms of what species (groups) should be protected where and when, to what level and with what level of certainty. For the context of EFSA's ERA's, which is primarily ERA in an agricultural context, establishment of these specific protection goals for endangered species requires a joint coordinated effort involving risk managers, risk assessors, scientists and other stakeholders.
- Different approaches can be followed to cover endangered species in ERA schemes for EFSA's remit. There is however not one single approach that suits all EFSA sectors (i.e. PPR, GMO, PLH and FEEDAP). For example, the surrogate species concept is frequently applied to assess GMOs, whereas a generic protection level in combination with a species-specific trait-based assessment (the vulnerable species concept) is more often used to assess PPPs.
- Trait-based approaches, in which species traits are being used as indicators of potential (increased) risk, provide promising opportunities for including endangered species in ERA

schemes. Further exploration and elaboration of the potential of this type of approaches is strongly recommended, i.e.:

  - Identification and validation of species traits that drive the ecological vulnerability of endangered species for different types of potential stressors, i.e. traits related to exposure, stressor sensitivity, recovery and sensitivity to indirect effects;

  - Development of a systematic procedure in which species traits are being used to obtain a qualitative and/or quantitative estimate of the environmental risk of potential stressors for endangered species;

  - Construction of a species trait database that can be used as a basis to assess the ecological vulnerability of endangered species for different types of potential stressors.

- The rapid advancements in “omics” and “in silico” techniques are resulting in large amounts of data which provide information about the molecular mechanisms and species traits that drive the sensitivity of organisms to potential stressors. Current practical and ethical limitations involved in testing endangered species in the field or the laboratory can be overcome if this type of information can be applied in a predictive way, i.e. to predict the sensitivity of species based on molecular traits regarding the phylogenetic relationships between endangered and non-endangered species of the same group.

- Mathematical models linking individual species traits and behaviours to populations, communities and landscapes provide a promising tool that can aid the risk assessment of potential stressors for endangered (if information on the actual impairment of the population is available at the ecologically relevant spatial scale) and other species.

- Because the coverage of endangered species in ERA schemes cannot *a priori* be limited to one particular spatial scale, risk assessment might need be conducted at different spatial scales. This also depends on the overlap between the sphere of influence of the potential stressor and the occurrence of the endangered species.

10. RECOMMENDATIONS FOR FUTURE STUDIES

The present opinion investigated the coverage of endangered species in current ERA schemes. Having looked at the available data, a number of questions and recommendations have been raised with the following priorities.

Trait-database

The trait-based approaches are a promising tool to cover (endangered) species in ERA schemes but before it can be operationalised, more research is necessary. The establishment of an integrated database can support the identification of relevant traits (e.g. though a systematic study to identify species traits that drive the vulnerability of endangered species), the centralisation of information and making it accessible and available to the public.

Since a trait-based approach can offer an alternative or complementary approach to include endangered species in ERAs, the SC recommends creating an integrated trait database with mainly significant traits. This is however not the sole responsibility of EFSA, but of all agencies that are involved in ERA (e.g. EEA, EMA, ECHA and/or other international organisations and/or commission services like JRC). This recommendation needs to be discussed with other organisations to construct a common framework and a solution should be found to work together on this topic.

It was shown during the development of this opinion that some highly physiologically specialised species lack certain detoxifying pathways or isoforms of enzymes. This insight may form the basis for a higher toxicological sensitivity of endangered species, since many of the endangered species are highly specialised. It is recommended to further explore this line of reasoning, e.g. in an explorative study in which the TK and TD traits of endangered species are compared with the TK and TD traits of other species, such as the standard species used in toxicity tests.

A comparison of demographic and recolonisation traits between endangered species and vulnerable non-endangered species should shed some light on the question whether endangered species exhibit traits that influence population growth rate relevant for both internal and external ecological recovery.

Protection goals

EFSA recommends to hold a stakeholder workshop for setting specific protection goals for endangered species. It would be necessary to develop a limited number of options for specific protection goals of endangered species, including an indication of their socio-economic consequences, that could serve as case-studies at the workshop.

For the purpose of this opinion, critical subpopulations are loosely defined as subpopulations that are essential for the survival of the endangered species in a particular area. When this concept is further operationalised, ecological criteria are needed to distinguish between ecologically critical and non-critical subpopulations, as well as (monitoring) data to assess the status of a subpopulation in a particular area.

Scenario development

When applying models for risk assessment, a crucial part is the scenario development specifying in what environmental context, defined by abiotic, biotic and agronomic parameters, the model is run. The GMP opinion (EFSA PPR Panel, 2014b) gives some general insights into scenario development. As there are many different combinations of environmental conditions that could be applied in the risk assessment, it would be essential to develop a set of realistic worst-case scenarios. The use of existing databases and modelling approaches provides additional tools that should be incorporated into ERAs. The focus here should be on population effects, about exposure, indirect effects and recovery.

Including more data

During the drafting of this opinion, the requirement for SSD data was discussed. These are readily available for old substances that are typically data-rich, but not for newer substances or biologicals such as GMOs. The potential of the available sensitivity data should be exploited fully to draw more robust conclusions on the positions of endangered species in the SSD than it was possible for this opinion. Newly generated data, from other stressors than PPP, should be included. Likewise, modelling approaches (including “omics” and “in silico” techniques) should be further advanced.

Regarding surrogates species, as demonstrated for PPPs in Section 5.1.2.2, using closely related species in toxicity tests does not necessarily increase the level of protection. However, it is advisable to identify what type of tests surrogates may yield more substantial gain in protection compared to standard test species.

Finally, the exposure to multiple stressors is currently not addressed in the individual ERA schemes, while endangered species, like other species, are exposed to multiple stressors (regulated or non-regulated; multiple routes of exposure, simultaneous or sequential). For the future, a more holistic approach should be aimed at. Steps into this direction could be: (i) consideration of multiple exposure routes and times of one stressor, (ii) consideration of multiple stressors of the same group (e.g. tank mixtures of PPPs, exposure from different fields) as well as different potential stressors, (iii) inclusion of additional (non-regulated) stressors into modelling scenarios (e.g. habitat availability, food levels).

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GLOSSARY

Adverse (environmental) effects: Any effect that causes harm to the normal functioning of plants or animals. Establishing what an adverse effect is and which effect is regarded as environmental harm is a complex process of also analysing and implementing policy objectives taking into account broader societal and relevant stakeholder values. It requires that risk managers define what is important to protect and the magnitude of the effect that is to be regarded as harmful or unacceptable.

Agricultural environment: Land used for crops, pasture, and livestock; the adjacent uncultivated land that supports other vegetation and wildlife; and the associated atmosphere, the underlying soils, groundwater, and drainage networks (Kattwinkel et al., 2012).

Alien species: According to the EU Directive on Invasive Alien Species: 'alien species' means any live specimen of a species, subspecies or lower taxon of animals, plants, fungi or micro- organisms introduced outside its natural range; it includes any part, gametes, seeds, eggs or propagules of such species, as well as any hybrids, varieties or breeds that might survive and subsequently reproduce, (see also invasive alien species).

Assessment endpoint: an explicit expression of the environmental value that is to be protected. Operationally, it is defined by an identified environmental entity of value that is susceptible to harm and an attribute that provides evidence of harm (Wolt et al., 2010).

Assessment factor: numerical adjustment used to extrapolate from experimentally determined (dose-response) relationships to estimate the exposure to an agent below which an adverse effect is not likely to occur.

Autoecology: is the ecology of a single species, that is the relations between that species and its environment, how the species affects the environment and how it is affected by the environment. Autoecology includes for example population ecology.

Biodiversity: the variability among living organisms from all sources including, inter alia, terrestrial, marine and other aquatic ecosystems and the ecological complexes of which they are part; this includes diversity within species, between species and of ecosystems.

Biomagnification: is the process whereby the tissue concentrations of a contaminant increase as it passes up the food chain through two or more trophic levels. It is a typical issue for persistent chemicals with a high affinity for fat tissue and/or that are poorly metabolised or excreted.

Case-by-case: is defined as the approach by which the required information may vary depending on the type of the potential stressor concerned, its intended use or impact and potential receiving environments, taking into account, *inter alia*, related stressors already in the environment (generalised from the GMO-specific definition in EC, 2001).

Community: an association of interacting populations, usually defined by the nature of their interactions or by the place in which they live (adapted from Ricklefs and Miller, 1999).

Delayed effects: Effects that occur sometime after exposure (Rand & Petrocelli, 1984).

Direct effect: effect that is mediated solely by the interaction between the specified receptor/target and the environmental stressor, i.e. when the receptor/target is exposed directly to the stressor and as a result the receptor/target exhibits a response or an ecological effect.

Ecosystem: a dynamic complex of plant, animal and microorganism communities and their nonliving environment interacting as a functional unit (MEA, 2003).

Ecosystem function: see Ecosystem processes.

Ecosystem processes: actions or events that result in the flow of energy and the cycling of matter (Ellis and Duffy, 2008). Examples of ecosystem processes include decomposition, production, water and nutrient cycling (MEA, 2003).

Ecosystem services: The benefits people obtain from ecosystems. These include provisioning services such as food and water; regulating services such as flood and disease control; cultural services such as spiritual, recreational, and cultural benefits; and supporting services such as nutrient cycling that maintain the conditions for life on Earth.

Ecosystem structure: attributes related to the instantaneous physical state of an ecosystem. There are several characteristics to describe ecosystem structure. For example, species population density, species richness or evenness, and standing crop biomass.

Effect: In general, an effect is something that inevitably follows an antecedent (cause or agent). A biological effect is the biological result of exposure to a causal agent.

Environment: natural environment, encompassing all living and non-living entities occurring naturally on earth or some region thereof. (Johnson et al., 1997).

Environmental harm: measurable adverse change in a natural resource or measurable impairment of a natural resource service which may occur directly or indirectly (EC, 2004).

Environmental risk assessment (ERA): the evaluation of the probability and seriousness of harmful (or adverse) effects to human health and the environment, whether direct or indirect, immediate or delayed, following exposure to a potential stressor.

Exposure: Exposure has been defined as the concentration or amount of a particular agent that reaches a target organism, system, or (sub)population in a specific frequency for a defined duration (WHO, 2004).

Exposure scenario: a set of conditions or assumptions about sources, exposure pathways, amount or concentrations of agents involved and exposed organisms, systems or (sub)populations (i.e. numbers, characteristics, habitats) used to aid in the evaluation and quantification of exposures in a given situation.

Feed additives: According to Commission Regulation (EC) No 1831/2003 feed additives are substances, micro-organisms or preparations, other than feed material and premixtures, which are intentionally added to feed or water in order to perform, in particular, one or more of the following functions: favourably affect the characteristics of feed or animal products, favourably affect the colour of ornamental fish and birds, satisfy the nutritional needs of animals, favourably affect animal production, performance or welfare and, and have a coccidiostat or histomonostatic effect (Article 5(3)).

Fitness (population fitness): The relative ability to survive and reproduce of a given genotype or phenotype conferred by adaptive morphological, physiological or behavioural traits.

Focal species: a representative subset of species, selected for testing purposes. Focal species are usually selected based on their ecological relevance, their likely exposure to the potential stressor under field conditions, their susceptibility to the potential stressor, and their testability (Hilbeck et al., 2013, Romeis et al., 2013). Ideally, focal species should have equal or greater sensitivity to the potential stressor than do the species they represent in the ERA and thus knowledge of the effects on these species provides reliable predictions about effects on many other species (Raybould et al., 2011).

Food web: a representation of the various paths of energy flow through populations in the community (Ricklefs, 1990).

Functional group: a collection of organisms with similar functional trait attributes and that are likely to be similar in their response to environmental changes and effects on ecosystem functioning (Tilman, 2001; Hooper et al., 2002).

Functional trait: a measurable property (e.g. mobility, feeding behavior, trophic level, and place in the food web) of an organism, which has demonstrable links to the organism's function (Lavorel et al., 1997; Harrington et al., 2010).

Genetic diversity: genetic variation between and within species. This can be characterised by the proportion of polymorphic loci (different genes whose product performs the same function within the organism), or by the heterozygous individuals in a population (Frankham and Briscoe, 2002).

Genetically modified organism (GMO): an organism, with the exception of human beings, in which the genetic material has been altered in a way that does not occur naturally by mating and/or natural recombination (EC, 2001).

Habitat: Ecological habitat of a species is the place where an organism normally lives, often characterised by a dominant plant form (e.g. forest habitat) or physical characteristic (stream habitat) (Ricklefs, 1990).

Hazard (harmful characteristics): the characteristics of a potential stressor that can cause harm to or adverse effects on human health and/or the environment.

In-crop area: surface covered by the crop plants including the space between the crop rows.

Indirect effect: An indirect effect involves effects being transmitted to the specified receptor through an indirect route involving one or more other, intermediary, receptors. A predatory non-target organism for example could be affected indirectly by a stressor in several ways, including effects of the stressor reducing the abundance of its prey species, its intra-specific or inter-specific competitors, its pathogens or its parasites.

In-field area: surface comprising the in-crop area and its boundaries that are *managed by the farmer* in the context of the crop management.

Intraspecific variability: variability relating to or occurring between members of the same species.

Invasive alien species (IAS) : plants, animals, pathogens and other organisms that are non-native to an ecosystem, and which may cause economic or environmental harm or adversely affect human health. The EFSA plant health panel assesses risks posed by invasive alien species that are harmful to plant health. Therefore, within the context of this opinion, the term IAS refers to invasive alien species that are harmful to plant health. Strictly, the term "invasive" refers to the tendency of a species to disperse and extend its spatial range, or colonize systems from which it was previously absent. An organism is "alien" if it does not naturally occur in a system or area.**Landscape:** an area comprising a system of interest (e.g. agricultural system) at a relatively large scale resulting in heterogeneity in space such as fields or habitat patches.

Life-history trait: Also referred as a demographic trait. A trait that influences the population growth rate and ultimately drives population densities and age distributions (Rubach et al., 2011).

Measurement endpoint: Measurable quality related to the valued characteristics chosen as the assessment (Suter et al., 1993). Within the context of ERAs that fall under the remit of EFSA this concerns a quantifiable response to a potential stressor that is related to the specific protection goal.

Metapopulation: population of populations of the same species connected through immigration and emigration (Levins 1969; Hanski and Gilpin, 1991).

Modelling: an attempt to describe the behaviour of a natural system or to predict the likelihood of an event occurring within a system; it may utilise mathematical formulas and computer simulations.

Non-target organism (NTO): An organism that is not intended to be affected by the potential stressor under consideration.

Off-crop area: area where the product is not intentionally applied.

Off-field area: Area outside the managed "in-field area".

Pest: the concept of pest organisms is anthropocentric and thus a pest is defined as any organism that is perceived by humans to interfere with their activities. Ecologically there are no such organisms as pests. Organisms in several phyla are considered to be pests: e.g. arthropods, nematodes, molluscs, vertebrates. In particular, any species, strain or biotype of plant, animal or pathogenic agent injurious to plants or plant products are called plant pests (FAO, 2005).

Plant Protection Product (PPP): A substance (or device) used to protect (crop) plants from damage by killing or reducing pest organisms or by mitigating its effects.

Population: A group of individuals of the same species.

Potential stressor: used as "environmental potential stressor" and meaning any physical, chemical, or biological entity resulting from the use of a regulated product or the introduction of an invasive alien plant species related to the food/feed chain that is assessed in any area of EFSA's remit and that can induce an adverse response in a receptor (Romeis et al. 2011). Potential stressors may adversely affect specific natural resources or entire ecosystems, including plants and animals, as well as the environment with which they interact (http://www.epa.gov/risk_assessment/basicinformation.htm).

Problem formulation: Phase of environmental risk assessment which includes a preliminary description of exposure and environmental effects, scientific data and data needs, key factors to be considered, and the scope and objectives of the assessment. This phase produces the risk hypotheses, conceptual model and analysis plan, around which the rest of the assessment develops (Raybould 2006; Wolt et al. 2010).

Protection goals: he objectives of environmental policies, typically defined in law or regulations. (Romeis et al. 2011).

Receiving environment: the range of environments into which the GMO(s) and their by-products will be released or may escape or be distributed to through active or passive spread and into which the recombinant DNA may 649 spread are defined as receiving environments (EFSA GMO Panel, 2013).

Recovery: Ecological recovery is the return of the perturbed ecological endpoint (e.g. species composition, population density) to its normal operating range.

Recovery option: Specific protection goal option accepting some population-level effects of the potential stressor if ecological recovery takes place within an acceptable time-period.

Regulated products: claims, materials, organisms, products, substances and processes submitted to EFSA for evaluation in the context of market approvals/authorisation procedures for which an ERA is required.

Risk: the combination of the magnitude of the consequences of a hazard, if it occurs, and the likelihood that the consequences occur.

Risk management: decision-making process involving considerations of political, social, economic and technical factors with relevant **risk assessment** information relating to the hazard.

Service providing unit: the systematic and functional components of biodiversity necessary to deliver a given ecosystem service at the level required by service beneficiaries (Luck et al., 2003; Vanderwalle et al., 2008).

Shannon entropy: The Shannon entropy (Shannon, 1948) is the first, and the most widely known, measure of uncertainty and is widely applied in ecology, e.g. as an index of species richness (Whittaker, 1972).

Sink population: A local sub-population within a spatially-structured population that does not produce enough offspring to maintain itself through future generations without immigrants from other populations.

Source population: A local sub-population within a spatially-structured population that produces an excess of offspring above those needed to maintain itself through future generations. The excess offspring provide a source of immigrants to other sub-populations.

Species sensitivity distribution: models of the variation in sensitivity of species to a particular stressor (Posthuma et al. 2002). They are generated by fitting a statistical or empirical distribution function to the proportion of species affected as a function of stressor concentration or dose. Traditionally, SSDs are created using data from single-stressor laboratory toxicity tests, such as median lethal concentrations (LC50s).

Specific Protection Goal: An explicit expression of the environmental value to be protected, operationally defined as an ecological entity and its attributes (Suter et al., 1993).

Sphere of influence: The sphere of influence of an potential stressor is more than the application area. It includes the fate of the (chemical) potential stressor (e.g. accumulation in food chains) and (spatial) propagation of indirect effects.

Surrogate species: A species selected for laboratory testing because it represents a taxonomic or functional group of organisms that should be addressed in the risk assessment (Romeis et al., 2011).

Threshold option: Specific protection goal option accepting no to negligible population-level effects of exposure to an potential assessed stressor.

Toxicodynamics: the process of interaction of chemical substances with target sites and subsequent reactions leading to adverse effects.

Toxicokinetics: the process of uptake of potentially toxic substances by the body, the biotransformation they undergo, the distribution of the substances and their metabolites in the tissues, and the elimination of the substances and their metabolites from the body.

Trait: A well-defined, measurable, phenotypic or ecological character of an organism, generally measured at the individual level, but often applied as the mean state of a species (McGill et al., 2006).

Uncertainty: Uncertainty is the inability to determine the true state of affairs of a system (Haimes, 2009) and it may arise in different stages of risk assessment due to lack of knowledge and to natural variability (EFSA SC, 2016c).

Voltinism: A trait of a species pertaining to its number of broods or generation per year or per season.

Vulnerable species: a vulnerable species is a species with a relatively high sensitivity for a specific stressor, high exposure and a poor potential for population recovery. It should be noted that this definition of vulnerability is limited to_the direct effects of toxic stressors. Vulnerability to indirect effects, e.g. propagated through disturbed predator-prey or competitive relationships, cannot be characterised by the triad of exposure, sensitivity and recovery. Vulnerability to indirect effects is related to dependability, i.e. whether a species depends, either directly or indirectly, on a species that is affected by the stressor at hand. Other pathways for indirect effects are related to behavioural change resulting e.g. in decreased predator avoidance or decreased competitive strength due to toxicant stress. Additionally, direct and indirect long-term effects need to be taken into account (Van Straalen, 1994).

ACRONYMS

ALMaSS	Animal, Landscape and Man Simulation System
AF	Assessment Factor
AOP	Adverse Outcome Pathway
BIOHAZ Panel	EFSA Panel on Biological Hazards
CEF Panel	EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids
CSM	Case-Specific Monitoring
EC	European Commission
ECx	Environmental Concentration where x % effect was observed/calculated
ECHA	European Chemical Agency
EEA	European Environmental Agency
EFSA	European Food Safety Authority
EMA	European Medicines Agency
EPA	US Environmental Protection Agency
ERA	Environmental Risk Assessment
ERO	Ecological Recovery Option
ETO	Ecological Threshold Option
EU	Europe Union
FEEDAP Panel	EFSA Panel on Additives and Products or Substances used in Animal Feed
FOCUS	FOrum for the Co-ordination of pesticide fate models and their USe
FWS	Fish and Wildlife Service
GD	Guidance Document
GM	Genetically Modified
GMO	Genetically Modified Organism
GMO Panel	EFSA Panel on Genetically Modified Organisms
GS	General Surveillance
FA	Feed Additive
FAO	Food and Agriculture Organisation of the United Nations
HCx	HCx hazardous concentration for x % of the species of a SSD
HT	Herbicid Tolerant
IAS	Invasive Alien Species
IPCS	International Programme on Chemical Safety
IPM	Integrated pest management
IR	Insect Resistant
IUCN	International Union for Conservation of Nature
JRC	Joint Research Centre
LC	Lethal Concentration
LC_{50}	Concentration required killing half the members of a tested population after a specified test duration
LD	Lethal Dose
LD_{50}	Dose required killing half the members of a tested population after a specified

	test duration
LOEC/L	Lowest Observed Effect Concentration/Level
NMFS	National Marine Fisheries Service
NAS	US National Academy of Science
NOEC	No Observed Effect Concentration
NOAEL	No Observed Adverse Effect Level; the maximum concentration of a substance that is found to have no adverse effects upon the test subject.
NTA	Non Target arthropod
NTO	Non Target Organism
OECD	Organisation for Economic and Co-operation Development
PEC	Predicted Environmental Concentration
PG	Protection Goal
PLH Panel	EFSA Panel on Plant health
PMEM	Post Market Environmental Monitoring
PNEC	Predicted No Effect Concentration
PPP	Plant Protection Product
PPR	Plant Protection Residue
PPR Panel	EFSA Plant Protection Residue Panel
PVA	Population Viability Analysis
QSAR	Quantitative Qtructure-Activity Relationship
RA	Risk Assessment
REACH	Registration, Evaluation and Authorisation of CHemicals
RM	Risk Management
SC	Scientific Committee
SCENIHR	Scientific Committee on Emerging and Newly Identified Health Risks
SCHER	Scientific Committee on Health and Environmental Risks
SCoFCAH	Standing Committee on the Food Chain and Animal Health
SPG	Specific Protection Goal
SPU	Service-Providing Units
SSD	Species Sensitivity Distribution
TD	Toxicodynamics
TK	Toxickinetics
ToR	Terms of Reference
UK	United Kingdom
US	United States
US-EPA	United States Environmental Protection Agency
WG	Working group
WHO	World health Organisation
WFD	Water Framework Directive

A. APPENDIX: EXAMINATION OF RELEVANT PARAGRAPHS FROM THE LEGAL FRAMEWORKS ON PROTECTED SPECIES

Below paragraphs are excerpt from original legal frameworks and *in italics the analysis made for this opinion*.

<u>EU Level:</u> Habitats Directive (COUNCIL DIRECTIVE 92/43/EEC of 21 May 1992 on the conservation of natural habitats and of wild fauna and flora).

1. Habitats (Natura 2000 network)

- Development of Natura 2000 network
- Avoid deterioration of habitats and significant disturbance of species in conservation areas
- Compensatory measures if a certain plan or project need to be carried out despite negative implication
- Land use planning and management to facilitate migration, dispersal and genetic exchange between Natura 2000 sites

2. Protection of species

Annex IV species = animal and plant species of community interest in need of strict protection

Article 12 (animals)

- Member States shall take the requisite measures to establish a system of strict protection in the natural range of Annex IV species – *It is noted that by mentioning only **Member States**, this clause is taken as directed to national level, not the EU level. – Regarding the term **natural range**, does that include in crop, in field? This would require legal interpretation as no further indications are found in this directive.*
    - No deliberate capture or killing- *It is not clear what is meant "**deliberate**" e.g. is it a deliberate action to kill an individual of a non-target protected species, if one knows that it could be killed? By using the term "killing", the level of protection seems to be set on the individual.*
    - No deliberate disturbance
    - No deliberate destruction of eggs
    - No deterioration or destruction of breeding sites or resting places. – *In this clause it is not specified that it has to be deliberate. Potentially, it is to be interpreted as deliberately. Spatial restriction if species has certain areas for reproduction (e.g. amphibian, semi-aquatic insects).*
- Monitor incidental killing and establish research or management to ensure that there is no significant negative impact – *It is not clear what this is.*

Article 13 (plants)

- Establish a system of strict protection in the natural range of Annex IV species
    - No deliberate destruction

Article 16 (derogation)

If

- no satisfactory alternative and
- maintenance of the populations of the species concerned at a favourable conservation status in their natural range - ***conservation status** of a species means the sum of the influences acting on the species concerned that may affect the long-term distribution and abundance of its populations within the territory referred to in Article 2; For endangered specie there is always /often in a non -favourable conservation status. The conservation status will be taken as '**favourable**' when: — population dynamics data on the species concerned indicate that it is*

maintaining itself on a long-term basis as a viable component of its natural habitats, and— the natural range of the species is neither being reduced nor is likely to be reduced for the foreseeable future, and — there is, and will probably continue to be, a sufficiently large habitat to maintain its populations on a long-term basis; For endangered species: consider always /often in a non -favourable conservation status.

Derogation possible

- to protect serious damage to crops, livestock, forests, fisheries, water, other types of property
- in the interest of public health and safety, other overriding public interests of social or economic nature

Report on the derogations to the EC

Article 22 (species introduction)

- regulate deliberate introduction of non-native species into the wild, so that there is no prejudice of natural habitats or wild native flora and fauna; assessment needed – *Is **non-native** important for GMOs ? "in the wild" is a key aspect: probably it means not in the agricultural landscape. Then the problem could be horizontal gene transfer to native species?*

National level :

Comparison to German Federal Nature Conservation Act

- specially protected species
    - no injure, killing etc.
- strictly protected species
    - no *considerable* disturbance during breeding, migration etc.
    - no damage etc. of *breeding sites*
- not only *deliberate* actions
- clearly on the individual level
- derogations for agriculture etc. allowed if conservation status is *not degraded* (hence weaker provision, as in Habitat Directive favourable status must be ensured)

B. APPENDIX: THE FIRST TIER HAZARD ASSESSMENT OF PPP AND ENDANGERED SPECIES

We are concerned here with testing for acute effects of chemicals (PPPs) on bird and aquatic species. Test results are either EC_{50}s (aquatic, counting LC_{50} as a form of EC_{50}) or ED_{50}s (birds). We distinguish between the measured sensitivity (EC_{50} or ED_{50}) and the true sensitivity which we denote by TEC_{50} or TED_{50}. By true sensitivity, we mean the average measurement which would be obtained if testing was carried very many times for the same chemical on the same species. The difference between true and measured sensitivity is that the latter includes a component of inter-test variation (Hickey et al.2012).

The issue with which we are concerned is where a single species, often a standard species, is tested and an assessment factor is then applied to arrive at a concentration or dose which should usually be protective of another species, perhaps an endangered species.

We want to answer a particular question: if x is the result (EC_{50} or ED_{50}) of testing one species on a chemical and we are interested in y which is the true sensitivity (TEC_{50} or TED_{50}) of a single other species, what is the probability p that y is less than x/AF where AF is some specified assessment factor suggested to be used for this purpose, usually 10 or 100. We are also interested in the obverse: what value of the AF would be required so that p is less than (say) 5%? In considering this issue, it is potentially important to recognise that x includes inter-test variation (ITV) whereas y does not.

Fundamental assumptions:

- The species in each database are representative of those we would like to consider
- The chemicals in each database are representative of those we would like to consider
- The test results for each chemical/species combination are representative.

Data used

The datasets used were those provided by EFSA at the start of the contract. As provided, data for aquatic species did not include information about censoring. However, those data were originally derived from the database referred to in (De Zwart, 2002), a large subset of which was published along with (Hickey et al.2012). Nearly all the data provided could be matched to records in that subset and censoring information retrieved. However, relatively few data were found to be censored and omitting or including those data made only small differences to results tabulated in the accompanying spread sheet. Consequently, the tabulated results are based on the data as provided by EFSA.

Records where a taxonomic description was not provided, for example “fish” or “crayfish”, were omitted. When comparing two records where, for one of the records, only the genus was provided, the species for that record was assumed to differ from the species for the record where the full Latin name was provided. When comparing two records where, for both records, only the genus was provided, the species for the two records was assumed to be the same. It was not possible in the time available to investigate alternative approaches to addressing this issue. When comparing records for the same genus, the analysis was restricted to records having complete Latin names for species.

For birds, the data provided by EFSA were used previously by (Luttik et al.2011) and included information about censoring. Those data were a subset of a larger database which included many additional censored data and chemicals for which only a very small number of species had been tested. It was not found that using the larger dataset or making adjustments for censoring led to substantially difference tabulated outcomes. Consequently, the tabulated results are based on the data as provided by EFSA. Species information for the bird data was provided only in the form of common names; it was possible to deduce the Latin name for 1038 of 1053 records and the 1038 records were used for all the reported analyses.

Methodology

Quantifying inter-test variation (ITV)

For each pair of test results for the same chemical-species combination, we take the ratio. Pooling all those pairs to form an empirical distribution gives us an approximation to the distribution of the ratio for repeated testing of a species on the same chemical. The possibility that such a distribution might be species dependent or chemical dependent was investigated in (Hickey et al.2012) but no evidence was found of such dependence.
In computing this empirical distribution, a chemical-species combination having k test results would contribute k(k -1) pairs but in reality, as for a standard deviation, it only contributes k -1 real degrees of freedom and so each pair is weighted 1/(k - 1) is computing the empirical distribution.

Random species tested

No adjustment for inter-test variation (naive empirical data analysis)

For each pair of test results for two different species on the same chemical, we take the ratio. Pooling all those pairs to form an empirical distribution gives us an approximation to the distribution of the ratio for measurements for two randomly chosen species for a randomly chosen chemical.
If we then compute the 95th percentile of this distribution, or equivalently the reciprocal of the 5th percentile, this gives us a value for the AF which would need to be applied to a single test result x so that x/AF would have a 95% probability chance of lying below the test result for a single other randomly chosen species.

There are four fundamental weaknesses to this approach:

- Both numbers in the ratio include inter-test variation (ITV). As discussed above, we should in principle want to include ITV for the tested species but not for the species whose sensitivity we wish to predict/cover. Consequently, the value we determine here for the AF is in that sense conservative: if instead of data on pairs of two test results we had data on pairs of one test result and one true sensitivity, we would expect to obtain a smaller AF,
- Each datasets is quite biased in the sense that there are many species tested for a relatively small number of chemicals and only a few species tested for the majority. Moreover, a small number of species were tested on many chemicals while others were tested on few chemicals.
- It ignores altogether the issue of non-exchangeability (EFSA PPR Panel, 2005; Craig et al., 2012): when many chemicals are considered, some species have a tendency to be more to the sensitive (or insensitive) end of the SSD.
- It cannot deliver uncertainty about the percentage of cases not covered by a specified assessment factor or uncertainty about the assessment factor required to cover a specified percentage. That would require a statistical modelling approach to the problem. Such statistical modelling was not possible within the time frame and resources available for this work.

We need also to be careful about exactly how we weight pairs of test results in computing the empirical distribution above. There are two issues to address:

- The datasets all contain some multiple outcomes for particular chemical-species combinations. Those multiple outcomes should not simply be averaged: we want to include ITV for x. Instead we need to weight them appropriately: if there are k test results for the same chemical-species combination, each should receive weight 1/k. When weighting pairs where the individual test results receive weights 1/k1 and 1/k2, the pair then receives weight 1/(k1k2).

- The first weighting issue was driven by variation in the number of test results for a single chemical-species combination. The second weighting issue is driven by variation in the number of species tested for a single chemical. If it could be assumed that all chemicals had the same SSD standard deviation (for log EC50 or log ED50), it would not matter that some chemicals had more species tested than others. There would be no need for further weighting adjustments. This is weighting scheme 1 in the tabulated results.

  However, there is evidence that the SSD standard deviation varies between chemicals EFSA PPR Panel, 2005); this is supported by the fact that some popular SSD calculations, for example (Aldenberg and Jaworska, 2000), estimate the standard deviation for a chemical without reference to data from other chemicals. If so, when considering y for a single species for a new chemical, we should want to weight chemicals equally when constructing the empirical distribution of pairs. The weight for a pair is then 1/(nk1k2) where n is the number of species tested on that chemical. This is weighting scheme 2 in the tabulated results.

Adjustment for inter-test variation

If we fit a distribution to ITV for a database, then we can in principle use that distribution mathematically to remove some of the unwanted variation from the empirical distribution of ratios of pairs used.

Efforts were made to estimate a distribution for ITV for each database using both fitting by eye and a Bayesian model generalised from the one used in (Hickey et al.2012) to allow a t-distribution model: distributions with heavier tails than normal distributions. In practice, it was found that the latter model was overly sensitive to particular features in the data, in particular to the presence of pairs having the same numerical outcome for the two tests which may well be due to rounding or the design of studies. Consequently, the results presented here were obtained for each database by:

- iterating the following process for fitting a distribution to ITV until a good match was found by eye between theoretical and empirical distributions:
  - specify the degrees of freedom and scale of a theoretical log-t-distribution for ITV
  - compute (using the rdistr package (Ruckdeschel et al., 2006) for R (R Core Team, 2014))) the corresponding theoretical distribution for log-ratios of pairs
  - compare the result to the original empirical distribution obtained for log-ratios of pairs for ITV

- iterating the following process for fitting a distribution to inter-species variation in true sensitivity until a good match was found by eye between theoretical and empirical distributions for differences of pairs of test results for different species for the same chemical:
  - specify the degrees of freedom and scale of a theoretical log-t-distribution for variation in true sensitivity y
  - compute (again using the rdistr package for R), using the proposed log-t-distribution for the true SSD and the log-t-distribution previously obtained for ITV, the corresponding theoretical distribution for log-ratios of pairs of test result for different species
  - compare the result to the empirical distribution for log-ratios of pairs of test result for different species obtained when making no adjustment for ITV using weighting scheme 1.

- Use the theoretical distributions for ITV and variation in true sensitivity to calculate mathematically the distribution of the ratio x=y and hence derive percentiles and percentages of interest.

Standard species tested

In this Section, we restrict attention to the situation where the test result x comes from a standard species. As standard species, we take *Oncorhynchus mykiss* for fish, *Daphnia magna* for crustaceans and investigate three possibilities for birds: bobwhite quail, mallard duck and Japanese quail. We perform only an analysis of the type in Section of the naïve empirical data analysis but restricting to pairs of test results where the first species is the selected standard species. The distribution-fitting process in the Section for adjustment of ITV was found to be unworkable with the reduced amount of data.

Species of the same genus tested

In this Section, we restrict attention to the situation where two species are from the same genus. We perform only an analysis of the type in Section of the naïve empirical data analysis but restricting to pairs of test results where both records have a complete Latin name.

Number of data available for calculations

In the Table below the number of data available for each calculation are presented.

Table 1: Number of data available for each calculation for different organisms groups

Inter-test variation	*Insects*	*Crustaceans*	*Fish*	*Birds*		
Number of chemicals	41	78	270	54		
Number of species	40	29	50	27		
Number of chemical-species combinations	152	225	698	226		
Mean number of tests per chemical-species combination	2.7	3.3	3.5	2.7		
Random species (with and without ITV adjustment)	Insects	Crustaceans	Fish	Birds		
Number of chemicals	53	86	375	65		
Number of species	175	110	170	70		
Mean number of species per chemical	14.6	8.6	5.0	10.0		
Standard test species (without ITV adjustment)	Insects	Crustaceans	Fish	Birds	Birds	Birds
Standard test species	Chironomus spec.	Daphnia magna	Oncorhynchus mykiss	bobwhite quail	mallard duck	Japanese quail
Number of chemicals	39	78	340	64	63	50
Number of other (not the standard) species	142	108	166	68	68	69
Mean number of other species per chemical	14.9	7.9	4.3	9.1	9.1	10.4
Species of same genus tested	Insects	Crustaceans	Fish			
Number of chemicals	34	75	85			
Number of genera	11	10	19			
Number of chemical-genus combinations	83	148	143			
Number of species	59	52	62			
Mean number of species per chemical-genus combination	3.7	2.3	2.2			

Remarks/comments

A few points need to be emphasised:

- All thresholds or percentages in the accompanying spread sheet are only estimates and are subject to undetermined levels of uncertainty if applied outside the context of the data used.
- A statistical modelling approach should enable one to quantify some of the uncertainties affecting those estimates, in particular sampling uncertainties due to the limited number of chemicals and species involved and the fact that some species and chemicals have tested many more times than others.
- A more important source of uncertainty is the representativeness of the dataset in terms of chemicals, especially modes of action, and of species and genera within each dataset species group. This would be more difficult to quantify.
- Other than the process of considering standard test species under Section standard species tested, no allowance/ adjustment was able to be made for non-exchangeability of species or genera.
- The nature of these calculations, being based empirically on pairs of test results, was reasonably well-suited to the endangered species situation where one wishes to consider the risk for a single chemical to a particular species based on a single test result for a species from the same group (insects/fish/crustaceans/birds).

They would not be suitable in situations where more species from the group had been tested or where one wanted to consider risk to more than one species. In those situations, the standard deviation of the SSD (log scale) would be of great importance. The analysis here (especially using weighting scheme 2) requires simply that the chemicals in the datasets are representative in this respect.

Assessment factors

In risk (and hazard) assessment often assessment factors[24] (AF) are used. The general idea is that the uncertainty in an assessment is accounted for by imposing a certain safety margin between exposure and hazard. The larger the uncertainty in an assessment the larger the assessment factor will be. Thus, e.g. a certain endpoint is multiplied with or divided by an AF to extrapolate from a laboratory study with for instance a bobwhite quail to a fish eating bird or from single-species laboratory data to a multi-species ecosystem ("to the real world"). In the literature (e.g. EC, 2003, a Technical Guidance Document on Risk Assessment) a number of uncertainties are identified which should be included in the assessment factor:

- Intra- and inter-laboratory variation of toxicity data;
- Intra- and inter-species variation (biological variance);
- Short-term to long-term toxicity extrapolation;
- Laboratory data to field impact extrapolation (additive, synergistic and antagonistic effects from the presence of other substances may also play a role here).

Sometimes the overall uncertainty factor is then derived by multiplying the single assessment factors.

For deciding whether a certain AF is also applicable for risk assessment of endangered species it is necessary to assess to which extent the standard AFs provide an adequate coverage of the above mentioned sources of variability and uncertainty.

For a number of groups of organisms, large enough databases with acute toxicity data are available to analyse some of the above mentioned sources of variability and uncertainty: aquatic insects, aquatic crustaceans, fish and birds (see appendix B for number of compounds and species available for each topic mentioned below).

In Table 2 the ranges are provided for which 50 and 90% of the re-test are expected to fall between for intra and inter-laboratory variation of toxicity data. The ranges are the smallest for bird species and the

[24] Often different names are used for an assessment factor, e.g. extrapolation factor, safety factor, uncertainty factor, trigger value, etc. In this document we will use the term assessment factor (AF).

largest for aquatic insect species. The range for 90% of the re-test for birds is 4 times smaller than for aquatic insect.

Table 2: Inter-test variation

	Insects	Crustaceans	Fish	Birds
50% of re-tests are expected to fall between	x/2.7 and 2.7x	x/2.4 and 2.4x	x/2 and 2x	x/1.5 and 1.5x
90% of re-tests are expected to fall between	x/24 and 24x	x/21 and 21x	x/14 and 14x	x/6.4 and 6.4x

In Tables 3 to 6 the results for intra-test variation are provided. A number of different approaches have been used:

A) An assessment when a random test species would have been the starting point for the risk assessment (Tables 3 to 5),
   a. Without adjustment for the inter-test variation with chemicals weighted by number of species (weighting scheme 1) and with chemicals weighted equally (weighting scheme 2),
   b. With adjustment for the inter-test variation with chemicals weighted by number of species and with chemicals weighted equally,

B) An assessment when the standard test species would have been the starting point for the risk assessment without adjustment of the inter-test variation with chemicals weighted by number of species and with chemicals weighted equally.

In Table 3 one can see, for example, that having obtained a test result x for a fish, it is estimated that there is a 10% chance that a test result for another fish lies below x/14 using weighting scheme 1 or below x/11 using weighting scheme 2.

Table 3: Random species tested: chance that a test result for another species lies below the toxicity value X divided by the values for a specific group of animals (no adjustment for inter-test variation)

Chance	Weighting scheme	Insects	Crustaceans	Fish	Birds
1.00%	1	x/690	x/2700	x/480	x/85
5.00%	1	x/67	x/180	x/44	x/16
10.00%	1	x/4.7	x/44	x/14	x/7.2
1.00%	2	x/1000	x/1600	x/420	x/75
5.00%	2	x/81	x/110	x/34	x/16
10.00%	2	x/23	x/33	x/11	x/7.3

There is no consistent pattern of differences in outcome using the two weighting schemes (see also next Tables) and so those differences should perhaps be seen as indicating some uncertainty.

The Table also shows that for achieving the same chance (for instance 5%) the smallest factor is found for birds and the highest factor for crustaceans: 16 versus 180 (weighting scheme 1) and 16 versus 110 (weighting scheme 2).

Table 4: Random species tested: chance that a test result for another species lies below the toxicity value X divided by the values for a specific group of animals (inter-test variation adjustment)

Chance	Weighting scheme	Insects	Crustaceans	Fish	Birds
5.00%	1	x/34	x/120	x/24	x/11
10.00%	1	x/13	x/26	x/8.5	x/5.4
5.00%	2	x/34	x/74	x/15	x/12

10.00%	2	x/13	x/19	x/6.2	x/5.5

In Table 4 the results for a random tested species adjusted for the inter-test variation are provided. In this case the values are all smaller than for the non-adjusted values in Table 3 which is to be expected since an important source of variation was removed. Again, the values for birds are the smallest and the values for the crustaceans are the largest.

Table 5: Standard species tested: chance that a test result for another species lies below the toxicity value X divided by the values for a specific group of animals (not adjusted for the inter-test variation)

Chance	Weighting scheme	Insects	Crustaceans	Fish	Bird	Bird	Bird
		Chironomus spec.	Daphnia magna	Oncorhynchus mykiss	Bobwhite quail	Mallard duck	Japanese quail
1.00%	1	x/1300	x/800	x/73	x/34	x/130	x/28
5.00%	1	x/140	x/83	x/10	x/9	x/45	x/8.2
10.00%	1	x/44	x/27	x/4.1	x/6	x/25	x/5.6
1.00%	2	x/1900	x/610	x/81	x/23	x/140	x/28
5.00%	2	x/230	x/77	x/10	x/9	x/51	x/7.9
10.00%	2	x/74	x/26	x/4.1	x/5.7	x/29	x/5.3

Table 5 provides the values for a standard tested species (not adjusted for inter-test variation). Here one can see, for example, that having obtained a test result x for rainbow trout (*Oncorhynchus mykiss*), it is estimated that there is a 10% chance that a test result for another fish lies below x/4.1 using both weighting scheme 1 and weighting scheme 2. The values for the fish assessment are considerably lower than the ones for crustaceans (*Daphnia magna*) and insects (*Chironomus spec*.). The values for the Bobwhite quail and the Japanese quail are comparable and approximately 4 times lower than for the Mallard duck.

Another way of looking at the result of the assessment is to calculate the percentages of ratios not covered by a specific assessment factor. For birds the default AF is 10 and for fish, crustaceans and insects the default AF is 100 (see Table 6). In case of random tested species, with or without adjustment for inter-test variation and for weighting either with scheme 1 or 2, the percentages not covered are less than 5% for fish and insects but only in one case for the crustaceans when using an AF of 100. For birds these percentages are greater than 5% (i.e. 5.4 to 7.3%) when using the official AF of 10.

In case of standard tested species, with or without adjustment for inter-test variation and for weighting either with scheme 1 or 2, the percentages are less than 5% for fish and crustaceans and 6.4 and 8% for insects when using an If the risk assessment for birds would be based on the Bobwhite quail or Japanese quail as the standard test species, the percentages for an AF of 10 would be lower than 5%, but not if the risk assessment would be based on the mallard duck when these percentages would be greater than 5% (i.e. 17.8 to 20%).

Table 6: Random species tested: percentage of ratios not covered by the specified assessment factor (not adjusted for inter-test variation). Values in bold are the values that belong to the official AF to be used in risk assessment.

Random species tested: percentage of ratios not covered by the specified assessment factor (no inter-test variation adjustment)

Assessment factor	Weighting scheme	Insects	Crustaceans	Fish	Bird

100	1	**3.8%**	**6.6%**	**3.0%**	0.8%
10	1	16.9%	19.5%	12.1%	**7.3%**
100	2	**4.5%**	**5.4%**	**2.7%**	0.7%
10	2	16.5%	17.6%	10.9%	**7.6%**

Random species tested: percentage of ratios not covered by the specified assessment factor (inter-test variation adjustment)

Assessment factor	Weighting scheme	Insects	Crustaceans	Fish	Bird
100	1	**2.4%**	**5.4%**	**2.3%**	0.9%
10	1	12.2%	16.4%	8.9%	**5.4%**
100	2	**2.4%**	**4.3%**	**1.6%**	0.9%
10	2	12.2%	14.4%	6.7%	**5.4%**

Standard species tested: percentage of ratios not covered by the specified assessment factor (not adjusted for inter-test variation)

Assessment factor	Weighting scheme	Insects Chironomus spec.	Crustaceans Daphnia magna	Fish Oncorhynchus mykiss	Bird Bobwhite quail	Bird Mallard duck	Bird Japanese quail
100	1	**6.40%**	**4.5%**	**0.7%**	0.0%	1.3%	0.0%
10	1	21.40%	16.0%	5.0%	**4.4%**	**17.8%**	**3.9%**
100	2	**8.00%**	**4.0%**	**0.6%**	0.0%	1.4%	0.0%
10	2	22.30%	15.7%	5.3%	**4.5%**	**20.0%**	**4.1%**

Discussion

In PPPs ERA, current regulatory practice in Europe is to use the result of the most sensitive tested species and to divide this value by a factor of 5-100, depending on the species tested and the endpoint considered (e.g. acute versus chronic effects). It has been suggested that these assessment factors of 5-100 cover interspecies differences in toxicity (EU, 2001), though it is unknown whether the actual numbers used are appropriate for this purpose (EC, 2002). The results presented above indicate that the assessment factors currently used in acute risk assessment of PPPs cover the toxicological sensitivity of 99.3-82.2% of the species, depending on the species considered. However, this does not include potential variation in the other dimensions of ecological sensitivity, i.e. exposure, recovery and indirect effects (Section 4.2). As such, it seems unlikely that no other extrapolation steps are required when using laboratory data to estimate effects in the field.

The lower Tier of the ERA is usually driven by the most sensitive test species or species group. For herbicides, the algae and macrophytes typically are the most sensitive group, while the crustaceans and insects typically are most sensitive for insecticides. In the research presented here, all available PPPs were used for the calculations. When the assessment would have been based on insecticides only, the outcome would have been slightly different and the percentages not covered by the AFs somewhat smaller.

The calculations presented above were based on acute toxicity data only. One could ask whether the results are also applicable to chronic toxicity data. However, no comparable large databases are available for chronic toxicity, and subsequently, similar calculations as presented above can currently not be performed for chronic data. However, Luttik et al. (2005) applied another approach to assess whether there is a difference in interspecies variation between acute and chronic toxicity data for one particular compound in a paper that was produced in response to a charge from the British Department of Environment, Food and Rural Affairs (DEFRA) to provide guidance to British and other EU regulators on the assessment of long-term risks to wild birds and mammals from their exposure to PPPs. They suggested that, in the absence of a strong rationale to the contrary, it should be assumed that reproductive data are at least as variable as acute data and that strategies developed for acute data could be applied to long term toxicity data as well. Considering only the two main bird test species for which reproduction data are available (Mallard and Northern Bobwhite), a comparison of the interspecies standard deviation for both acute and reproduction data suggested that the two are equally variable. In the same paper, an analysis of a very limited data set also suggested that this conclusion holds regardless of which endpoint is triggered in the reproduction study (Luttik et al., 2005).

Conclusions

Risk assessment based on the standard aquatic test species and an AF of 100 appears to provide varying levels of protection: fish appear to be the best protected group (only 0.7% of the ratios are not covered by the AF), followed by crustaceans (4.5%) and insects (6.4%). Risk assessment based on the standard bird species, i.e. Bobwhite quail and Japanese quail, and an AF of 10 appears to provide almost the same level of protection, respectively 4.4% and 3.9%. These percentages would be 17.8% for the Mallard duck. The level of protection for fish seems to be higher than for birds. Choosing a random insect species for each test rather than the standard test species of *Chironomus* might provide a better level of protection. The percentages not covered might decrease from 6.4% to 3.8%.

If the aim of the risk assessment is for example to protect at least 95% of the species in any taxonomic group, it appears that AF is consumed by the uncertainty from the between species variability where a standard species is tested: for bird assessment based on testing one of the two quail species and for the insect and crustacean species (percentages not covered are close to 5%). This means that other sources of uncertainty are not covered anymore by the AFs. For fish, there is still some room left for other uncertainties. It is evident that in case an AF is not covering the uncertainty for a general risk assessment it is also not covering the uncertainty in a risk assessment for endangered species.

Use of surrogate species

Using the same database as outlined above in this Appendix, the toxicological sensitivity of closely related species was compared to explore whether closely related species can serve as a surrogate for endangered species. It was assumed for these calculations that a species in the same genus can be considered a closely related species. The ratios in toxicity values between different species within one genus were calculated as a proxy for the variation in toxicological sensitivity between closely related species.

Table 7 shows for example that having obtained a test result x for a fish species, it is estimated that there is a 5% chance that a test result for a different species in the same genus lies below x/15 using weighting scheme 1 or 2. For crustaceans species this would be x/38 and for insect species x/20.

Table 7: Chance that a test result for a different species in the same genus lies below the toxicity value X divided by the values for a specific group of animals (e.g. for a chance of 1% for insects below x/170) (no adjustment for inter-test variation)

Chance	*Weighting scheme*	*Insects*	*Crustaceans*	*Fish*
1.00%	1	x/170	x/660	x/390
5.00%	1	x/20	x/38	x/15
10.00%	1	x/9.4	x/11	x/6.6
1.00%	2	x/750	x/640	x/380
5.00%	2	x/21	x/38	x/15
10.00%	2	x/10	x/11	x/6.5

In case of using an AF of 100 for the 3 groups of aquatic species the percentages of ratios not covered by the specified assessment factor are 1.6% for insects, 3.2% for crustaceans and 1.9% for fish (see Table 8). If the standard test species are being used (instead of species in the same genus), these ratios are respectively 0.7, 4.5 and 6.4% (see Table 7). This suggests that testing a closely related fish species will generally not provide a better outcome than testing the Rainbow trout. For crustaceans and insect, the results suggest that testing a species from the same genus may result in a more conservative assessment. When applying an assessment factor of 10 to closely related test species, between 6.4 and 11.3% of the ratios would not be covered (see Table 8).

Table 8: Species tested from same genus: percentage of ratios not covered by the specified assessment factor (no adjustment for inter-test variation)

Assessment factor	*Weighting scheme*	*Insects*	*Crustaceans*	*Fish*
100	1	1.6%	3.2%	1.9%
10	1	9.5%	11.3%	6.4%
100	2	1.9%	3.1%	1.8%
10	2	10.3%	11.2%	6.5%

Conclusions

Testing of surrogate species, which is not current practice in PPPs, could slightly improve the outcome of the risk assessment but the gain in knowledge is only marginal. Even when using a surrogate species (a closely related species from the same genus) for testing, to reach a protection level of 95%, one would have to use a safety factor of 100 (in the case of crustaceans, insects and fish). This means that, when using a surrogate species, the AFs can not be substantially lowered; they have to be in the same range as for the standard test species.

Note: The weakness of the methods used is that it is purely empirical. Consequently it is vulnerable to biases in a database and provides no measure of uncertainty for the results. It would in principle be desirable to attempt a statistical modelling approach to include uncertainty ranges around the outcomes. An immediate benefit would be some indication of the robustness of the numbers provided in the Tables above. A suitable statistical approach would additionally provide an indication of uncertainty due to the amount of data available for each analysis. In principle, subject to some assumptions, it might also be possible to account for uncertainty due to the fact that some chemicals and some species have been tested many more times than others. Like the existing approach, it would fundamentally assume that the tested chemicals and species are representative and that the test data are representative for each chemical-species combination which has been tested.

C. APPENDIX: SPECIES TRAITS AND (TOXICOLOGICAL) SENSITIVITY

The (toxicological) sensitivity of endangered species to an assessed stressor cannot be tested in a laboratory setting. However, if sufficient knowledge is available about the generic mechanisms that govern the interactions between stressor and the organism, it may be possible to describe and predict these interactions based on a limited number of stressor and species characteristics. Over recent years, our mechanistic understanding of the interactions between stressors and organisms has increased enormously, particularly for chemical substances. It is the result of the enormous boom in analytical techniques at the molecular level, e.g. in vitro cell lines, (eco)genomics, metabolomics, transcriptomics and proteomics. This trend towards more mechanistic understanding feeds a future perspective where the need for testing with whole organisms becomes obsolete and the prediction of (toxicological) sensitivity becomes a largely theoretical exercise based on *in vitro* test systems and *in silico* methods (NRC, 2007). If this perspective becomes reality, the problem that endangered species cannot be tested experimentally may resolve.

This Appendix summarises recent developments in mechanistic understanding of toxicological sensitivity, with a focus on the role of species traits. Examples are given illustrating how species traits influence toxicological sensitivity and might be used to assess toxicological sensitivity in prospective risk assessment. Distinction is made between the toxicokinetic and toxicodynamic phase of toxicological sensitivity. The internal dose of the toxicant depends on processes in the toxicokinetic phase, while the expression of toxicity is mediated via receptor interactions within toxicodynamics (Spurgeon et al., 2010).

Species traits related to toxicokinetics

The processes which determine the internal concentration of the toxicant at the target site are often referred to as ADME (absorption, distribution, metabolism and excretion). Consequently, any trait affecting these processes can help explain the toxicological sensitivity of the organism. There are numerous species traits affiliated with toxicokinetic processes; mostly morphological and physiological traits (Rubach et al., 2011; De Man, 2014). For example, in the case of metals, bioaccumulation differences among taxa can be largely explained by species-specific physiological traits related to ionoregulation and digestive processes (Spurgeon et al., 2010; Luoma & Rainbow, 2005). Accumulation of organic toxicants has been shown to be related to organism size and lipid content (Hendriks et al., 2001). Neutral organic compounds are usually assumed to be taken up primarily via passive uptake, while charged organic compounds and metals are mostly assumed to be assimilated via uptake channels or carrier proteins (Spurgeon et al., 2010). The so-called ABC-transporters (ATP-binding cassette transporters) are involved in the transport of a wide range of xenobiotic molecules across extra and intra cellular membranes (Buss & Callaghan, 2008) and play an important role in controlling the internal concentrations of a wide range of substrates within cells. As for loss rates, profound differences among species have been reported in numerous studies, but little is known about the traits that drive these loss rate differences on a molecular level (Rubach et al., 2011). It is expected that phylogenic comparative studies between species may eventually help to predict the ability of a taxon to eliminate toxicants on a molecular level. The elimination of toxicants also depends on the presence of specific excretory organs like the kidney and the gall. Once a chemical has entered the systemic circulation, it can interact with a range of metabolic pathways. Various enzymes and proteins are involved in these biotransformation pathways, e.g. metallothioneins, transporters (e.g. p-glycoproteins, organic anion transporters), phase I enzymes (e.g. cytochrome P450s (CYPs) and esterases), phase II enzymes (e.g. UDP-glucuronosyltransferase, methyl-S-transferases and glutathione-S transferases), protein chaperones (e.g. the heat shock protein family), antioxidant defence enzymes, and mitogen activated kinase signalling associated proteins (Causton et al., 2001; Korsloot et al., 2004). Together, these systems provide a network of responses which can contribute to the detoxification of chemicals, and in some cases also the production of toxic metabolites. When biotransformation is faster than efflux, toxicokinetics is no longer a simple partitioning process between two phases. Therefore, any trait that determines biotransformation is potentially important for explaining the variability in intrinsic sensitivity between species. The presence and translation rates of all these enzymes reflect the biotransformation potential and can differ even between closely related species. For example, Rust et al. (2004) investigated the relative ability of 11 species of near-shore benthic invertebrates to metabolise and bioaccumulate benzo[a]pyrene (B[a]P), a typical polycyclic aromatic hydrocarbon (PAH). After 7 days of exposure to sediments spiked with radiolabeled B[a]P, metabolites comprised between 6.1% to 85.7% of total accumulated B[a]P, with individual species from the same phylogenetic groups showing large differences in their ability to metabolise this PAH. Recent approaches for prediction of biotransformation potential have investigated the use of mechanistically-based QSARs to describe patterns of metabolic processes in mammals and predict metabolic rates (Pirovano et al., 2012, 2014).

Regarding metabolism, a large body of evidence from the literature demonstrates that a number of taxa have evolved with particular sets of xenobiotic metabolising enzymes. In Section 4.2.1 and 4.2.2 it was highlighted that there can be considerable differences in the metabolic capacities between species (e.g. cats and other mammals, and the organochlorine examples in birds). It was also clear that such differences have a large effect on how well the test data from a selected test or available surrogate species will cover a potentially sensitive endangered species upon which direct testing is unfeasible. For the purpose of this opinion, bees and cats are here discussed in detail to illustrate the concept of how molecular sequencing information can inform the prospective risk assessment of a species for invertebrates and vertebrates, respectively.

Honey bees

Recent sequencing of the honey bee *(Apis mellifera)* genome has revealed that it lacks major detoxification enzymes, and possesses only about half as many glutathione-S-transferases (GSTs), cytochrome P450 monooxygenases (CYP) and carboxyl/cholinesterases (CCEs) compared to other insects. Comparing the genome of the parasitic juwel wasp (*Nasonia vitripennis*) to the honey bee, has revealed that the wasp has twice as many CYPs than the honey bee with 92 CYP isoforms encoded in its genome. From an evolutionary perspective, eusociality in bees and the high level of nest homeostasis insulate the queen from exposure to toxins making CYP-mediated detoxification less critical compared with other insects. Additionally, bees have a long evolutionary history of consuming processed nectar and bee bread resulting in a specialised exposure to phytochemicals and a low exposure to other environmental toxins, reducing the need for detoxification enzymes (Claudianos et al., 2006; Johnson, 2008). These deficiencies in detoxification enzymes have been hypothesised to be responsible for the sensitivity of the honeybee to insecticides and have been reviewed in recent EFSA opinions and scientific reports (EFSA PPR Panel, 2012; EFSA, 2014c).

Cats

Cats (Felidae) have evolved as hypercarnivorous mammals with major deficiencies in a number of detoxifying conjugation enzymes such as UDP-glucuronosyltransferase (UGT; UGT1A6 and UGT1A9), glycine conjugation enzymes, N-acetyltransferase-2 (NAT-2) and thiopurine methyltransferase (TMPT). Recently, the phylogenetic timing of the gene inactivation of UGT in cats and felids (UGT1A6) has been established to have taken place between 35 and 11 million years ago. From an evolutionary perspective, it has been hypothesised that mutations of the major species-conserved phenol detoxification enzymes (i.e. UGT1A6) co-evolved with hypercarnivory because hypercarnivory results in minimal exposure to plant-derived phenol toxicants. This mutation has also been shown in two other Carnivora species, such as brown hyena (*Parahyaena brunnea*) and northern elephant seal (*Mirounga angustirostris*). Domestic cats (*Felis catus*) and other felids show remarkable sensitivity to the adverse effects of phenolic drugs including acetaminophen (UGT deficiency) and aspirin (deficiency in glycine conjugation) as well as structurally-related toxicants. In contrast, UGTs responsible for detoxification of endogenously generated bilirubin (UGT1A1) are fully functional in felids. Further work is needed to establish whether these preliminary findings can be generalised to all Carnivora (Shrestha et al., 2011).

Species traits related to toxicodynamics

Several frameworks have been proposed over the years to capture our improved mechanistic understanding of the processes involved in chemical toxicity, such as (1) toxicity pathway, (2) mode and mechanism of action, and (3) adverse outcome pathway. The US National Research Council (NRC, 2007) defined a toxicity pathway as a ''*cellular response pathway that, when sufficiently perturbed, is expected to result in an adverse health effect*''. However, the NRC focuses almost exclusively on initiating events and proximal cellular responses that can be measured and modelled *in vitro*, instead of the adverse outcome which is implicit in this definition. Thus, within this framework, the linkage of pathway disruption to adverse outcomes is regarded as part of the science base required to implement the vision, but the pathway itself is at the cellular level.

Mode of action has been defined by the WHO as "*a biologically plausible sequence of key events leading to an observed effect, supported by robust experimental observations and mechanistic data*". MoA describes the key cytological and biochemical events – that is; those that are both measurable and necessary to the observed effect – in a logical framework (Boobis et al., 2006; WHO, 2007; Meek et al., 2014; EFSA, 2014c). MoA has been proposed as a framework to classify toxicity in different classes. As an example, Verhaar et al. (1992) proposed a scheme to classify acute aquatic toxicity using four distinct MoA classes: 1. Narcosis: chemicals are baseline toxicants and assumed to be inert, MoA is assumed to be mediated by chemical lipophilicity; chemical diffusion into biological membranes and disruption of their functions; 2. Polar narcosis: chemicals are slightly more toxic than the baseline toxicants; 3. Reactive chemicals displaying a higher (excess) toxicity compared with their lipophilicity which is assumed to result from unselective, covalent interactions with biomolecules; 4. Specific toxicity where the MoA is mediated through interaction with specific receptor molecules (Verhaar et al., 1992; Segner, 2011). It is the interaction between substance properties and organism traits that ultimately determines the MoA in an organism. Consequently, the MoAs are more complex to depict in a trait-based manner, because of the multiple MoAs that may differently affect target and non-target organisms.

More recently, the Adverse Outcome Pathway (AOP) concept has been proposed as an evolving concept to link chemical exposure to toxicological and ecological effects and to move from an empirical approach to more mechanistic approaches (Ankley et al., 2010). In its original form, an AOP has been defined as "a sequence of events from the exposure of an individual or population to a chemical substance, through to a final adverse (toxic) effect at the individual level (from a human health perspective) or population level (from an environmental perspective)" (Ankley et al., 2010; Meek et al., 2014; OECD, 2013, EFSA, 2014c). The US-EPA has defined AOPs as "the mechanistic or predictive relationship between initial chemical-biological interactions and subsequent perturbations to cellular functions sufficient to elicit disruptions at higher levels of organisation, culminating in an adverse phenotypic outcome in an individual and population relevant to risk assessment" (Ankley et al., 2010). The AOP brings together molecular, physiological and ecological knowledge on taxa-specific traits describing vulnerability and life history at different levels of biological organisation to predict toxicity (Ankley et al., 2010). Thereby, AOP aims to understand the processes that e.g. link the binding of a toxicant to a certain receptor up to the ultimate effects on the community level. Although the definition of the AOP concept includes population and ecosystem level effects, in practice it mainly focuses on processes at the molecular, tissue and organism levels.

Recently, applications of AOPs in human and ecological risk assessment have taken international dimensions with the launching of the Adverse Outcome Pathway Knowledge Base (AOP-KB; https://aopkb.org/background.html) as a partnership between the Organisation for Economic Co-operation and Development (OECD), the U.S. EPA, European Commission - Joint Research Center (JRC), the US Army Corps of Engineers - Engineering Research and Development Center, the World Health Organization (WHO), the International QSAR Foundation, ILSI-HESI, Altamira, LLC, and the US FDA CFSAN. The AOP-KB enables the scientific community, in one central location, to share, develop and discuss their AOP related knowledge. For the moment these database focus on the molecular events and in the future these may be extended to also cover for more ecological events.

Recently, Villeneuve et al. (2014) stated that development of AOP knowledge is a critical scientific activity to support the development and application of alternative methods for chemical risk assessment. The authors propose a generalised strategy in six steps to develop AOPs: 1) Define the purpose/scope of the AOP development activity; 2) Assemble a conceptual model of the known biology for the system of interest; 3) impose pragmatic priorities when needed; 4) link key events via biological plausibility and weight of evidence into hypothesised AOPs; 5) where necessary, conduct research to fill in critical research gaps; and 6) catalogue the assembled information and weight of evidence to support use by risk assessors/regulators and research communities.

Determination of the presence or absence of receptor targets in species using molecular and sequencing techniques has been proposed as a potential tool for prospective risk assessment in several studies. Below follow some recent examples illustrating the potential of this approach.

Conservation of drug targets

A recent study by Gunnarsson et al. (2008) showed how the sensitivity of 16 different species, ranging from vertebrates (zebrafish) via invertebrates (e.g. Daphnia) to plants (green algae), to specifically acting human pharmaceuticals could be linked to the degree of similarity in the molecular receptor targets present for the given pharmaceuticals. By linking the information on human drug targets from the DrugBank database with comparisons of whether a given species had the receptor for a drug or not (based on gene sequence ontology using simple BLAST and Gene Ontology Classification searches) a species potential sensitivity to a drug could be assessed. Not surprisingly, the presence of receptors was highest in vertebrates and lowest in plants, with zebrafish having targets for 86% of the 1318 drug targets tested, and Daphnia and green algae having targets matching only 61% and 35%, respectively. This study shows that by using genetic sequence information only and comparing the presence or absence of targets, or even in time using *in silico* 3D folding of proteins to derive binding affinities between chemicals and species specific enzymes or targets, it might be possible to make judgements on how representative a given test or surrogate species might be for a given endangered species.

Conservation of neurotoxicity targets

Garcia-Reyero et al. (2011) used a transcriptional network approach to compare and contrast the neurotoxic effects of hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) used on military training sites amongst five phylogenetically disparate species: rat (Sprague-Dawley, *Rattus norvegicus*), Northern bobwhite quail (*Colinus virginianus*), fathead minnow (*Pimephales promelas*), earthworm (*Eisenia fetida*), and coral (*Acropora formosa*). Pathway enrichment analysis indicated a conservation of RDX impacts on pathways related between all species but coral. The authors concluded that as evolutionary distance increased common responses decreased with impacts on energy and metabolism dominating effects in coral. A neurotransmission related transcriptional network based on whole rat brain responses to RDX exposure was used to identify functionally related modules of genes, components of which were conserved across species depending upon evolutionary distance. Overall, the meta-analysis using genomic data of the effects of RDX on several species suggested a common and conserved mode of action of the chemical throughout phylogenetically remote organisms.

Extrapolation of sensitivities across species

Lalone et al. (2013) proposed a strategy to combine molecular sequence information (primary amino acid sequence alignments) from databases for a range of non-target species with the identification of specific molecular chemical targets (e.g. analyses of conserved functional domains), the perturbation of which may lead to adverse outcomes. They covered a broad phylogenetic range of species, including vertebrates, invertebrates, plants, bacteria, and viruses. This approach supports the extrapolation of toxicity data across different species for ecological risk assessment, particularly for regulated substances and environmental contaminants with known modes of action. Bioinformatic approaches are employed to automate, collate, and calculate quantitative metrics associated with cross-species sequence similarity of key molecular initiating events (MIEs) of defined adverse outcome pathways. The approach is illustrated in three case studies, dealing with the actions of: (1) 17-ethinyl estradiol on the human (*Homo sapiens*) estrogen receptor; (2) permethrin on the mosquito (*Aedes aegypti*) voltage-gated para-like sodium channel; and (3) 17-trenbolone on the bovine (*Bos taurus*) androgen receptor. The authors foresee, after further refinement, practical and routine utility for this molecular target similarity-based predictive method particularly in the case of limited testing possibilities.

Being able to use molecular and sequencing information to predict the specific sensitivities of species might overcome current limitations in prospective risk assessment, such as the limited testability of certain species. The possible lack of representation by the test species can be addressed either through adjusting the assessment factors used in cases where the test species is proven not to have the target receptors that are found in the endangered species. Or better, the information on “ontology based” receptor (or metabolic enzyme) groupings for specifically acting chemicals can be used to select test species that represent all the “groupings” for general ERA, or specifically to ensure that the tests data is available to make educated estimates of risk for the endangered species. However, it should be kept in mind that taxonomic proximity is not a sufficient condition to assume comparable toxicological sensitivity. As was shown in the earthworm example of Section 4.2.2, the affinity of a chemical for a target receptor may vary significantly between closely related species.

Considering that it cannot be deduced from the existing literature that endangered species have *a priori* different sensitivity, and considering the scarcity of testing possibilities with endangered species, the SC sees great potential for the application of molecular and sequencing techniques to detect the presence or absence of receptor targets and to predict the toxicological sensitivity of (endangered) species. The SC strongly encourages further exploration of these techniques and their potential for use in prospective risk assessments.