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Scientific Opinion on the state of the science on pesticide risk assessment for amphibians and reptiles

Author

Abstract

Following a request from the European Food Safety Authority, the Panel on Plant Protection Products and their Residues developed an opinion on the science to support the potential development of a risk assessment scheme of plant protection products for amphibians and reptiles. The coverage of the risk to amphibians and reptiles by current risk assessments for other vertebrate groups was investigated. Available test methods and exposure models were reviewed with regard to their applicability to amphibians and reptiles. Proposals were made for specific protection goals aiming to protect important ecosystem services and taking into consideration the regulatory framework and existing protection goals for other vertebrates. Uncertainties, knowledge gaps and research needs were highlighted.

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51

52 **Summary**

53

54 **Introduction**

55 The PPR Panel was tasked to provide a scientific opinion on the state of the science on pesticide
56 risk assessment for amphibians and reptiles. Concerns had been raised that the current risk
57 assessment of pesticides may not sufficiently cover the risk to amphibians and reptiles. The
58 opinion should provide the scientific basis for potentially developing a guidance document for
59 pesticide risk assessment for amphibians and reptiles.

60 Amphibians and reptiles do occur in agricultural landscapes, some species resident and some
61 migrating through. Amphibians often breed in water bodies in or adjacent to agricultural fields.
62 Laboratory, field and survey studies have linked pesticides with harm to amphibians. Especially,
63 few existing studies on terrestrial stages of amphibian have shown that currently approved
64 substances and authorized pesticides do cause mortality in frogs and toads at authorized field
65 rates. Even when including possible interception by crop plants, deposited residues are
66 expected to lead to high risks for amphibians. There are few studies on reptiles, but those that
67 exist suggest that pesticides can cause harm and that further investigation is needed.

68 In addition to ecotoxicological concerns, amphibians are the most endangered group of
69 vertebrates with faster decline rates than mammals and birds. Many of the European reptile
70 species are threatened, with 42% of the reptile species exhibiting a declining population trend.
71 The majority of species in both groups are protected species under European regulation.

72 The Panel concludes that exposure of amphibians and reptiles to pesticides does occur, and
73 that this exposure may lead to decline of populations and harm individuals, which would be of
74 high concern. Therefore, a specific environmental risk assessment (ERA) scheme is needed for
75 for these groups.

76

77 **Ecology/Biology of Amphibians and Reptiles**

78 Amphibians and reptiles are two phylogenetically distinct groups that show unique anatomical
79 and physiological features compared with fish, birds and mammals. One common physiological
80 feature of amphibians and reptiles is poikilothermy which differentiates them from birds or
81 mammals. Sensitivity and exposure to pesticides, affected by poikilothermy through its
82 influence on physiology, growth, development, behaviour or reproduction may be shared, but
83 other factors e.g. skins with increased permeability in amphibians, may also have a large
84 influence on risks associated with pesticides. Potential for overspray, dermal exposure by
85 contact with pesticidal active substances on soils or plants, and oral uptake of pesticides
86 through ingestion of contaminated materials exist for both groups. Exposure of amphibians and
87 reptiles when inhabiting a treated area can be prolonged, especially in the case of territorial
88 reptile species or of amphibian aquatic stages.

89 The amphibian life cycle has a major influence on exposure, which is difficult to predict from
90 data generated from other taxa. Amphibians possess some structures typical of higher
91 vertebrates that do not occur in fish (e.g. the Müllerian ducts as precursors of sexual organs).
92 Impacts of pesticides on these structures cannot be identified through assessment based on
93 fish toxicity endpoints and require specific assessment at specific, sensitive time windows in the
94 amphibian's aquatic development.

95 Based on ecological, biological and population distribution traits, a list of potential focal species,
96 that are also suitable to develop population models to support specific protection goals is
97 suggested. Selection based on traits leading to potential high exposure and sensitivity to
98 pesticides is proposed. Regulatory testing of adequate numbers of species representing diverse
99 taxa that exhibit a considerable range of important life-histories and ecologies is required.
100 These species are the great crested newt (*Triturus cristatus*), the natterjack toad (*Epidalea*
101 *calamita*), the common treefrog (*Hyla arborea*), the Hermann's tortoise (*Testudo hermanni*),
102 the sand lizard (*Lacerta agilis*) and the smooth snake (*Coronella austriaca*).

103

104 **Spatial Aspects**

105 Pesticide exposure depends on behaviour of individuals. Realistic risk assessments should take
106 spatial behaviour within a season into account, which is particularly important for migrating
107 amphibians. Population structure and spatio-temporal dynamics can have other important
108 implications for pesticide impacts on amphibian and reptile populations. There is considerable
109 evidence that many amphibians exist in unstable spatially sub-structured populations of various
110 types (e.g. mainland-island), which may be sensitive to pesticide disturbance. Spatial dynamics
111 necessary to support spatially-structured population in the long-term is dependent on landscape
112 structure. Therefore, for inclusion of both the spatial and temporal implications of pesticide
113 usage, and to take the ecological state of the population into account, a systems approach to
114 ERA is recommended.

115

116 **Population Dynamics and Population Modelling**

117 Population dynamics informs the risk assessment primarily through a description of changes in
118 animals' distribution and abundance in space and time. This is justified from basic principles.
119 For the modelling of these dynamics to be useful for the risk assessment, trading off generality
120 for the realism of the systems approach will have to be addressed. The system approach
121 integrates environment, ecology and pesticide use and fate, providing baseline population
122 states against which the impact of the use of the pesticide is assessed. Multiple and varied
123 baseline scenarios may be needed to ensure that the realistic worst-case baseline situation is
124 represented.

125 An illustrative model of Great Crested Newt is presented, demonstrating potential uses in
126 amphibian ERA. Models such as this can help to translate toxicity data to population modelling
127 endpoints at landscape-scales. However, landscape structure, farming assumptions, and
128 weather conditions can be important factors influencing overall population level effects and
129 must be considered carefully in regulatory scenarios. Endpoints from population modelling that
130 can be used in the risk assessment and in support of Specific Protection Goal (SPG) definitions
131 are population impact on abundance and occurrence, as well as changes in total population size
132 with time expressed as relative population growth rates. These endpoints facilitate the
133 assessment of impacts, possible recovery and long-term population viability.

134 To assess risk, landscape-scale spatially-explicit mechanistic models for the six focal species
135 need to be developed and tested. This will provide support for the general risk assessment
136 framework suggested below. If possible, to address the complications of poikilothermy and
137 mobility, a TK/TD modelling component might be directly integrated into the behavioural
138 simulation. Simulation results should be included in lower-tiers as look-up tables of pre-
139 simulated regulatory scenario results. These models can then be also be used for higher tier
140 risk assessment and to support the setting of tolerable magnitude of effect for the protection
141 goals.

142

143 **Specific Protection Goals**

144 SPG Options were developed based on the legislative requirements in place for non-target
145 vertebrates. The need to encompass the endangered status of a great proportion of amphibian
146 and reptile species and the importance of amphibians and reptiles as drivers of valuable
147 ecosystem services in agricultural landscapes was also taken into account. Ecosystem services
148 considered were the provision of genetic resources and biodiversity, maintenance of cultural
149 services, provision of food and pharmaceutical resources, support of nutrient cycling and soil
150 structure formation, regulation of pest and disease outbreak, invasion resistance and the
151 support of food webs.

152 It is proposed that SPG options be agreed on the individual level for the survival of adult
153 amphibians and reptiles; risks to the long-term persistence of populations should be considered
154 for all other impacts. Attributes of population persistence relate to the assessment of

155 abundance/biomass of amphibian and reptile species, but also to the landscape occupancy of
156 these species, and to changes in population growth rates. The limits of operation for
157 amphibians and reptiles in agricultural landscapes were considered to be negligible effects on
158 mortality and small effects of up to months on population impacts for both groups.

159

160 **Toxicological endpoints and effect assessment**

161 A range of toxicological responses related to population fitness in amphibians and reptiles have
162 been shown in laboratory experiments to be potentially useful as test endpoints (e.g. impaired
163 embryo/larval survival, developmental rate, gonadal differentiation, spermatogenesis,
164 oogenesis, fertility rate, and behaviour). Possible endpoints for reproductive and endocrine
165 toxicity testing in amphibians and reptiles include changes in sex-ratio and ovotestis frequency,
166 reproductive organ development and fertility, use of biomarkers for estrogenic compounds, and
167 secondary sex characteristics such as sexually dimorphic characteristics or sexual behaviour.

168 For amphibians there are standardized tests available, of which the following are more often
169 performed: a) the Larval Amphibian Growth and Developmental Assay (LAGDA), b) the
170 Amphibian Metamorphosis Assay (AMA), and c) the Frog Embryo Teratogenesis Assay –
171 *Xenopus* (FETAX). Of these, LAGDA is the most extensive test with an experimental design that
172 allows detection of disrupted metamorphosis as well as sexual development in the model
173 species *Xenopus laevis*. None of the above tests, however, cover the reproductive ability of
174 amphibians. A full life cycle test with amphibians (e.g. with *Xenopus tropicalis* which has a
175 shorter generation time than *Xenopus laevis*) could be very useful in a risk assessment context
176 because it enables the identification of impaired reproductive function following exposure during
177 a sensitive window of development.

178 For reptiles, there are no existing standard test guidelines; there is also a lack of toxicity data
179 for this group of vertebrates. This makes it very difficult to compare the toxicological sensitivity
180 among different reptile species. Efforts should be made to investigate the toxicity of active
181 substances and plant protection on reptiles in order to close these knowledge gaps in future.

182 Differences in sensitivity among life stages, especially within amphibians, should be considered
183 when determining the toxicity of pesticides, since the morphological and physiological
184 differences among them are considerable. Regarding terrestrial amphibian life stages, no
185 agreed guideline exist. However, tests to detect toxicity of pesticides via dermal exposure
186 routes have been carried out, consisting of housing animals in a terrarium and applying the
187 chemical at a realistic rate with a device simulating a professional pesticide application. The
188 Panel stresses the importance of research efforts in the identification of in-vitro test endpoints,
189 in order to minimize animal testing. However, dermal exposure routes are particularly crucial for
190 terrestrial stages of amphibian, since the skin has vital functions in gas and water exchange.
191 These actively steered processes might be difficult to be mimicked in-vitro.

192

193 **Exposure Routes**

194 As a general approach, Exposure Assessment Goals and associated Ecotoxicologically Relevant
195 Exposure Quantities (EREQs) in exposure relevant environmental matrices provide the basis for
196 calculating Predicted Exposure Quantities (PEQs) in the field. EREQs enable a coherent linking
197 between exposure in ecotoxicological experiments and exposure in the field. A final decision on
198 EREQs is possible after agreement on the ecotoxicological effect assessment for amphibians
199 and reptiles (e.g. in test protocols).

200 The main routes of exposure for amphibians in the aquatic system are via contact to pond
201 water and sediment and to a lesser extent via oral uptake. Main entry routes for pesticides into
202 ponds in agricultural areas are spray-drift deposition, runoff or drainage. Sediment may
203 accumulate pesticide residues and in such cases exposure of tadpoles by uptake of sediment
204 may be an important route.

205 The analysis of the dimensions of Spanish and Swiss amphibian ponds and ponds in the UK
206 demonstrated that the large majority (70-90%) of them are considerably shallower and smaller

207 than the FOCUS ponds, used at present in the EU registration procedure. Therefore, we expect
208 peak concentrations in FOCUS ponds not to be conservative estimates for those in the analysed
209 ponds. For peak concentrations in FOCUS ditches and streams the working group was unable to
210 make a general statement on their conservativeness compared to those in the analysed ponds.
211 In view of the higher flow-through rates in the FOCUS ditches and streams the pesticide
212 concentrations are expected to lower rapidly and thus they probably represent underestimates
213 of the chronic exposure in the analysed ponds. The FOCUS scenarios for use in amphibian ERA
214 therefore need to be considered and this may entail the gathering of data via surveys of
215 amphibian use of water bodies along with chemical monitoring. It is important to note that
216 small surface waters are not routinely monitored and thus chemical monitoring should be
217 extended.

218 In their terrestrial environment dermal exposure via direct overspray and contact to residues on
219 soil and plant surfaces are important exposure routes as well as oral uptake of contaminated
220 food.

221 The main exposure routes for reptiles are food intake, contact to residues on soil and plants
222 and contact of eggs to contaminated soil. As reptiles have a high site fidelity, dermal uptake
223 may be more important for reptiles than amphibians although their skin is less permeable than
224 the skin of amphibians.

225

226 **Coverage of Amphibians and Reptiles by Existing RA**

227 It is important to distinguish between the *predictability*, i.e. the coverage of existing test results
228 with other non-target organisms as a surrogate for toxicological sensitivity of amphibians and
229 reptiles and the *protectivity* of existing risk assessment procedures as a surrogate for the
230 protection of amphibians and reptiles toward risks from PPP intended uses.

231 The potential of relying on other vertebrates as surrogates for amphibians and reptiles to cover
232 toxicity of pesticides is compromised by some particular biological processes typical of these
233 animals, including metamorphosis in amphibians or hormone dependent sex determination and
234 reproductive organ development in both amphibians and reptiles. Thus, impacts of pesticides
235 need to be assessed for specific, sensitive time windows within the animals' development.

236 Exposure through water:

237 Several studies indicate that the acute endpoints for aquatic life stages of amphibians (eggs,
238 embryos, tadpoles and adults) are lower than the acute endpoints for fish in about 30% of the
239 cases. Therefore, if a higher percentage of all cases should be covered, an extrapolation factor
240 needs to be applied on the acute fish endpoint if it has to be used in the risk assessment of
241 amphibians. Uncertainty with regard to representativeness of *Xenopus laevis* for European
242 amphibian species and species sensitivity distribution needs to be addressed further to suggest
243 extrapolation factors.

244 No conclusion can be drawn for the coverage of the chronic sensitivity of amphibians by fish
245 because of limitations in comparability of chronic studies and endpoints observed in those
246 studies. Furthermore, the chronic fish studies do not address relevant sub-lethal endpoints
247 effects on metamorphosis, reproduction or immunosuppression in amphibians. No data and
248 thus no comparison in toxicity was possible for reptiles in the aquatic system.

249 Oral and dermal exposure in terrestrial environment:

250 The oral exposure estimates from the screening steps in the risk assessment for birds and
251 mammals may cover the oral exposure estimate for amphibians and reptiles. In order to
252 estimate oral exposure, allometric equations as in the bird and mammal risk assessment could
253 be applied with amphibian and reptile specific parameters. One existing model is the US-EPA T-
254 herps model, which would need to be adjusted for European species. Whether the risk to
255 amphibians and reptiles is covered by the risk assessment of birds and mammals depends on
256 the differences in toxicological sensitivity and assessment factors applied.

257 The comparisons of the daily dietary exposure and dermal exposure from overspray (assuming
258 100% uptake) give an indication that both exposure pathways are of high importance for

259 amphibians and reptiles and hence both should be addressed in the risk assessment. However,
260 the risk from dermal exposure is not assessed for birds and mammals. Therefore, protection of
261 reptiles by the risk assessment for birds and mammals is highly uncertain.

262 The exposure model for workers or alternatively the dermal exposure models for birds from US-
263 EPA TIM could be used to estimate the systemic exposure via dermal uptake in terrestrial
264 stages of amphibians and reptiles from contact to residues on plants or soil after adjusting with
265 amphibian and reptile specific factors such as the dermal absorption fraction (DAF), the surface
266 area of the animal, and foliar contact rate. For the time being 100% dermal absorption of
267 substances is suggested. It may be possible to refine this value once data on dermal absorption
268 become available for different active substances. Data need to be generated on the body
269 surface area in contact with the soil and in contact with plant surfaces when they move, the
270 speed of movement and time when they are actively moving versus resting.

271 It is recommended that experiments are performed to analyse the quantities taken up by the
272 animals by the various routes of dermal contact to understand how these quantities add to the
273 systemic exposure of the animals. Moreover, the effects of pesticides on the skin of amphibian
274 as an organized organ actively regulating water and gas exchange should be investigated.

275

276 **General Risk Assessment Framework**

277 The general risk assessment framework suggested is based on a tiered approach but is adapted
278 to take account of parallel lines of assessment for local and landscape scale assessment which
279 takes into account long-term population risks.

280 In general, data are needed on the chronic toxicity of pesticides for amphibians, starting from
281 the exposure in the aquatic stages up to and including reproductive stages. The determination
282 of effects of pesticides terrestrial stages via the dermal route of exposure is a central
283 requirement for amphibians. Effects determinations in juvenile frogs are needed until
284 development of surrogate in-vitro tests is sufficiently advanced. For reptiles, toxicity data for
285 both acute and chronic endpoints are lacking and there is insufficient data to support mammals
286 or birds as surrogates for toxicity testing. Consequently, research is needed to allow any
287 emerging relationships to existing tests (e.g. bird testing), to be sufficiently supported. All
288 addressed endpoints should be determined in simple experiments allocated at the lower
289 assessment tier. Inclusion of further animal testing at higher tiers (e.g. multi-species tests or
290 field studies), is not recommended. Higher assessment tiers should rely on refinement of
291 exposure options.

292 The risk assessment scheme comprises an evaluation of effects at the local scale and long-term
293 effects at the landscape scale. At local scale, a risk assessment for all relevant environmental
294 compartments in which different life-stages occur would be performed. After an assessment of
295 acute and chronic effects at local scale, the risks of intended pesticide uses have to be assessed
296 at the landscape scale. At landscape-scale, all life-stages and compartments should be
297 combined in a single risk assessment. The landscape scale also covers single population long-
298 term risk assessment over years of pesticide use. This should be performed in a first step using
299 pre-run computer models that address the long-term repercussions of the effects of year-on-
300 year use of pesticides on amphibian and reptile populations.

301 Within each compartment, the impact of pesticides on amphibians and reptiles resulting from a
302 combination of the main exposure routes should be performed - as the different exposure
303 routes are considered equally important at present. It is suggested that the outcome of
304 exposure to pesticides by several routes is addressed in order to combine the risks of the main
305 routes. As a pragmatic worst-case approach for the first-tier risk assessment, combination of
306 the relevant terrestrial exposure routes following the approach used for mixture toxicity is
307 suggested.

308 Unlike other non-target groups, recovery may not be considered as an option for amphibians
309 and reptiles since no long-term impact on populations is likely to be allowed. However, short-
310 term recovery e.g. by local density-dependent compensation during larval stages may still need
311 to be considered as part of an integrated population assessment.

312 It is suggested that management options to mitigate risks from pesticide use on amphibians
313 and reptiles identified at lower tiers are considered and exhausted before higher tier
314 assessment is performed, especially when higher tier approaches should include animal testing.
315 Mitigation options would need to be locally specified to be successful.

316 Two main areas where uncertainty needs to be generally addressed in the risk assessment of
317 amphibians and reptiles are the calibration of a risk assessment scheme and the treatment of
318 additional uncertainties in the assessment (e.g. use of surrogates). The aim of developing the
319 local and landscape-long-term assessments and supporting these with further data collection
320 and ideally short-term use of toxicity testing is to reduce these uncertainties as quickly as
321 possible.

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507

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508

509 1. Introduction

510 1.1. Background and Terms of Reference as provided by the 511 requestor

512 The PPR panel is tasked with the update of the Guidance Document on Terrestrial Ecotoxicology
513 under mandate M-2009-0002. The Guidance Documents that are still in place were developed
514 under Directive 91/414/EEC¹. A public consultation on the existing Guidance Documents was
515 held by EFSA in 2008 in order to collect input for the revision of the aquatic and terrestrial
516 Guidance Documents (EFSA 2009a). The following points were most often mentioned in the
517 comments for updating the Guidance Documents:

- 518 – Considerations of the revision of Annexes II and III of Directive 91/414/EEC,
- 519 – Consideration of the new Regulation (EC) 1107/2009.²
- 520 – Harmonisation with other directives and regulations (biocides, REACH)
- 521 – Clearly defined protection goals
- 522 – Multiple exposure
- 523 – Inclusion of additional species in the risk assessment (e.g. amphibians, reptiles, bats,
524 molluscs, ferns, mosses, lichens, butterflies, grasshoppers and moths)
- 525 – More guidance on statistical analysis
- 526 – Preference of ECx over NOEC values in the risk assessment
- 527 – To consider all available information from workshops (EUFRAM, ESCORT, PERAS and
528 other SETAC workshops)
- 529 – Endocrine disruption
- 530 – Consideration of all routes of exposure
- 531 – Bee risk assessment
- 532 – Non-target arthropods risk assessment
- 533 – Soil organism risk assessment

534 The comments received in the stakeholder consultation will be consulted on again during the
535 revision of the Guidance document.

536 A survey on the needs and priorities regarding Guidance Documents was conducted among
537 Member States Authorities and a final list was compiled in the Pesticide Steering Committee
538 meeting in November and December 2010.

539 The following topics were indicated as priorities for the update of the terrestrial Guidance
540 Document:

- 541 – Assessment of impacts on non-target organisms including the ongoing behaviour
- 542 – Impact on biodiversity
- 543 – Impact on the ecosystem

¹ Council Directive 91/414/EEC concerning the placing of plant protection products on the market OJ L 230, 19.8.1991, pp. 1-32.

² Regulation (EC) No 1107/2009 of the European Parliament and of the council of 21 October 2009 concerning the placing of plant protection products on the market and repealing Council Directives 79/117/EEC and 91/414/EEC. OJ L 309/1,24.11.2009, pp.1-50.

- 544 – Effects on bees
- 545 – Effects on amphibians and reptiles
- 546 – Linking exposure to effects and ecological recovery
- 547 – The use of field studies in the risk assessment and guidance for interpretation of field
548 studies
- 549 – Revision of non-target arthropod risk assessment (ESCORT II)
- 550 – Guidance for risk assessment in greenhouses
- 551 – Definitions of environmental hazard criteria (POP, PBT, vPvB) that will serve as a cut-off
552 criteria according to the new regulation. Guidance on what studies, test conditions and
553 endpoints should be used in determining whether the cut-off values have or have not
554 been met. The Commission will consider the respective competencies of institutions
555 regarding this topic and will check whether it takes the lead in this area.
- 556 – Definition of hazard criteria in relation to endocrine disruption and guidance on what
557 studies, test conditions and endpoints should be used in determining whether the cut-
558 off values have or have not been met. The Commission has the lead in developing
559 these criteria. It is expected that the Commission will consult EFSA on the final report in
560 October 2011. The outcome of these activities should be incorporated in the Guidance
561 Documents.
- 562 Generic questions that arose during the peer-review expert meetings should also be taken into
563 consideration in the update of the guidance document. The pesticides unit provided a
564 compilation of general reports. One of the points mentioned was that more detailed guidance is
565 needed for the risk assessment of non-target plants (e.g. sensitivity of test species, use of
566 species-sensitivity distributions, exposure estimates).
- 567 Regulation (EC) 1107/2009 states that the use of plant protection products should have no
568 unacceptable effects on the environment. The regulation lists in particular effects on non-target
569 species, including their ongoing behaviour and impact on biodiversity and the ecosystem.
- 570 The assessment of effects on ongoing behaviour and biodiversity are not explicitly addressed
571 under the existing Guidance Documents and appropriate risk-assessment methodology needs to
572 be developed.
- 573 The expertise needed in the different areas of terrestrial ecotoxicology ranges from in-soil
574 biology, non-target arthropods, bees and other pollinating insects, terrestrial non-target plants,
575 amphibians and reptiles, and modelling approaches in the risk assessment.
- 576 This justifies the need to split the activity in several separate areas due to the complexity of the
577 task and in order to make most efficient use of resources.
- 578 A separate question was received from the European Commission to develop a Guidance
579 Document on the Risk Assessment of Plant Protection Products for bees and to deliver an
580 opinion on the science behind the risk-assessment guidance. This question will be dealt with
581 under mandate M-2011-0185 (to be found on efsa.europa.eu).
- 582
- 583 EFSA tasked the Pesticides Unit and the PPR Panel with the following activities, taking into
584 consideration Regulation (EC) 1107/2009, stakeholder comments and the recommendations and
585 priorities identified by Member States:
- 586 • Scientific Opinion on the state of the science on pesticide-risk assessment for
587 amphibians and reptiles
- 588 • Public Consultation on the draft Scientific Opinion on the state of the science on
589 pesticide risk assessment for amphibians and reptiles
- 590 • EFSA Guidance document on pesticide risk assessment for amphibians and reptiles, to
591 be delivered within two years after agreement on specific protection goals

- 592 • Public consultation on the draft EFSA Guidance document on pesticide risk assessment
593 for amphibians and reptiles

594

595 **1.2. Interpretation of the Terms of Reference**

596 The PPR panel is tasked to provide a scientific opinion on the state of the science on pesticide
597 risk assessment for amphibians and reptiles. In order to provide a scientific basis for a future
598 development of a guidance document, the panel suggests first addressing the following
599 questions in the current opinion :

- 600 1. Do amphibians and reptiles occur in agricultural landscapes?
- 601 2. Are amphibians and reptiles exposed to pesticides?
- 602 3. Are amphibians and reptiles adversely affected by pesticides?

603 As a result of affirmative answers to the three questions above (see chapters 1.3,1.4 below and
604 chapter 2), these specific topics were addressed in the current opinion:

- 605 1. Possible specific protection goal options for consideration by risk managers (in
606 particular for long-term, population-level effects)
- 607 2. Consideration of endangered species
- 608 3. Overlap of occurrence of amphibians and reptiles and pesticide applications in
609 agricultural landscapes.
- 610 4. Consideration of other stressors in a landscape context
- 611 5. Toxicological endpoints relevant for amphibians and reptiles
- 612 6. Potential coverage of the risk to amphibians and reptiles by the risk assessment for
613 other groups of organisms including human risk assessment.
- 614 7. Use of endpoints from other groups of organisms
- 615 8. Recommendations for testing in risk-assessment context *vs.* recommendations for
616 testing in research context to elaborate the basis for risk assessment in order to avoid
617 testing for each product.
- 618 9. Suggestions for the development of aquatic and terrestrial exposure assessment
619 methodology.
- 620 10. Identification of future research needs.

621

622 **1.3. General considerations on the need for investigating** 623 **pesticide impacts on amphibians and reptiles**

624 Loss of biodiversity and its consequences for ecosystem services provided to humans is of high
625 concern and has led to initiatives such as the convention on biological diversity. The EU
626 pesticide regulation makes specific reference to “no unacceptable” effects on biodiversity as a
627 decision criterion for approval of pesticides.

628 Vertebrate biodiversity is decreasing rapidly. Amphibians are the most endangered group of
629 vertebrate species with faster decline rates than mammals and birds (Hoffmann et al 2010,
630 IUCN 2008). About 20% of the European reptile species are threatened and the population
631 trend shows a decline for 42% of the reptile species (Cox and Temple, 2009). A worldwide
632 analysis of threatened reptile species resulted in an estimate of 15-36% of threatened species
633 (Böhm et al. 2013).

634 Exposure to xenobiotic chemicals is hypothesised to be one of the causes of declines of
635 amphibian and reptile species (e.g. Alford 2010, Todd et al. 2010). Other important stressors
636 are habitat destruction, diseases, invasive species and over-exploitation. These stressors

637 interact and can cause much more severe effects in combination e.g. regarding pesticides and
638 susceptibility to predation (e.g. Rylea et al. 2003). The quality and configuration of the habitats
639 in which amphibians and reptiles live are of high importance, for example in modulating
640 exposure and effects for amphibian population during migration (e.g. Lenhart et al. 2015). The
641 impact of pesticides may be altered by exposure to fertilisers and to other stressors in the
642 agricultural environment, which makes linking effects of single active substances observed in a
643 laboratory studies to field effects challenging (Mann et al. 2009). Although there is published
644 evidence showing that endocrine disrupting chemicals will also have some detrimental effects
645 on amphibians or reptiles (Safholm et al, 2014), very little is known about the effects of
646 pesticides at environmentally relevant concentrations (Wagner et al., 2014).

647 Therefore, identification of evidence for an impact of a chemical on wildlife needs to consider
648 laboratory studies and field observations and to interpret them in a landscape-specific context.

649 Amphibian and reptile species do occur in agricultural landscapes (Fryday and Thompson 2009,
650 Fryday and Thompson 2012). Some species move through fields during their migratory phase
651 (Berger et al. 2015) and some species such as crested newt even prefer agricultural fields to
652 off-field habitats (Cooke 1986). Amphibians often breed in water bodies (ponds, streams) in
653 agricultural areas and are thereby exposed to pesticides expected to occur in such waters.
654 Several pesticides have been detected in water and sediments of breeding ponds e.g. in the
655 United States in the µg/l-range (Battaglin et al., 2009; Fellers et al., 2013; Smalling et al., 2015;
656 Battaglin et al., 2016). The scarcity of monitoring data in small, standing waterbodies in the EU
657 has been criticised (Aldrich et al., 2015) as such waters are not routinely monitored under the
658 Water Framework Directive (WFD)³. Action has, however, been taken in different member
659 states, e.g. in Germany within the National Action Plan on sustainable use of pesticides
660 ("Kleingewässermonitoring", coordinated by the German Environment Agency). Unpublished
661 preliminary data from several small standing ponds suitable for amphibians in an agricultural
662 area in Switzerland seem to indicate that the concentrations of several PPPs are within the
663 same range of concentrations measured in flowing surface waters (Wittmer et al., 2014). The
664 use of in-field areas for foraging and laying eggs in some reptile species has also been
665 demonstrated (e.g. Wisler et al 2008).

666 There is overlap between pesticide applications and occurrence of amphibians and reptiles in
667 agricultural landscapes (e.g. Berger et al. 2015) and concerns have been raised that the current
668 risk assessment may not sufficiently cover amphibians and reptiles (e.g. Brühl et al. 2013,
669 Weltje et al. 2013).

670 **1.4. Specific evidence of pesticide impacts and need for action**

671 The works cited above give the overall picture that amphibians and reptiles, which are
672 vertebrate groups with a high occurrence of threatened species, are present in agricultural
673 fields, because they use them as habitats, breed in associated water bodies or cross them
674 during migration at time of PPP use. But is this co-occurrence of PPPs and the animals a
675 concern in reality? There is recent evidence from both field and laboratory studies indicating
676 that the use of PPPs poses a risk to reproduction and survival in amphibian and reptile
677 populations (e.g. Brühl et al. 2013).

678 **1.4.1. Amphibians**

679 **Aquatic stages**

680 Studies have shown lethal, teratogenic (deformation), endocrine, reproductive, behavioural,
681 immunosuppressive or genotoxic effects of pesticides on amphibians. Indirect effects have also
682 been observed e.g. the perceived palatability of gray treefrog tadpoles, which are normally
683 noxious to fish predators, has been altered by the exposure of fish to carbaryl (Hanlon and

³ Directive 2000/60/EC of the European Parliament and of the Council of 23 October 2000 establishing a framework for Community action in the field of water policy. OJ L 327/1, 22.12.2000, pp. 1–72.

684 Parris, 2013). It has to be stated, though, that a number of studies seem to contradict each
685 other – whereas one study observed an effect in the laboratory, another study did not observe
686 the same effect in a different laboratory or in a mesocosm study. Tested species, morphology,
687 exposed life stage, pre-exposure, duration of exposure and observation, type of effect, type of
688 replicates as well as type of active substance, single, in mixtures or formulated and
689 concentration tested all contribute to these variations (Shuman-Goodler and Propper, 2016;
690 Wagner et al., 2016a; Wagner et al., 2016b; Jones and Relyea, 2015; Biga and Blaustein, 2013;
691 Wagner et al., 2013; Egea-Serrano et al., 2012; Jones et al., 2009). Effects may be aggravated
692 in studies owing to confounding factors such as UV, predators, parasites, pH or fertilizers.
693 Monitoring of endocrine and reproductive disruption in wild amphibian populations is hampered
694 at present by a lack of validated biomarkers. Several field studies demonstrate increased
695 incidences of gonadal intersex (the presence of ovarian follicles within the testicle) in male
696 amphibians inhabiting agriculture intensive areas (Hayes et al 2003; McCoy et al 2008;
697 McDaniel et al 2008). Interestingly, male amphibians inhabiting habitats characterized by an
698 increasing degree of agricultural activity displayed a gradual reduction in the display of
699 secondary sex characters i.e. reduced forelimb size and nuptial pad size (McCoy et al 2008).
700 These findings may indicate an impact of anti-androgenic chemicals. Anti-androgens act by
701 diminishing the action of androgens, either through androgen receptor antagonism or by
702 changing steroid hormone metabolism. Several widely used pesticides (e.g. imidazoles) were
703 recently shown to have anti-androgenic activity in vitro (Orton et al 2011). Laboratory studies
704 have shown that environmentally relevant concentrations of the pesticide atrazine (not
705 approved in Europe) can severely impair reproductive development and output in amphibians
706 i.e. *Xenopus laevis* and *Lithobates pipiens* (Hayes et al 2002; 2010). Davidson et al. (2001,
707 2002) reported a correlation on a larger scale between pesticide usage and amphibian decline
708 in the Sierra Nevada Mountains in California owing to pesticide use on agricultural land upwind.

709 The conflicting results emphasize the importance of examining the effects in natural settings,
710 where indirect effects can also be observed. See Lehman and Williams (2010) for a review of
711 the effects of current-use pesticides on amphibians. So far, some substances have been
712 highlighted in the literature to be of great concern with regards to toxicity to amphibians such
713 as organophosphates, organochlorines, carbamates and pyrethroids (Mann et al., 2009;
714 Shuman-Goodier and Propper, 2016). Phosphonoglycines and triazines did overall not show
715 negative effects on swim speed and activity of aquatic vertebrates (amphibians and fish) in a
716 meta-analysis (Shuman-Goodier and Propper, 2016). It seemed that shorter exposure times
717 (pulse exposure) of pyrethroids caused larger effects on activity. The question is whether
718 authorised pesticides cause adverse effects on amphibians and reptiles at concentrations
719 considered safe.

720 In laboratory settings, effects on *Hyla intermedia* from Gosner stage 25 to completion of
721 metamorphosis (GS 46) were observed in a long-term exposure (78 days) laboratory study
722 (Bernabo et al., 2016) with pyrimethanil and tebuconazole at regulatory acceptable
723 concentrations. The regulatory acceptable concentrations (i.e. the concentration that drives the
724 aquatic risk assessment) derived from the standard surrogate species are for pyrimethanil RAC
725 = 8 µg/l (NOEC = 80 µg/l for *O. mykiss* based on a 100 d long early life study) (UBA 2016). At
726 5 and 50 µg/l of pyrimethanil survival was significantly decreased (56% and 44% for
727 pyrimethanil), the incidence of deformity increased (23% and 9% for pyrimethanil), and the
728 time to complete metamorphosis was delayed by 2.4-4.4 days. Effects on survival and
729 deformity occurred in a nonlinear relationship before the onset of the metamorphic climax,
730 which has also been observed before for chlorothalonil and atrazine, possibly due to the
731 endocrine-disruption potential of these substances before the metamorphic climax.

732 **Terrestrial stages**

733 Experimental findings by Belden et al. (2010), Brühl et al (2013, 2015) and by notifying
734 companies point to significant risks for amphibian in their terrestrial life stages exposed to
735 intended uses of PPPs. The active ingredients tested are amongst the most used in Europe and
736 pesticides were applied according to field rates that are currently authorized. These findings are
737 further described here by way of example, in order to clarify the PPR Panel's initial concerns

738 and the rationale behind the analysis of coverage and possible major deficits in the current
739 assessment schemes regarding the risks for amphibians and reptiles.

740 Belden et al. (2010) treated tadpoles and juveniles of *Bufo cognatus* (Great Plain Toad) with an
741 aerosol spray of PPPs with fungicidal mode of action (or water in the controls) while contained
742 in aquaria. Juveniles were placed on soil, tadpoles in water mixed with fungicide spray. The
743 chosen concentrations for every tested fungicide were the authorized label rate ('Med' in Figure
744 1:), one tenth of the label rate ('Low') and 10 times the authorized rate ('High'). The fungicides
745 contained the active substances pyraclostrobin (Headline), propiconazole with trifloxistrobin
746 (Stratego) and propiconazole with azoxystrobin (Quilt) in different percentages (see Belden et
747 al. 2010 for further details).

748 Significant levels of toxicity were noted for two out of three fungicides. All concentrations of the
749 fungicide Headline resulted in 100% tadpole mortality and the medium and highest
750 concentrations resulted in significant toxicity to juveniles (Figure 1:).

751 Since mortality occurred mostly within the first 24 hours after spraying, the authors concluded
752 that "thus, juveniles exposed in a normal spraying event, such as in a field undergoing fungicide
753 application, will likely not survive. Furthermore, tadpoles in a wetland directly sprayed or
754 exposed to spray drift at 10% of the application rate will likely not survive". The water
755 concentrations in the low rate compared roughly to a calculated realistic worst-case
756 environment concentrations in surface waters not oversprayed and without further refinements
757 (FOCUS step 1 at intended uses in Europe). The authors concluded further that comparative
758 acute sensitivity was to be expected for fish and crustacean species, but that no similar
759 comparison was possible for aerial exposure of juvenile toads. It was argued that behavioural
760 patterns vary among species, but that the tested species is active during the day and spends
761 much of its time above ground, potentially resulting in full exposure. Further, potential exposure
762 might vary with age, but newly morphed individuals of all amphibian species in the investigated
763 area Great Plains are present above ground during daylight hours (Belden et al. 2010).

764

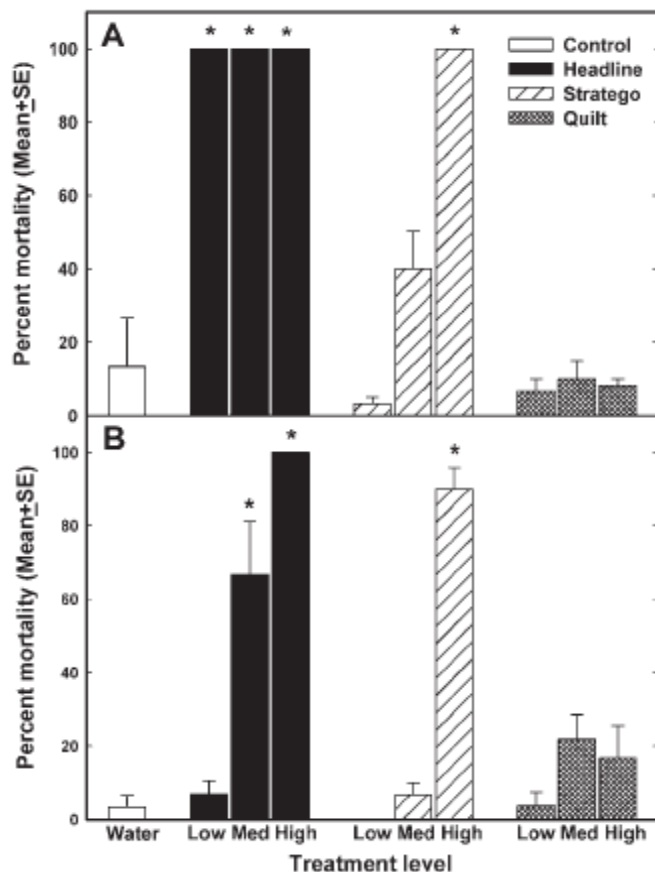


Figure 1: Mean percent mortality (\pm standard error) of *Bufo cognatus* tadpoles (A) and juveniles (B) 72 h after a single exposure to either Headline, Stratego or Quilt fungicide at maximum label rate for corn (Med), or 0.10 label rate (Low) or 10 x label rate (High). From Belden et al. (2010)

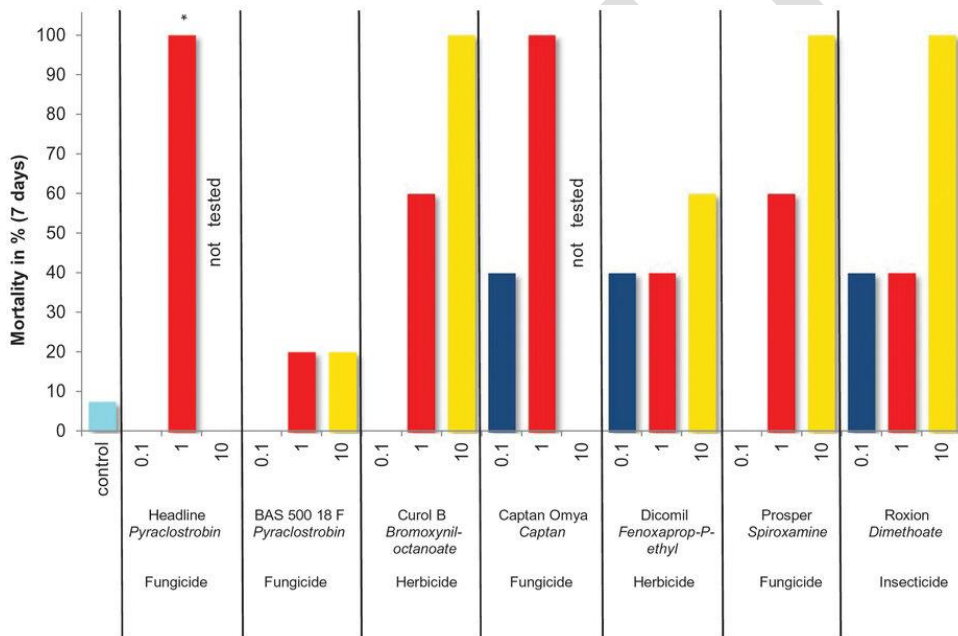
786

787 Brühl et al. (2013) mimicked exposure in a terrestrial environment where juvenile frogs were
788 directly oversprayed by authorised field rates. The effects of seven PPPs (four fungicides, two
789 herbicides and one insecticide) on juvenile European common frogs (*Rana temporaria*) were
790 investigated. The selected PPPs are regularly employed in cereals and orchard in Central Europe
791 (Germany and Switzerland). For one of the PPPs containing the active substance pyraclostrobin,
792 a formulation of known toxicity was also tested (Headline EC, Belden et al. 2010) in addition to
793 another type of formulation with the same active substance (BAS 500 18 F).

794 The tested rates for all PPP were those authorized for the intended uses (label rate 1x), a tenth
795 of the label rate (0.1x) and ten times the recommended label rate (10x; see Figure 2:). The
796 test set up was again a realistic worst-case scenario for terrestrial exposure of juvenile frogs
797 leaving breeding ponds in spring. The frogs were exposed to PPP overspray in terrestrial
798 microcosms with natural soils, and for the following seven days also to residues of the applied
799 PPP in the soil matrix.

800

801



802

803 **Figure 2:** Mortality of juvenile European common frogs (*Rana temporaria*) after seven days
804 following an overspray exposure for seven pesticides at 0.1x, 1x and 10x the label rate
805 (formulation name, active substance and class are given). From Brühl et al. (2013)

806 As a result of the exposure, acute mortality ranged from 100% after one hour to 40% after
807 seven days at the recommended label rate of currently authorized PPP intended uses (Figure 2:
808). Three PPPs out of seven caused a mortality of 40% after seven days at the lowest rate tested
809 (10% of the authorized rate). PPPs with the same active but varying in formulation type
810 showed pronounced differences in acute toxicity for this amphibian species: one formulation
811 caused 100% mortality after one hour, while another formulation with the same concentration
812 of active substance caused only 20% mortality in the rate corresponding to 10x the authorized
813 rate. The relation between the juvenile frog mortality and some specific parameters (e.g.
814 content of naphtha-compounds as co-formulants, log Pow of the active substance) as well as
815 additional toxicity data (fish toxicity, inhalation toxicity, potential for eye irritation) was further
816 investigated (Brühl et al., 2015). The calculations of simple linear regressions revealed no
817 statistically significant relationship for the majority of the investigated parameters, which may
818 be due to the low number of pesticides investigated. The only relationship that proved to be
819 statistically significant was the one detected between values of product-inhalation toxicity and

820 the toxicity to *R. temporaria*. Furthermore, the inclusion of skin sensitization as categorical
821 variable increased the statistical significance of the correlation.

822 In the study set-up of Brühl et al. (2013), it could not be determined whether the active
823 substance itself or effects of co-formulants determined the final toxicity of PPP for amphibian
824 terrestrial stages. Further data submitted by notifiers to EFSA and national authorities for active
825 substance and PPP authorization confirm that the the active substances can drive the toxicity of
826 PPP, that the formulation type can modulate this toxicity (see Table 1), that interception by
827 plants reduces the exposure of these animals in-field and overspray can be seen as a realistic
828 worst-case exposure scenario.

829 For the one active substance that was formulated in different products A, B and C, acute toxicity
830 values for *Rana temporaria* exposed in an overspray scenario differed by a factor 6 to 7 (see
831 Table 1:). Here, the formulation type also differed between the tested products, not only the
832 composition of the co-formulant system. Formulation B was a slow-release capsule suspension
833 and C water-dispersible granules.

834 Interestingly, data are also available for the blank formulation without active ingredient of
835 product A as an emulsifiable concentrate. The results of the tests with product A and its blank
836 formulation show that the active substance itself is the driver of the product toxicity and not the
837 co-formulants, since no effect could be detected at the highest tested rate of the blank
838 formulation, while at the same rate exposure to the product resulted in 70% mortality of the
839 juvenile frogs.

840 The question arises why different PPP with different formulation types might have different
841 effects if is the active substance that causes the observed mortality. Apparently, the dynamic of
842 the exposure of the organisms to the active substance is modulated by the type of formulation,
843 most clearly seen in the lower toxicity of the slow-release encapsulated formulation. Co-
844 formulants of the emulsifiable concentrate might possibly enhance the skin passage of the
845 active substance without being toxic themselves (see Table 1:). It appears that, for this active
846 substance, the available amount over time and the dosage form determine its toxic effect *via*
847 the dermal route for terrestrial stages of amphibians.

848

849 **Table 1:** Toxicity of three PPPs with the same active ingredient but different formulation
850 type expressed as Toxicity to Exposure Ratio (TER) between the mean lethal rate
851 (LC_{50}) or rate causing no mortality (LC_0) and the intended field-application rate. The
852 test organism was the amphibian *Rana temporaria* in a realistic worst-case
853 overspray scenario. EC emulsifiable concentrate; CS capsule suspension; WG
854 wettable granules. Adapted from information submitted for pesticide registration.

Formulation	Formulation type	TER LR_{50} / field rate [g a.s./ha]	TER LR_0 / field rate [g a.s./ha]
A	EC	0.38	0.25
blank formulation A	EC	>> 0.39	≥ 0.39
B	CS	2.80	0.80
C	WG	> 2.04	0.92

855

856 Contact with contaminated soil also delivers an important exposure path for the juvenile frogs,
857 although less crucial compared with overspray (see formulation A in Table 1: and 0). If the PPP
858 spray residues were allowed to dry up shortly before juvenile frogs were introduced, then the
859 observed effects were higher than if animals were introduced after four hours. Nevertheless,
860 calculated toxicity to exposure ratio remained low also for this exposure route, showing a high
861 toxicity of the formulation to juvenile frogs. Refinement steps are not presented at this stage,
862 but would need to reduce exposure by a factor of 10-40x in order to reach a TER of 10 on
863 acute mortality of juvenile amphibians.

864 **Table 2:** Toxicity of a PPP in different test-exposure scenarios, expressed as Toxicity to
 865 Exposure Ratio (TER) between the mean lethal rate (LC₅₀) or No Observed Effect
 866 Rate (NOAER) and the intended field-application rate. Test organism was the
 867 amphibian *Bufo bufo* placed on soil directly after spraying with the intended rate or
 868 several hours afterwards. EC emulsifiable concentrate. Adapted from information
 869 submitted for pesticide registration.

Formulation	Test set up	TER LR ₅₀ / field rate [g a.s./ha]	TER NOAER / field rate [g a.s./ha]
A	Animals introduced shortly after spray residue on soil dried up	> 1.2	0.6
	Animal introduced four hours after spray application to soil	> 1.2	≥1.2

870

871 In conclusion, several of the tested PPPs show strong effect on the survival of amphibian
 872 terrestrial life stages at label rates or even less. The tested products and similar formulations
 873 (apart from Headline[®] for the US market) have been authorized for the market and have
 874 passed the assessment of the risks posed by their intended uses for all non-target organism
 875 groups currently considered. Moreover, the concerns raised might even increase, considering
 876 that the exposure tested in all studies above is short-term and mortality was the main endpoint
 877 assessed. Also when taking into account possible exposure refinement (e.g. plant interception),
 878 risk might still be high, deeming for the time being a ratio between acute toxicity and predicted
 879 exposure (TER) of at least 10 as acceptable. As Belden et al. (2010) pointed out, "in an actual
 880 application, longer-term exposure, chronic effects, and less tolerant species are all likely to
 881 occur".

882 It has been shown (see Table 1:) that the toxicity of the active substance itself can be the
 883 driver of the observed mortality for amphibian terrestrial life stages. Since the amount of
 884 available data is very poor, it cannot be concluded at the moment that this would be the case
 885 for all tested PPPs. Interaction between toxicity of active substance, the co-formulant system
 886 and the formulation type might interact in modifying the resulting impact on amphibians.

887 1.4.2. Reptiles

888 Direct evidence regarding protectiveness of the current risk-assessment scheme on reptiles is
 889 missing, which is in partly due to the scarcity of studies in this context. Rauschenberger *et al.*
 890 (2007) suggest that parental exposure to organochlorine pesticides (OCP) may be contributing
 891 to low clutch viability in wild alligators (*Alligator mississippiensis*) inhabiting OCP-contaminated
 892 habitats in central Florida. Rodenticides as baits may be taken up when softened by rain by
 893 lizards and cause adverse effects (Spur, 1993). Hall (1980) stated that reports of reptilian
 894 mortality from pesticide applications are numerous enough to establish the sensitivity of reptiles
 895 to these materials. Reports of residue analyses demonstrated the ability of reptiles to
 896 accumulate various contaminants. But the significance of the residues to reptilian populations
 897 remained unknown.

898 Recent data on cypermethrin in lizards (Chen et al., 2016) indicate that metabolic rates are
 899 strongly affected by external temperature and this may increase the elimination half-life of
 900 pesticides (in this study, cypermethrin). Some reports on anticoagulants also show that these
 901 compounds are poorly metabolized by reptiles. The susceptibility of reptiles to anticoagulants is
 902 not known precisely but it appears that they may accumulate these compounds to a greater
 903 extent than other, more susceptible species such as mammals. Evidence from rodent-
 904 eradication programs in tropical islands confirms that reptiles (gecko) contained residues of
 905 brodifacoum in liver samples but did not display any evidence of poisoning (Pitt et al., 2015).
 906 Exposure experiments on Floreana lava lizards (*Microlophus grayii*), Geckos and snakes from
 907 the Galapagos archipelago were designed to reveal toxic effects on blood clotting. All animals
 908 were given brodifacoum-poisoned prey over a 5-day period and followed for three weeks. None
 909 of them displayed any evidence of abnormal coagulation (Fischer 2011).

910 Only Sánchez et al. (1997) conducted a field test in order to evaluate the impacts of the
911 application of a parathion-based formulation (pesticidal active ingredient no longer approved for
912 used in the EU) on giant Canary lizards (*Gallotia galloti*). These authors reported serum
913 butyrylcholinesterase inhibition in lizards after field application of the insecticide, but did not
914 assess any of the endpoints commonly used in pesticide risk assessment (e.g. mortality or
915 reproduction). A recent laboratory study was conducted on wall lizards (*Podarcis muralis*) using
916 soil-pesticide concentrations derived from authorized application rates of three different
917 formulations (based on either folpet, glufosinate ammonium or fenpyroximate). Incubation of
918 eggs in soils treated with folpet at authorized field rates caused reduced embryonic growth,
919 whereas incubation in soils treated with glufosinate ammonium significantly reduced post-
920 hatching growth of individuals, which in lizards has been related to reduced survival chances
921 later in life. (Ortiz-Santaliestra et al., unpublished).

922 1.4.3. Conclusions and structure of the Opinion

923 Summarizing the above, there is considerable evidence that active substances and authorized
924 PPPs in Europe do have toxic impacts on amphibians and reptiles. Especially for the terrestrial
925 life stages of amphibians, risk assessment based on effects on groups of non-target organisms
926 as currently assessed seem not to cover the risk of exposure to active substances or PPPs *via*
927 the dermal route of exposure.

928 The PPR Panel therefore considers that initial suspicion is given for a thoughtful examination of
929 actual risk-assessment schemes, in order to provide the fundamentals for an operational
930 assessment of active substances and PPPs. The PPR panel recommended already in the
931 scientific opinion on the update of the data requirements (EFSA 2007) that an appropriate risk-
932 assessment approach for amphibians should be developed. The aim is to ensure that those
933 products are authorized that have no unacceptable effects on non-target species, biodiversity
934 and the ecosystem as required by current legislation (EU 1107/2009).

935

936 The current opinion aims at providing the scientific basis for developing a future risk-
937 assessment scheme and covers the following topics:

- 938 – Ecology and biology of amphibians and sources of environmental exposure, section 2,
939 p. 23 (include hyperlink)
- 940 – Definition of spatial aspects to be considered in the risk assessment, section 3, p.53
941 (include hyperlink)
- 942 – Population dynamics and modelling approach to support the setting of Specific
943 Protection Goals (SPG), section 4, p. 57 (include hyperlink)
- 944 – Specific protection goal options for amphibians and reptiles, sections 5 and 6, p. 76
945 (include hyperlink) and p. 81 (include hyperlink)
- 946 – General framework for developing a risk assessment scheme, section 7, p. 93 (include
947 hyperlink)
- 948 – Uncertainties in the risk assessment for amphibians and reptiles, section 7.11, p. 112
949 (include hyperlink)
- 950 – Toxicological endpoints and standard tests relevant for amphibians and reptiles, section
951 8, p. 120 (include hyperlink)
- 952 – Exposure assessment in the environment, section 9, p. 136 (include hyperlink)
- 953 – Coverage of amphibians and reptiles by existing risk assessment schemes for other
954 groups of organisms, section 10, p. 160 (include hyperlink)

955

956

957

958 **2. Ecology/biology of amphibians and reptiles and sources of**
959 **environmental exposure to pesticides**

960 Although amphibians and reptiles are studied together under the same branch of zoology (i.e.
961 herpetology: animals that creep), they are very different animals with multiple biological and
962 ecological characteristics extremely divergent between them. A description that defines these
963 two groups in common is that they are poikilothermic tetrapods. Poikilothermy is the condition
964 by which the internal temperature of an organism is subjected to wide fluctuations as a
965 response of changes in environmental temperature. Poikilothermy is one of the most important
966 aspects that make amphibians and reptiles different from other surrogate species like birds and
967 mammals, which are homeothermic (i.e. their body temperature remains almost constant,
968 regardless of environmental temperature).

969 **2.1. Role of poikilothermy in environmental physiology and**
970 **pollutant exposure**

971 Poikilothermy determines many aspects of amphibian and reptile environmental physiology, and
972 is a key factor in most of the characteristics that differentiate these animals from homeothermic
973 tetrapods. These include metabolic rate, oxygen consumption and energetic expenditure, which
974 in amphibians and reptiles are directly associated with fluctuations in environmental
975 temperature (and therefore in body temperature) and play an important role in defining the
976 potential toxic effects of an exposure to a chemical substance. Increased metabolic rates
977 involve increased energetic demands and respiratory rates (Halsey and White 2010), which can
978 account for an increment of the chemical oral uptake or inhalation. For example, Avery (1971)
979 described an increment of the daily food-intake rate in green lizards (*Lacerta viridis*) during
980 sunny days compared with partly cloudy ones. Moreover, animals tend to move more frequently
981 as their metabolic activity increases, although this is not a fixed rule (e.g. basking reptiles have
982 high metabolic activity but remain motionless). If animals move more frequently, the chances of
983 chemical uptake grows. Although metabolic rate seems therefore associated with increased
984 chances of chemical exposure, toxicants are more readily metabolized by more metabolically
985 active organisms, which in turn reduces the risks of suffering toxic effects at the physiological
986 level, as demonstrated by Talent (2005) with *Anolis carolinensis* exposed to pyrethrins. Toxicant
987 metabolism, however, has an associated energy cost that can alter the relationship between
988 metabolic and energetic investment in homeostasis, thus compromising other essential
989 biological functions like growth, development, immunity or reproduction. As far as has been
990 described, the mechanisms of pollutant metabolism and detoxification in amphibians and
991 reptiles are not different from those of other vertebrates in terms of components (e.g. Katagi
992 and Ose 2014). The main determinant of the ability of these animals to transform and/or
993 eliminate toxic substances from their bodies will be the rate at which metabolic processes work,
994 which in turn depends on temperature. Therefore, poikilothermy constitutes a key issue,
995 making chemical uptake, toxicokinetics and toxicodynamics in amphibians and reptiles
996 somewhat different from what pertains in birds and mammals.

997 Homeothermic organisms spend most of the energy that they ingest as food in temperature
998 regulation (Kronfeld-Schor and Dayan 2013). By contrast, poikilothermic animals, which use
999 little or no energy to maintain body temperature, can invest most of the energy available from
1000 metabolism for other purposes such as growth. This major energetic investment in new body
1001 tissues determines some aspects of amphibian and reptile growth that have ecological
1002 importance. Poikilothermy allows growth rate to be adapted to the availability of resources in
1003 each territory and period of time, in such a way that growth is ratchet-like rather than uniform
1004 (Andrews 1982). This adaptability results in amphibians and reptiles being commonly present in
1005 habitats subjected to extreme environmental conditions.

1006 Besides being adaptable to environmental conditions, growth in amphibians and reptiles is
1007 considered to be indeterminate, which means that organisms continue growing after sexual
1008 maturity. This is in contrast to species with determinate growth that stop growing once sexual
1009 maturity is reached (Seben 1987). Indeterminate growth is also possible because of the great
1010 energetic investment in body tissues (Congdon et al. 2012), and is probably one of the reasons
1011 why amphibians and reptiles constitute an important part of the biomass in the ecosystems

1012 where they are present (e.g. Gibbons et al. 2006), sometimes in locations with low availability
1013 of resources. Growth is therefore a sensitive endpoint during the entire life of individuals.
1014 Nevertheless the relevance of potentially toxicity impaired growth will probably be higher during
1015 pre-adult stages, when growth rate determines survival probabilities in later life (Semlitsch et al.
1016 1988, Galán 1996). In turn, amphibian and reptile communities are, because of the high
1017 biomass, important components of trophic nets; as consumers, they ingest large amounts of
1018 food, often with little specificity in the food choice, and consequently play a role as sentinels of
1019 the nutrient composition of the ecosystems. As prey, they constitute a major resource for top
1020 predators, and are therefore key elements in the transfer of energy and chemical substances
1021 across the food chains.

1022 In spite of all the similarities or common characteristics derived from poikilothermy that
1023 differentiate amphibians and reptiles from birds and mammals, both groups are so different that
1024 they require separate sections to explain most of the aspects of their general biology and
1025 ecology.

1026 **2.2. Main aspects of ecology and biology of amphibians**

1027 **2.2.1. Origin and diversity**

1028 Amphibians include more than 7000 known species (AmphibiaWeb 2016), with the highest
1029 species richness located in tropical regions. Living amphibians are grouped in three orders:
1030 anurans (toads and frogs, ~6500 species), caudates (newts and salamanders, ~680 species)
1031 and caecilians (~200 species), the latter being absent from Europe. Amphibian diversity in the
1032 European Union includes a total of 89 species (53 anurans and 36 caudates, Sillero et al. 2014),
1033 of which 23.6% (17% of anurans and 33% of 12 caudates) are recognized by the International
1034 Union for the Conservation of Nature as endangered (i.e. listed within the categories of Critically
1035 Endangered, Endangered or Vulnerable for their global conservation status); this percentage
1036 can be locally higher if national or regional red lists are considered. In evolutionary terms,
1037 amphibians include the most ancient tetrapods, which appeared as fossils during the Devonian
1038 (360 million years ago), being the first vertebrates colonizing the terrestrial environment
1039 (Duellman and Trueb 1994). However, the fact that part of amphibian life cycle takes place in
1040 the aquatic environment makes amphibians not totally independent from the water.

1041 The diversity of amphibians is patent in their body sizes and shapes. Anurans have a
1042 characteristic tailless morphology, with a robust body where head and trunk form a continuous
1043 unit and hindlimbs are usually much longer than the body, which is an adaptation to saltatory
1044 (hopping or leaping) locomotion. Not all anurans hop, however; some simply walk. Caudates
1045 have elongated, more or less cylindrical, bodies, thus with a higher surface area to volume ratio
1046 than anurans. They exhibit heads differentiated from the rest of the body, relatively long tails
1047 and short limbs, both hind and front pairs being of similar length.

1048 **2.2.2. Anatomy and function of skin**

1049 The dependence of amphibians on water is not only reflected in their life cycle. Amphibian
1050 anatomy, and in particular the characteristics of their integument, makes water balance a
1051 critical issue for these organisms. Amphibian skin lacks any kind of specialized structure of
1052 protection compared with other groups of terrestrial vertebrates, being very permeable to the
1053 diffusion of water and chemical agents. Therefore, skin is the main route of both water uptake
1054 and loss in amphibians. Chemical uptake of pollutants through amphibian skin has been
1055 suggested to be dependent on the octanol/water partition coefficient ($\log K_{ow}$) of each substance
1056 (Quaranta et al. 2009), although data obtained from live individuals indicated soil-partition
1057 coefficient was a better predictor than $\log K_{ow}$ in determining bioconcentration factors of
1058 pesticides (Van Meter et al. 2014).

1059 The anatomy of amphibian integument has been extensively studied (Barthalmus and Heatwole
1060 1994). The outer layer of amphibian skin is the epidermis, which is only a few cell layers thick
1061 (generally 2-3 cell layers in larvae and 5-7 in adults). In terrestrial stages, cells of the outer cell
1062 layer keratinize and die, forming the *stratum corneum*, which confers some sort of protection

1063 against excess water loss and injury. The innermost cell layer of the epidermis is called *stratum*
1064 *germinativum* and is continuously dividing to replace the outer layers. Thus, *stratum corneum* is
1065 periodically shed. During the yearly activity period, inter-moult period can range from several
1066 days to few weeks, in a process mainly controlled by the hormonal system. The possibility that
1067 shed skin is used as a matrix for pollutant elimination in amphibians has not been explored. The
1068 moulting frequency does not seem to be species- but environment-dependent. Paetow et al.
1069 (2012) observed that northern leopard frogs (*Lithobates pipiens*) individuals infected with
1070 *Batrachochytrium dendrobatidis* showed a higher moulting frequency than non-infected ones,
1071 which could be interpreted as a mechanism to control pathogen loads; in the same study, frogs
1072 were also exposed to different levels of atrazine, which was found to have no effect on
1073 moulting frequency.

1074 The dermis is behind the epidermis, separated from it by a thin basement membrane. The
1075 dermis is considerably thicker than the epidermis. The outermost region receives the name of
1076 *stratum spongiosum* and is formed by different structures, including glands, nerve ends, blood
1077 vessels or pigment cells, whereas the innermost part of the dermis, known as *stratum*
1078 *compactum*, is a tight net of connective tissue. The thickness and permeability of the skin vary
1079 from larval to post-metamorphic stages but, even within adult forms, there are also some
1080 variations depending on whether they predominantly occupy aquatic, ground terrestrial or
1081 arboreal habitats as adults. These habitat-dependent variations might influence dermal uptake
1082 of pollutants (Van Meter et al. 2014).

1083 Tegumentary glands play important roles in amphibian relationships with the environment
1084 (Barthalmus and Heatwole 1994); the abundant and evenly distributed mucous glands protect
1085 skin from desiccation. Holocrine glands are responsible for the secretion of antimicrobial
1086 substances, and granular glands secrete poisonous substances to repel predator attacks. These
1087 poison glands are often concentrated in the body parts most commonly targeted by predators,
1088 such as head and neck, and in many toad and salamander species can form macroglands on
1089 both sides behind the head known as paratoid glands. The internal mechanisms of activation of
1090 all these glands is not totally known, although the endocrine system is known to play an
1091 important role. Environmental stress affects glandular activity in the skin and can compromise
1092 the capabilities of organisms to keep skin moisture and water balance, or to defend them from
1093 pathogenic or predator attacks. Skin secretions could be another way of eliminating
1094 pollutants from the body in amphibians, though this possibility has not been investigated. If so,
1095 differential composition of glandular secretions could favour elimination of chemical substances
1096 with different physico-chemical properties, but no research has been conducted in this context.

1097 **2.2.3. Water balance and gas exchange**

1098 In aquatic stages, water balance is generally not a problem; actually, permeability of amphibian
1099 skin to the water is up to 12 times higher in aquatic than in the terrestrial stages (Galey et al.
1100 1987), which contributes to increased water diffusion, and probably also increased dermal
1101 uptake of contaminants during the aquatic life. Terrestrial forms must, however, adopt
1102 mechanisms to avoid excessive water loss. On the one hand, several behavioural mechanisms
1103 like avoiding activity during high temperature or irradiation hours are common (e.g. Pough et
1104 al. 1983). In addition, amphibians show a so-called water-absorption response (Hillyard et al.
1105 1998). The pelvic patch is an area of the posterior part of the ventral zone where skin is
1106 especially permeable to water because of its high degree of vascularization. The water-
1107 absorption response consists of pressing moist surfaces with the pelvic patch in such a way that
1108 a large volume of water can be absorbed in a short time. This results in potential for pesticide
1109 diffusion to be also higher through ventral than through dorsal skin (Kauffman and Dohmen
1110 2016). On the other hand, the mostly granular skin of the dorsal and cephalic regions, which
1111 are the most exposed to air and solar irradiation, makes water permeability considerably lower
1112 than that of the pelvic patch. Some physiological adaptations also help terrestrial amphibians to
1113 maintain water balance, like the reduction of urinary water elimination by decreasing the rate of
1114 glomerular filtration and accumulating large volumes of water in the bladder (Geise and
1115 Linsenmair 1986). For this reason, mechanisms of osmoregulation, which are mostly controlled
1116 by hormones (McCormick and Bradshaw 2006), are critical in maintaining water balance.

1117 As mentioned above, metabolic rate in amphibians is strongly temperature-dependent, with a
1118 more or less linear relationship between the metabolic rate and the body temperature. Besides
1119 temperature, other factors like health or nutritional status, or the exposure to environmental
1120 pollutants, can affect metabolism as well. The metabolic demands under different situations are
1121 fulfilled in part thanks to the integrated involvement of the different respiratory organs
1122 (Shoemaker 1992). Skin is an important respiratory organ in amphibians; in small individuals,
1123 where the surface area to volume ratio is high, skin breathing covers an important part of the
1124 necessities derived from the basal metabolism. In large animals, with a higher metabolic rate
1125 and a lower surface area to volume ratio, skin loses importance compared with lungs as the
1126 main organ of gas exchange. Some adaptations may, however, appear in large-bodied animals
1127 to increase gas exchange through the skin, like increasing skin vascularization or skin surface
1128 area by means of additional folds (Czopek 1965).

1129 **2.2.4. Description of the reproductive system**

1130 Amphibian reproduction is characterized by a great variation with respect to breeding,
1131 fertilization and embryo development. Most amphibian species have external fertilization and
1132 embryo development, but embryo development inside the mother's body (ovoviviparity (inside
1133 egg) and viviparity) is observed in anuran and gymnophionan species. Despite the variation in
1134 embryo development, the reproductive anatomy of the three amphibian orders (Anura, Caudata
1135 and Gymnophiona) shares the basic anatomical features. The male amphibian reproductive
1136 system consists of a pair of testes with adjacent fat bodies, a system of efferent ducts, Wolffian
1137 ducts (serving as ureters and sperm ducts), and the cloaca. The female amphibian reproductive
1138 system is composed of two ovaries with connected fat bodies, a pair of oviducts, and a cloaca.
1139 There are, however, anatomical structures that are unique to certain species such as the
1140 Bidder's organ, which is present in the anuran family Bufonidae. The Bidder's organ is a
1141 structure that develops from the most anterior part of the gonads in both sexes. It resembles
1142 an ovary and contains immature oocytes but the function of this organ remains poorly
1143 understood (Ogielska 2009). Another unique feature is the sperm-storage glands called
1144 spermathecae localized in connection with the cloaca in females of most newt and salamander
1145 species (Ogielska 2009). A third unique feature is observed in females of the viviparous
1146 caecilian *Typhlonectes compressicauda*, which have a specialized, placenta-like structure in the
1147 oviduct (Ogielska 2009).

1148 *Gonadal sex differentiation*

1149 In most amphibians the differentiation of the gonads into ovaries or testes starts during the
1150 larval period (Lofts 1974). The process of gonadal development is similar in the three
1151 amphibian orders, but the majority of data originate from studies on anurans (Ogielska 2009).

1152 In the most common amphibian model, the African clawed frog (*Xenopus laevis*), the sexual
1153 differentiation of the gonads starts around Nieuwkoop-Faber (NF) stage 56 (Tinsley and Kobel
1154 1996; see section 8.1 for details on developmental stages), whereas in the western clawed frog
1155 (*Xenopus tropicalis*) the gonadal sex differentiation has been reported to start around NF stages
1156 46-50 (El Jamil et al. 2008, Piprek and Kubiak 2014). Before these stages, the gonads are
1157 sexually indifferent i.e. there is no morphological difference between testes and ovaries. During
1158 ovarian differentiation, a cavity is formed in the centre of the gonad and the primordial germ
1159 cells are located in the surrounding cortex. In the male gonad, the germ cells are located in the
1160 medulla and no cavity is formed (Witschi 1971). The ovarian cavity is therefore used as an early
1161 histological marker to discriminate between ovaries and testes.

1162 Little is known about the mechanisms for sex differentiation in amphibians. All amphibians have
1163 genetic sex determination, the heterogamety appearing either in males (XY system) or females
1164 (ZW systems) depending on the species (Witschi 1971). The sex differentiation of the gonads
1165 is, however, a plastic process compared with higher vertebrates. Experiments in anurans show
1166 that hormone exposure can override the genetic mechanisms and induce phenotypic sex
1167 reversal resulting in a skewed sex ratio compared with the control group (reviewed in Hayes
1168 1998). For instance, exposure to exogenous androgens, oestrogens or progestogens during
1169 critical periods of larval development causes complete or partial sex-reversal of the gonads
1170 (Hayes 1998). Cytochrome P45019 (cyp19, aromatase) mediates biotransformation of

1171 androgens into oestrogens. As sex hormones are important for gonadal differentiation in
1172 amphibians, this enzyme is supposed to have a role in sex determination. Aromatase is thought
1173 to be involved in gonadal differentiation in lower vertebrates as inhibition of aromatase in
1174 embryos of birds, reptiles and fish induces testicular differentiation (Elbrecht and Smith 1992,
1175 Piferrer *et al.* 1994, Pieau *et al.* 1999). The prevailing hypothesis is that aromatase is involved
1176 in amphibian gonadal differentiation but its specific role remains to be elucidated (Kelley 1996,
1177 Urbatzka *et al.* 2007, Jansson *et al.* 2016). As explained below, temperature is an
1178 environmental factor that regulates gonadal sex differentiation in reptiles. In amphibians
1179 (anurans and caudates) experiments have shown that extreme (close to lethal) temperatures
1180 can influence gonadal sex differentiation (Hayes 1998), but this does not seem to be a relevant
1181 factor for natural sex determination.

1182 *Müllerian and Wolffian duct development*

1183 The Müllerian ducts are present in both sexes in early life stages of amphibians, reptiles, birds
1184 and mammals, whereas they are lacking in teleost fish. In females, these ducts develop into the
1185 reproductive tract (i.e. the oviduct in amphibians, reptiles and birds, and the oviduct, uterus,
1186 and vagina in mammals). In males, the Müllerian ducts usually regress during early life stages.
1187 However, in males of Gymnophiona the Müllerian ducts do not degenerate but instead develop
1188 into glandular organs (Ogielska 2009). The growth and differentiation of the Müllerian ducts are
1189 under the control of hormones including oestrogens and progesterone. While it has been shown
1190 that gonadal differentiation starts during the larval stages in many amphibian species, there is
1191 little information on the ontogenetic development of the Müllerian ducts in amphibians. In
1192 *Xenopus*, the Müllerian ducts begin to form shortly before metamorphosis around NF stage 64
1193 (*X. laevis*: Witchi 1971; *X. tropicalis*: Jansson *et al.* 2016). The differentiation of the ducts into
1194 oviducts occurs in parallel to an increment of plasma levels of ovarian hormones. The oviducts
1195 are involved in egg formation in amphibians and are therefore crucial to their reproductive
1196 success.

1197 The Wolffian ducts extend from the kidney to the cloaca and serve both as ureter and sperm
1198 duct in male amphibians. Enlarged regions of the Wolffian ducts serve as sperm-storage sites
1199 close to the cloaca (Ogielska 2009).

1200 *Oogenesis*

1201 Oogenesis is the process by which female germ cells undergo meiosis and differentiation into
1202 mature oocytes. The germ cells differentiate into oogonia, which proliferate before they enter
1203 meiosis. They are referred to as immature or primary oocytes as they enter the prophase of the
1204 first meiosis. The oocytes are then arrested in meiotic prophase during the whole process of
1205 folliculogenesis until gonadotropin-induced signals trigger them to resume meiosis prior to
1206 ovulation (reviewed in Hammes 2004). In most mammals, the early germ-cell differentiation
1207 into primary oocytes is restricted to foetal life. In contrast, amphibians have a continuous
1208 oogenesis with germ cells differentiating into oocytes throughout life (Al-Mukhtar and Webb
1209 1971). When the immature oocyte progresses beyond the early diplotene stage of meiotic
1210 prophase and becomes surrounded by follicular cells, it is referred to as a follicular oocyte.
1211 During folliculogenesis, the amphibian oocyte increases in size due to incorporation of the yolk
1212 precursor protein vitellogenin. Oogenesis is regulated by endocrine and paracrine control
1213 mechanisms involving steroid synthesis by the follicular and theca cells as well as the oocyte
1214 itself, reviewed by (Konduktorova and Luchinskaya 2013).

1215 *Spermatogenesis*

1216 The frog testis consists of seminiferous tubules with germ-cell nests in different maturation
1217 stages. The germ cells develop synchronously within each nest. The nests are produced when a
1218 Sertoli cell connects to a primary spermatogonium. The spermatogonium enters mitosis and
1219 produces a cluster of secondary spermatogonia enclosed by the Sertoli cell (Pudney 1995).
1220 Secondary spermatogonia transform into primary spermatocytes that undergo meiotic division
1221 into secondary spermatocytes. Transformation then proceeds into round and elongated
1222 spermatids and finally to spermatozoa. The last step in spermatogenesis is spermiation, i.e.
1223 when the nest wall is ruptured and spermatozoa move into the lumen of the seminiferous
1224 tubule (Pudney 1995). The process of spermatogenesis is complex and depends on endocrine

1225 and local controlling mechanisms that are not yet fully understood (Pierantoni et al. 2002,
1226 Sasso-Cerri et al. 2004).

1227 *Bidder's organ*

1228 Bidder's organ is a structure that develops from the most anterior part of the gonads in
1229 anurans belonging to the family Bufonidae. Bidder's organ resembles an ovary and contains
1230 immature oocytes. The function of this organ remains poorly understood (Ogielska 2009).

1231 *The larynx and sound production calling*

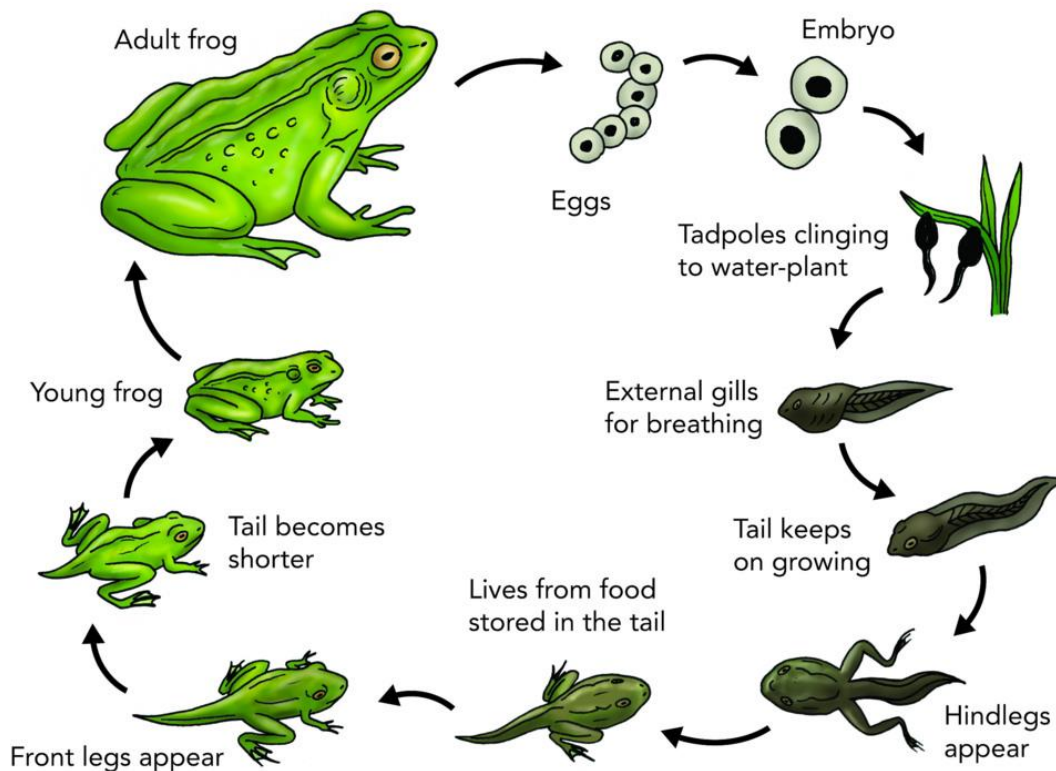
1232 Communication by sound production occurs in all three amphibian orders. The vocalization
1233 system (including larynx and associated structures) and calling behaviour have been well
1234 characterized in several anuran species including *X. laevis* (Duellman and Trueb 1994). While
1235 the overall structure of the respiratory tract is similar between terrestrial species and aquatic
1236 species such as *Xenopus*, the larynx of the latter is specialized to produce sound underwater
1237 (Kelley 1996).

1238 **2.2.5. Life history and reproduction**

1239 The common reproductive strategy involves an aquatic egg in which the embryo develops to a
1240 larval form, also aquatic. At the end of the larval stage, individuals undergo a metamorphosis
1241 process during which they transform into a terrestrial juvenile, morphologically and anatomically
1242 similar to the adult form (Figure 3:). In temperate and cold-temperate regions, the amphibian
1243 breeding cycle is strongly seasonal. In most of EU territory, the breeding season begins at some
1244 point in spring, shortly after animals emerge from overwintering. Aquatic stages then develop
1245 during most of the spring and summer to emerge as terrestrial forms in summer or autumn,
1246 before the temperatures drop again. There are, however, many exceptions to this general
1247 scheme; some species, or certain populations of some species, do not lay eggs but are either
1248 viviparous or ovoviviparous, giving birth to more or less developed larvae (Wake 1993). Some
1249 populations have larvae that hibernate and do not emerge until the next spring (e.g. Gilbert and
1250 ter Harmsel 2016) or, in areas with mild winters and extreme temperatures in summer (which
1251 in Europe would correspond to the southernmost areas of the Mediterranean basin), an
1252 aestivation period followed by an autumnal or winter breeding season (e.g. Gomez-Rodriguez et
1253 al. 2012). The seasonality of the life cycle of amphibians results in the presence of periods
1254 during which animals rely on accumulated reserves. These periods are critical from a
1255 toxicological perspective, as accumulated pollutants could also be mobilised as part of the
1256 consumption of internal reserves (James et al. 2004), as has been described in fish and
1257 homeothermic vertebrates (Daley et al. 2014). Especially long resting periods could take the
1258 availability of internal reserves to the limit, resulting in animals awaking in a very impoverished
1259 condition, but also with a high potential of having mobilised significant amounts of accumulated
1260 pollutants.

1261 At the beginning of the breeding season, adults migrate from their refuges to water bodies (see
1262 *Habitat and movements* section below) where eggs are laid. Amphibians generally use
1263 courtships to attract mates; this strategy ensures that both male and female gametes have
1264 achieved the maturation stage and are ready for fertilization, as well as placing both gametes in
1265 close proximity before gamete transfer and eventual fertilization (Vitt and Caldwell 2014).
1266 Fertilization is external in most anuran species and in a few caudate groups (reviewed in detail
1267 in Duellman and Trueb 1994); the male sheds the sperm on the eggs as they are being
1268 released through the female's cloaca. In anurans, the male grabs the female in a posture
1269 known as amplexus; the part of the body where males grab the female varies among species,
1270 resulting in different types of amplexa (e.g. inguinal, axillar, cephalic). In caudates with external
1271 fertilization, male and female cloacae are normally close at the end of the courtship and sperm
1272 is deposited on top of the egg mass during or right after release. Most caudates, as well as
1273 caecilians and a few frog species have internal fertilization, either by cloacal contact (some
1274 frogs), male intromitting organs (some frogs and all caecilians) or through a spermatophore
1275 that is picked up by the females (salamanders). In salamanders with internal fertilization,
1276 females may storage sperm for long times (up to several years); this is supposed to allow

1277 females to select the time of fertilization and further egg laying in order to optimize embryonic
1278 development conditions.



1279

1280 **Figure 3:** Representation of the life cycle of a frog exhibiting the amphibian common
1281 reproductive mode (taken from Siyavula Education. Image under License Creative
1282 Commons Attribution 2.0 Generic. Retrieved on November 29th 2016) from website at
1283 <https://www.flickr.com/photos/121935927@N06/13578724373>,

1284

1285 Amphibian eggs are encased in an envelope formed by several layers of mucoprotein and
1286 mucopolysaccharide (Viarengo and Falcone 1977). The envelope, upon release and contact with
1287 the medium water, acquires a gelatinous texture that contributes to protect eggs from
1288 mechanical damage and from other threats like predation, ultraviolet radiation and uptake of
1289 some environmental chemicals (Ward and Sexton 1981, Marquis et al. 2006). Some species
1290 provide additional parental care to their eggs by carrying them on their backs or rear legs (e.g.
1291 genus *Alytes*) or even inside a dorsal skin pocket (e.g. genus *Pipa*), wrapping them with aquatic
1292 plant as in many newt species, or making foam nests inside which not only embryos but also
1293 larvae can develop (observed in leptodactylid species) (Duellman and Trueb 1994). Maternal
1294 deposition of pollutants to the eggs is known to exist in amphibians, although the patterns
1295 influencing this process have not been investigated in depth. Metals, trace elements and
1296 persistent organic pollutants can be transferred from maternal bodies to the eggs (e.g.
1297 Kadokami et al. 2004, Metts et al. 2013), which can sometimes result in reduced offspring
1298 viability (Metts et al. 2013). Maternal deposition of pollutants into the eggs could be a way of
1299 detoxification (Kadokami et al. 2004), preserving maternal organisms at the expense of
1300 offspring; at the population level, this could be beneficial in those species relying more on adult
1301 survival than offspring survival, which is typical of so-called r-strategists (see Annex A for how
1302 to assess such effects using sensitivity or elasticity analysis).

1303 Although most amphibians have very large clutch sizes, this parameter size may vary among
1304 species within an extremely wide range from a few tens to several thousands of eggs per
1305 female. Following a general rule in the animal kingdom, species that use more energy in
1306 producing large amounts of ova put less energy into ensuring offspring survival; this is the case

1307 in many toads that may lay several thousands of eggs per female. At the other end of the life-
1308 history continuum, those species showing parental care are the ones with smaller clutch sizes;
1309 for example, females of midwife toads of the genus *Alytes* lay only a few tens of eggs per year,
1310 but these eggs are then carried by adult males until hatching, which can contribute to reduce
1311 embryonic mortality.

1312 Larvae possess hatching glands that secrete proteolytic enzymes, which degrade the gelatinous
1313 capsules to facilitate their breakage (Nokhbatolfoghahai and Downie 2007). Hatching in
1314 amphibians normally occurs early in embryogenesis, such that most of the aquatic development
1315 is accomplished by a free-living larva. There are, however, some species with direct
1316 development in which hatching happens at the end of development, and what emerges from
1317 the egg is not a larva but a fully formed juvenile morphologically similar to the adult. In these
1318 species, there is no real metamorphic event (Duellman and Trueb 1994).

1319 Larvae of most amphibian species are aquatic, with a very thin skin consisting of only two or
1320 three epidermal layers, highly vascularized to facilitate its role as a gaseous exchange organ.
1321 The skin is not the only respiratory surface in amphibian larvae; this function is shared with the
1322 gills, which are external during the initial stages of larval development (McDiarmid and Altig
1323 2000). Caudate and caecilian larvae retain external gills throughout the entire larval period, but
1324 in most anurans, gills become covered by the operculum as development progresses. In these
1325 species, one or two spiracles remain open in the operculum to allow the flux of water. The
1326 location of the spiracle(s) varies among species. The presence of external gills during part or all
1327 the larval development has the function of providing increased surface area for gas exchange;
1328 such an increase of the surface area to volume ratio can also lead to an increased rate of
1329 chemical absorption in the water. That is the reason why newly hatched individuals are
1330 commonly identified as the most sensitive ones among amphibian aquatic stages (Ortiz-
1331 Santalieu et al. 2006).

1332 Larvae of caudates and caecilians are morphologically similar to adults. In caudates, the four
1333 limbs are developed at the very beginning of the larval development. In contrast, anuran
1334 tadpoles are very different from adult forms. Their main functions are feeding and growth, for
1335 which they present large coiled intestines and specialized oral apparatus consisting of a disc
1336 with several rows of keratinized labial teeth and sometimes jaw sheaths, everything covered
1337 with papillae (Altig 2007). Morphological variations exist across tadpoles on the basis of their
1338 diet, foraging strategy and type of habitat. Limbs in anuran tadpoles are developed at the end
1339 of the larval period; hindlimbs are the first to emerge, whereas forelimbs, although developed
1340 at the same time as hindlimbs, remain covered by the operculum and do not emerge until
1341 metamorphosis (Duellman and Trueb 1994).

1342 The duration of embryonic and larval development also varies across species, although it is
1343 strongly determined by environmental conditions. Amphibians show a phenotypic plasticity that
1344 allows for adjusting developmental pace to the conditions of the environment (e.g. Richter-Boix
1345 et al. 2006). This plasticity results, for example, in accelerated larval development with
1346 increased temperatures, which could allow for larvae to complete development and
1347 metamorphosis before ponds desiccate. Several studies have postulated the negative impact
1348 that pollutant-related alterations of developmental rates could exert on this phenotypic
1349 plasticity, and consequently on the ability of amphibian larvae to respond to changing
1350 environmental conditions. In experimental conditions, there are many studies demonstrating
1351 that exposure to environmental pollutants decelerates developmental rate. Besides the impact
1352 that this effect may have on phenotypic plasticity, a longer time to complete development also
1353 involves a prolonged exposure to the pollutants in the aquatic environment, which results in a
1354 positive feedback potentially enhancing negative effects of pollution on embryonic and larval
1355 development. Overall, duration of larval development can range from less than 20 days to
1356 several years. This plasticity in larval development can result in larvae of a given species
1357 reaching metamorphosis, not only at different times, but also with variable sizes. As long as the
1358 conditions in the water remain good (e.g. enough food and no desiccation, predation or
1359 infection risks), larvae can continue growing to reach metamorphosis with a larger body size,
1360 which is generally associated with increased juvenile success (e.g. Cabrera-Guzmán et al.
1361 2013). In polluted environments, however, the amount of energy spent in toxicant metabolism

1362 cannot be allocated to growth, which results in reduced growth rates and loss, at least partially,
1363 of the theoretical advantage of prolonging larval life to metamorphose with a larger body size.

1364 The larval period finishes with the metamorphosis. Metamorphosis is a key process in the life
1365 history of amphibians in which larvae transform into juvenile, adult-shaped forms. The
1366 morphological and anatomical reorganization is especially intense in anurans. Metamorphosis
1367 involves not only morphological changes, but also a series of physiological modifications of
1368 almost all systems necessary to transform an aquatic organism into a terrestrial one (Gilbert
1369 and Frieden 1981). Metamorphosis is mostly regulated by thyroid hormones (Kikuyama et al.
1370 1993), with all the processes happening during metamorphosis resulting from differential
1371 exposure of the involved tissues to the thyroxine hormone (TH). Some other hormones like
1372 corticosterone and prolactin also play a role in regulation, acting as inhibitors during larval life
1373 and disappearing towards the end of the larval period to allow TH to initiate the process of
1374 metamorphosis (Etkin and Gona 1967). The action of TH release is in part responsible for the
1375 developmental plasticity of amphibians. Several environmental factors can promote the release
1376 of TH, thus affecting the timing of beginning of metamorphosis and consequent emergence of
1377 the individual from its aquatic habitat. Like overwintering or aestivating periods, metamorphosis
1378 is toxicologically critical because of the potential mobilisation of reserves, and therefore of
1379 pollutants, accumulated during the larval period (Sparling et al. 2006). Metamorphosing
1380 individuals do not eat but, in contrast with what happens during resting periods, maintain a
1381 high activity and metabolic rate, which results in a quick consumption of the body reserves. The
1382 proportional body mass loss during metamorphosis could be a direct indicator of the risk of
1383 suffering toxic effects because of mobilisation of accumulated substances.

1384 Age at sexual maturity varies from a few months, especially in tropical species, to up to seven
1385 years in large salamanders (Vitt and Caldwell 2014). In general, the age at sexual maturity is
1386 subjected to a trade-off between early maturation (which relates to reduced offspring size and
1387 increased chances of predation of adults) and breeding and maturing at a larger size (which
1388 results in increased pre-adult mortality and reduced number of reproductive events throughout
1389 the entire lifespan), although this can be modulated by the fact that size at sexual maturity
1390 does not necessarily correlate with age but also with juvenile growth rate (Halliday and Verrell
1391 1988). In addition, maximum lifespan is generally correlated with age at sexual maturity in such
1392 a way that individuals attaining the reproductive status during the first or second year of life
1393 rarely live more than five years, whereas those animals reaching sexual maturity at older ages
1394 can live up to 25 years (Vitt and Caldwell 2014).

1395 **2.2.6. Habitat and movements**

1396 Amphibians are widely distributed across the Earth, being present on all continents but the
1397 Antarctic and occupying a great variety of habitats if water bodies are available for breeding.
1398 Over the last 25 years, evidence has grown pointing to a global decline of amphibian
1399 populations: the main reasons, according to the Global Amphibian Assessment, are habitat loss
1400 and environmental pollution (IUCN et al. 2008). In agricultural areas, where these two factors
1401 co-occur, the presence of amphibians is well known, and in Europe, up to 38 amphibian species
1402 (43% of the amphibian diversity) are identified by the IUCN as inhabiting arable lands. Although
1403 the majority of amphibian populations inhabiting agroecosystem seem to prefer off-field sites
1404 (e.g. Miaud and Sanuy 2005, Oromi et al. 2010), the occupation of arable areas can
1405 occasionally be dominant, as observed by Cooke (1986) with adult great crested newts (*Triturus*
1406 *cristatus*) preferring mature wheat fields to marsh or woodlands. In other cases, the use of
1407 arable fields is restricted to particular activities like feeding (Oldham and Swan 1992) or moving
1408 (see below).

1409 The spatial ecology and habitat selection of amphibians are very important in determining the
1410 chances of exposure of their terrestrial stages to environmental pollution, especially in
1411 agricultural areas. Home ranges in amphibians are generally small, and a particularity of
1412 amphibian spatial ecology is that, in many species, home ranges change with the season. For
1413 most European species, animals tend to concentrate around water bodies during breeding
1414 seasons, whereas the rest of the activity period they occupy terrestrial environments where
1415 they search for food. Terrestrial feeding habitats may also coincide with resting areas during

1416 inactive periods (Indemauro et al. 2009). This creates a very variable spatial pattern of chemical
1417 exposure, as seasonal movements may result in animals travelling across areas with potentially
1418 different degrees of contamination (Regosin et al. 2005). Another peculiarity of amphibian
1419 spatial behaviour is the high degree of site fidelity, especially in what refers to breeding
1420 habitats. Year-to-year faithfulness to breeding sites has been reported as 88-98% in wood frogs
1421 (*Lithobates sylvaticus*) and up to 100% in spotted salamanders (*Ambystoma maculatum*)
1422 (Vasconcelos and Calhoun 2004). In the event of a continuous or repeated occurrence of
1423 pollutant in the habitat, this pattern will presumably have a major influence on the exposure of
1424 breeding adults, as well as of aquatic stages, to contaminants, not only because of repeated
1425 exposures over time but also because it will restrict the capacity of searching for alternative,
1426 unpolluted sites.

1427 Amphibian breeding migrations from wintering sites to breeding points, together with the return
1428 trip at the end of the season, and emergence and dispersal of juveniles after metamorphosis
1429 constitute the most typical movement events in amphibians. Breeding migrations are directional
1430 and have clearly defined destinations. Migrations can run over the shortest distance to the
1431 target point with or without little selection of the habitat to cross on the way (e.g. Pilliod et al.
1432 2002), which increases chances of crossing less suitable habitats; alternatively, migrations can
1433 run through more suitable corridors (Hartel and Demeter 2005), reducing chances of travelling
1434 across less suitable habitats. Dispersal movements, on the contrary, are usually not pre-defined,
1435 although they tend to orientate non-randomly towards the most suitable habitat patches
1436 (Vasconcelos and Calhoun 2004). Besides these aspects, a particularity of both types of
1437 movements is that they occur massively, with an important proportion of the adult or juvenile
1438 populations moving at the same time. In terms of risks from pollution, if there is spatial and
1439 temporal overlap of sources of pollution and presence of amphibians, these phases of the life
1440 cycle are critical because of the important effects that could occur at the population level.
1441 Amphibian-breeding migrations may happen over short (e.g. few hundred metres as generally
1442 seen in newts; Schabetsberger et al. 2004) or they can be rather long (e.g. >4 km for *Epidalea*
1443 *calamita*; Miaud et al. 2000). Equally, post-emergence, dispersal movements can also go over
1444 1.5 km (Sinsch 1988).

1445 Some amphibians are known to cross crop fields in spring, while migrating towards breeding
1446 ponds (Miaud et al. 2000, Miaud and Sanuy 2005, Kovar et al. 2009, Lenhardt et al. 2013),
1447 temporally and spatially overlapping with periods of pesticide application (Berger et al. 2012,
1448 2013, Lenhardt et al. 2015). The risk of coincidence with pesticide application is nevertheless
1449 very variable; the percentage of amphibian populations moving over agricultural sites coinciding
1450 with pesticide application could vary from 20% to almost 90%, depending on the species, crop
1451 types and years, according to Berger et al. (2011). Amphibian-breeding migrations can be
1452 facilitated if animals use arable lands. At the beginning of the activity period, when animals
1453 migrate towards the breeding sites, vegetation cover in crop fields is very low, which favours
1454 easy and quick displacement of animals. Berger et al. (2011) observed use of recently ploughed
1455 fields for dispersal of some species including the common toad (*Bufo bufo*), although during
1456 daytime resting periods, animals tended to look for refuge in densely vegetated areas.
1457 Individuals of other species like the spadefoot toad (*Pelobates fuscus*), however, dug
1458 themselves to find shelter, and loose, ploughed fields facilitate this strategy. Individuals may
1459 therefore stay inside crop fields for entire days during their breeding migrations. In other cases,
1460 migrating toads have been observed to move along pasture corridors, avoiding arable lands
1461 (Hartel and Demeter 2005). Crossing arable fields during breeding migrations seems therefore
1462 to be dependent on habitat structure, temporal variations in the vegetation cover and other
1463 characteristics of different habitat patches, and particular preferences of each species or
1464 population.

1465 2.2.7. Feeding ecology

1466 Adult amphibians, as well as caudate larvae, are mostly carnivorous and feed generally on small
1467 arthropods. The type of preferred prey is normally related to the body size of the animal (e.g.
1468 Labanick 1976), in such a way that the net energy obtained in each feeding event is optimised
1469 as a function of the prey size (related to gross energy income) and the effort necessary to
1470 capture and manipulate it (energy spent in the activity itself). This leads to inconsistencies

1471 between the preferred prey if they are considered either in terms of number of ingested items
1472 or in terms of ingested biomass. In general, amphibian diet is opportunistic (Duellman and
1473 Trueb 1994, but see Simon and Toft 1991), and the diet composition seems to respond to the
1474 type of prey available within the optimal prey sizes in each case. Newt and salamander larvae
1475 feed mostly on zooplankton. Anuran larvae, on the contrary, are vegetarian and feed mainly on
1476 periphyton, grazing on sediments, although other feeding modes like filtering phytoplankton or
1477 skimming the scum at the water surface are also very common among anuran tadpoles
1478 (McDiarmid and Altig 2000).

1479 Feeding behaviour, like diet composition is highly variable. Among carnivorous forms, aquatic-
1480 feeding individuals are generally active predators, whereas terrestrial individuals may show
1481 either active search or ambush (i.e. "sit-and-wait") strategies. Because of the differences in
1482 energy expenditure between the ambush and active modes, active foragers should tend to
1483 compensate the greater energy loss through a less specific diet (Schoener 1971).

1484 Estimating food-intake rate in amphibians is complicated. The amount of ingested food as well
1485 as the time spent feeding fluctuates daily (Larsen 1992), which is probably a consequence of
1486 the environmental dependence of physiological activity and therefore of nutrient necessities,
1487 associated with poikilothermy. Larssen (1992) estimated a yearly uptake of 142.4 kJ for a male
1488 common toad weighing 30g. Estimating a daily food-intake rate from this value is, however,
1489 difficult because of the aforementioned fluctuations among days, even within a given activity
1490 period. Assimilation efficiency is a key factor, not only to infer food-intake rate, but also to
1491 estimate the likelihood of ingested pollutants being absorbed in the intestine. The data show
1492 how energy gain is a function of the type of prey ingested, with mealworms appearing as the
1493 most profitable prey (assimilation efficiency >90%; Dimmitt and Ruibal 1980), followed by flies
1494 (79-91%; Bobka et al. 1981, Grafe et al. 1992), crickets (73.7%; Smith 1976), and beetles (65-
1495 66%; Dimmitt and Ruibal 1980). The majority of these studies on nutritional physiology
1496 coincide in pointing an inverse relationship between temperature and gut retention time, which
1497 results in the expected higher assimilation efficiency at lower temperatures (but see Smith
1498 1976). Higher assimilation efficiency at lower temperatures allows amphibians to reduce the
1499 number of feeding events, which is consistent with their reduced activity, movement and
1500 metabolism as temperatures drop.

1501 **2.3. Main aspects of ecology and biology of reptiles**

1502 **2.3.1. Origin and diversity**

1503 Although amphibians constitute the initial evolutionary step of the colonization of terrestrial
1504 environment by tetrapods, they were unable to become independent from the water as most of
1505 them need the aquatic environment to complete their life cycle. The early amniotes, ancestral
1506 to all reptiles, became able to reproduce in the absence of water and developed a skin
1507 protection against evaporative water loss, completing the process of land colonization initiated
1508 by the ancestor of amphibians. These forms appear as fossils during the Carboniferous (320
1509 million years ago) and gave rise to the different lineages resulting not only in all past and
1510 present forms of reptiles, but also of birds and mammals (Carroll 1969).

1511 Extant reptiles belong to three major clades: archosaurians, which include crocodylians as well
1512 as birds, testudines (turtles) and lepidosaurians (squamates and tuataras). Crocodylians, with 25
1513 living species, and tuataras (a single species) are not present in Europe. Testudines comprise
1514 346 extant species (seven of them in the European Union, excluding the Macaronesia, northern
1515 African sites, and overseas territories; Sillero et al. 2014), whereas living squamates are divided
1516 into three suborders: amphisbenians or blind snakes (196 species, two in the EU), saurians,
1517 including lizards, skinks, geckos, iguanas, etc. (6263 species, 76 in the EU), and ophidians or
1518 snakes (3619 species, 35 in the EU). Turtles have bodies typically covered by a shell formed
1519 from the fusion of the tegument and the thoracic skeleton, with a lower (plastron) and upper
1520 (carapace) parts that normally fit together. Within this general uniformity of shapes, turtles
1521 have a wide ecological range, from marine to fully terrestrial species (tortoises), including forms
1522 associated with freshwater environments (terrapins). Body shapes and sizes in squamates are
1523 much more diverse than in any other reptile group, from very tiny geckos to large snakes, from

1524 limbless forms to animals with robust legs, like iguanas, and from species living in deserts to
1525 semiaquatic or arboreal forms. In parallel with this morphological variability, surface area to
1526 volume ratios are very different among species (e.g. long, slim snakes will have higher surface
1527 area to volume ratios than large lizards or chameleons with more compressed shapes).
1528 Interestingly, some reptile groups do not seem to follow Bergmann's rule that predicts larger
1529 body sizes (and therefore reduced surface area to volume ratios) in species living in colder
1530 climates (Bergmann 1847). The particular necessities of reptiles with regards to heat gain (see
1531 *Thermoregulation and gas exchange* section below) cause this trend to be reversed in lizards
1532 and snakes (Ashton and Feldman 2003), which leads to species in temperate areas (more
1533 favourable for agriculture) tending to show lower surface area to volume ratios than species in
1534 cold areas.

1535 **2.3.2. Anatomy and function of skin**

1536 Although the skin of reptiles is structurally similar to that of amphibians, several important
1537 differences can be found (Lillywhite and Maderson 1982). The reptilian epidermis has a higher
1538 number of cell layers than in amphibians, which results in a thicker section of keratinized cells.
1539 Furthermore, reptiles are unique in producing β -keratin, which is hard and brittle and combines
1540 with the more elastic and pliable α -keratin typical also of other vertebrates. The skin in reptiles
1541 is usually modified in scales, which share the characteristic of being keratinized epidermal parts,
1542 but have different structures and names depending on the taxonomic group and the body
1543 region. Scale surfaces are formed by β -keratin, whereas sutures of separation between scales
1544 are formed by α -keratin. Epidermal growth patterns are also variable among groups; in
1545 lepidosaurians, the *stratum germinatum* divides in a cyclic manner, in such a way that two
1546 epithelial layers are superposed in the outermost part of the integument. In crocodiles and
1547 turtles, skin growth is continuous, only with the corresponding periods of arrest during
1548 hibernation or aestivation. This variation in epidermal growth patterns also results in differences
1549 in the process of skin shedding or ecdysis. Thus, whereas in lepidosaurians skin is shed all at
1550 once or in large patches, with a very uniform periodicity (interrupted however by a variable
1551 resting phase in the process of cell differentiation), in crocodiles and turtles, small flakes of the
1552 skin are continuously being shed (Irish et al. 1988, Maderson et al. 1998). Shed skin from some
1553 snakes has been analysed for pollutant presence in order to estimate whether this could be a
1554 way of toxicant elimination, finding detectable levels of metals and POPs (Jones et al. 2005,
1555 Jones and Holladay 2006). It is difficult, however, to establish how important skin shedding
1556 might be as a detoxification mechanism in reptiles without a more detailed monitoring of
1557 internal concentrations in animals.

1558 The dermis of some reptilian species has osteoderms, bony plates that underlay scales and are
1559 disposed in layers, with an outer, spongy layer formed by porous bone and an inner, compact
1560 layer formed by dense bone tissue (Lillywhite and Maderson 1982). In most cases, osteoderms
1561 are simply attached among them forming an additional protective layer; in some cases, they
1562 can fuse with pieces of the skeleton, like vertebrae, ribs and sternum in turtles, to form rigid
1563 shells (Hirashawa et al. 2013). The presence of glands in reptilian skin is common, although in
1564 a lower number and diversity of forms than in amphibians. The major roles of these glands are
1565 for the secretion of pheromones and impermeable waxes (Quay 1972).

1566 Because of these highly keratinized structures, reptilian skin is commonly viewed as a barrier
1567 against dermal uptake of contaminants (Snodgrass et al 2008). Weir et al. (2010), however,
1568 pointed out that permeability of the skin to pollutants would be more likely affected by lipid
1569 content than by keratin content of the skin. Reptile skin normally has a high lipid content
1570 (Pough et al. 2004), which will prevent diffusion of hydrophilic contaminants but allow
1571 absorption of lipophilic ones.

1572 **2.3.3. Thermoregulation and gas exchange**

1573 Metabolic rate in reptiles is temperature dependent, as in amphibians. There is, however,
1574 decoupling, in some lizards and snakes, between both parameters over the range of preferred
1575 temperatures, in such a way that metabolic rate keeps invariant across a window of 3 to 5°C
1576 (Bartholomew 1982). A notable difference from amphibians in this context is the relative

1577 importance of skin as a respiratory organ, which in reptiles is really low, the vast majority of gas
1578 exchange being done through lungs.

1579 Thermoregulation is the process by which the organism exchanges heat with the environment,
1580 and is a key factor to all physiological functions. Thermoregulation may have the function of
1581 warming up or cooling down the body, depending on the environmental conditions. Evaporative
1582 water loss through the skin of amphibians must be minimised, therefore activity at higher
1583 temperatures is normally lower than in reptiles (Tracy and Christian 2005), for which water loss
1584 through the skin is generally not an issue. Critical thermoregulation in amphibians is generally
1585 focused on cooling down during warm periods, whereas gaining heat is usually the function of
1586 thermoregulation in reptiles. Reptiles are usually active during sunny, warm days and, within
1587 these, during the hours when temperatures are close to their optimal. This means that, in
1588 temperate areas, daily activities in spring and autumn are normally unimodal (with a single
1589 active period in the central part of the day) whereas activity in summer is bimodal (with two
1590 active periods in the morning and late afternoon, avoiding the very high temperatures of the
1591 central part of the day). This pattern is of course subjected to variations depending on the
1592 environmental conditions of each specific location. Heat gain is achieved through two
1593 mechanisms: heliothermy consists of gaining heat by basking in sun, and thigmothermy
1594 consists of gaining heat by conduction from warm surfaces not necessarily exposed to the
1595 sunlight. Although most species have relatively broad ranges of temperature of activity, the
1596 preferred temperature range is narrow, and the closer that animals are to this temperature, the
1597 better their physiological functions work (reviewed by Seebacher and Franklin 2005).

1598 **2.3.4. Life history and reproduction**

1599 Reproductive modes in reptiles can be broadly divided into two major groups: oviparity and
1600 viviparity. The former is the most common mode in the group, including all crocodylians, turtles,
1601 tuataras and most squamates. Viviparity occurs in approximately 20% of squamates (Vitt and
1602 Caldwell 2014). Viviparity in reptiles appears as an adaptation to cold climates, with short
1603 periods of appropriate conditions for activity and development of offspring. The timing of the
1604 life cycle of European reptiles is determined by the seasonality of the weather. Mating and
1605 fertilisation typically happens in winter, egg laying in late spring or early summer, and hatching
1606 in late summer. As in the case of amphibians, the climatic particularities of each location may
1607 lead to variations from this general pattern. Viviparous species inhabiting cold areas, for
1608 instance, mate right before hibernation, gestation progresses during winter and spring, and
1609 births occur in early summer.

1610 As in amphibians, most reptile species display a breeding courtship before copulation (Moore
1611 and Lindzey 1992). Fertilization in reptiles is internal. Fertilisation happens by cloacal apposition
1612 in just one genus (*Sphenodon*). Otherwise, males possess intromitting organs, either a single
1613 penis (turtles and crocodylians) or two hemipenes (squamates). Reptilian embryos are protected
1614 by eggshells that limit their pre-hatching growth. Reptilian eggshells are, however, permeable
1615 to water diffusion, and water is used in yolk metabolism (Packard et al. 1982). The uptake of
1616 water by eggs means that soil contaminants can also be absorbed, potentially affecting
1617 embryonic development. Typical clutch sizes in reptiles vary from the 3-4 eggs in the smallest
1618 lizards to the ~30 that some turtles can lay. Within each species, the number of eggs a female
1619 produces shows a trade-off with the size of offspring, which ultimately relates to juvenile
1620 survival probabilities. Annual reproductive productivity in lizards has been analysed in detail by
1621 Meiri et al. (2012); these authors calculated annual reproductive output on the basis of clutch
1622 size, egg mass, and number of clutches per year, and found that it correlated with parental
1623 body size in an allometric way, which would suggest that the proportion of energy spent in
1624 reproduction is fairly constant across species. The models developed in that study to analyse
1625 the effects of environmental factors on reproductive production suggested that reduced body
1626 size, oviparity, and sit-and-wait species would be more productive than their counterparts.
1627 Equally, productivity would increase in non-insular, non-fossorial, diurnal species inhabiting
1628 warmer areas with higher net primary productivity.

1629 Nesting-site selection is important because of the major physiological role that environmental
1630 conditions like temperature, water or oxygen availability play in development of eggs. As in

1631 amphibians, temperature influences growth rate of embryos, but in some groups it also
1632 determines the sex (see below). The presence of water around the egg is, unlike in amphibians,
1633 disadvantageous, as it can create a barrier to gas diffusion and affect embryo respiratory
1634 physiology (Kennet et al. 1993). As noted above, however, water is used in yolk metabolism,
1635 and eggs require some sort of moisture to develop properly. Communal egg-laying is relatively
1636 frequent in reptiles (Doody et al. 2009). This communal behaviour may have an important
1637 influence on embryonic exposure to contaminants if, as shown in some studies with turtles and
1638 snakes inhabiting agricultural areas (see *Habitat* section below), animals show some preference
1639 for nesting in soils subjected to chemical applications. The process of hatching may be relatively
1640 fast or it may last for several hours, and this plasticity usually allows for achieving some sort of
1641 hatching synchrony within each nest (Spencer et al. 2001). In some species of turtles, a
1642 temporal separation has been observed between hatching and nest emergence, with hatchlings
1643 remaining inside the nest up to several months (Costanzo et al. 2008). This strategy could also
1644 increase exposure chances in those cases where nests are made in potentially contaminated
1645 soils.

1646 Sex determination in reptiles is not always chromosomal as in the other vertebrates (Bull 1980).
1647 Some reptile species, mainly crocodiles, tuataras and turtles but also some saurians, have
1648 temperature-dependent sex determination (Valenzuela and Lance 2004). This means that sex is
1649 determined as a function of the temperature of incubation of the egg. The mechanisms of
1650 temperature sex determination are not fully understood. The influence of the temperature on
1651 the activity of sexual hormones and on enzymes like aromatase, which regulates the activation
1652 of these hormones, has been proposed as a mechanism; more recently, some authors have
1653 suggested that such enzymatic regulation of sexual hormones could be accomplished by
1654 epigenetic mechanisms (Goldberg et al. 2007, Zhang and Ho 2011). Both the pivotal
1655 temperature (i.e. that leading to a balanced sex ratio) and the sex ratio resulting from
1656 increasing or decreasing temperatures varies across species, and the temperature range over
1657 which sex determination happens in nature is relatively narrow. In some cases, temperature-
1658 dependent sex determination coexists with elements of genetic sex determination within the
1659 same species or even population. The way in which the two mechanisms interact to end up in a
1660 given phenotypical sex is unknown.

1661 Inter-specific variation in age at sexual maturity, as well as its correlation with lifespan, is
1662 similar to what has been described for amphibians. Some lizards of the genus *Anolis* are
1663 sexually mature at 2-4 months of age but do not usually live more than four years (Andrews
1664 1976). On the other hand, some terrestrial turtles need more than one decade to reach sexual
1665 maturity and can live as long as 70 years (Grubb 1971).

1666 2.3.5. Habitat

1667 Home ranges in reptiles are normally better defined than in amphibians. Territoriality is not
1668 uncommon, especially for guarding mates and nesting areas, but also for defending a territory
1669 particularly good in terms of resources. As in the majority of animals, home-range size in
1670 reptiles is directly related to body size and inversely related to resource availability in the area
1671 (e.g. Simon 1975). Seasonal changes in home-range location in reptiles are not so marked as in
1672 amphibians (with the exception of marine turtles). Some individuals can move during the
1673 breeding season in search of areas with loose soils that favour nesting, and semiaquatic species
1674 move to upland areas for nesting, but the majority of continental reptilian species are quite
1675 sedentary. This makes the pattern for chemical exposure of sedentary species likely to be very
1676 stable; for those populations inhabiting agricultural areas (e.g. Madsen 1984, Jofre et al. 2016),
1677 exposure will be almost chronic, whereas individuals inhabiting non-exposed areas will have
1678 little chance of contact with chemical pollution.

1679 Patterns governing dispersal of juveniles or emigration of adults when population densities
1680 become too high (Lambin et al. 2001) have been scarcely studied in reptiles, but seem to
1681 depend on intrinsic factors such as body condition and also environmental cues, including
1682 habitat quality (Vignoli et al. 2012). Dispersal corridors for reptiles have been mostly studied for
1683 freshwater species; in terrestrial species, poor-quality habitats contribute to population
1684 fragmentation, and in agricultural areas the existence of corridors connecting patches of high-

1685 quality habitat seems essential to maintain reptile populations (Jelinek et al. 2014). On the
1686 other hand, in populations inhabiting agroecosystem, the preference for nesting in loose soils
1687 has been seen as the cause of some snakes and turtles increasing their presence inside crop
1688 fields during egg laying and incubation (Kaufmann 1992, Wisler et al. 2008), as cultivated soils
1689 are normally easy to manipulate for building nests. For these animals moving inside crop fields,
1690 exposure is not restricted to eggs; dermal exposure of adults may occur through direct over-
1691 spray during pesticide application or contact with contaminated soils, including granules or
1692 treated seeds if present on soil surfaces. Dermal exposure by contact with water can also
1693 happen in puddles or pools inside fields; in warmer months, it is common that reptiles take
1694 baths and even dive where water is available (e.g. Gollman and Gollmann 2008) to help
1695 thermoregulation processes. In addition, some species like terrapins or water snakes (genus
1696 *Natrix*) are semiaquatic and spend long periods of time in water bodies. Thus, water
1697 contamination by runoff, drift or deposition of atmospheric contaminants not only has the
1698 potential to affect amphibians but also some reptiles.

1699 **2.3.6. Feeding ecology**

1700 Reptilian feeding ecology is as diverse as the group itself (Vitt and Caldwell 2014). Focusing on
1701 European species, turtles are mostly phytophagous, although they have a scavenger component
1702 in their diets, especially terrapins. Among lizards, we can find a wide gradient from the mostly
1703 herbivorous (e.g. genus *Gallotia*) to the mostly carnivorous (e.g. genus *Anguis*) species. Snakes
1704 are typically carnivorous, active predators. As in other groups, there is a relationship between
1705 prey and predator sizes, but in reptiles the prey size relative to predator size is particularly
1706 large, and this is especially noticeable in some snakes that are capable of feeding on very
1707 voluminous prey. Reptiles have two characteristics that allow them to feed on relatively large
1708 prey: a wide mouth relative to the cranium width, and skull kinesis (i.e. the capacity to
1709 articulate, to a certain degree, the bones that form the skull) (Iordansky 1989). In addition,
1710 snakes present a very sophisticated feeding apparatus with very kinetic jaws whose left and
1711 right halves can move independently. Because of these characteristics, the feeding apparatus of
1712 snakes can accommodate very large prey compared with their own size (Gans 1961). Physical
1713 capture of a large prey is always difficult, and snakes have prey-capture mechanisms of prey
1714 immobilisation, like constriction or venoms, adapted to the handling of very large prey. Another
1715 consequence of eating relatively large prey is that they provide large amounts of energy all at
1716 once, which translates in a reduction of the number of feeding events. Snakes feeding on large
1717 prey may then not eat for several days or even weeks, relying on the energy obtained from a
1718 single meal. Some studies have even found seasonal adaptations of the digestive system of
1719 snakes as a function of prey-resource availability (Santos and Llorente 2008). This feeding
1720 regime is possible, not only because of their ability to ingest large prey but also because
1721 metabolic rate can fall in poikilothermic animals.

1722 Food-intake rate in reptiles can be estimated from information on daily energy expenditure.
1723 Friday and Thompson (2009) compiled the information available for 67 species, and calculated
1724 allometric equations relating body mass and daily energy expenditure. Then, considering the
1725 diet composition for the different species (i.e. arthropods and soil invertebrates for small
1726 lacertids to small vertebrates for snakes) and the energy and relative moisture content in each
1727 prey type, food-intake rate can be estimated, although no empirical data have been collected to
1728 validate such estimations. The reported assimilation efficiency values for lacertids are within the
1729 range described for small birds and mammals feeding on animals (71-89%; Avery 1971, 1975,
1730 Christian et al. 1996). An important aspect of the reptilian feeding ecology is the retention time.
1731 As mentioned above, because of the ability of feeding on relatively large prey, feeding events
1732 can be very occasional especially in snakes. This feeding regime should expectedly involve a
1733 high assimilation efficiency, and especially after ingesting large meals, this will require long gut
1734 retention times. As reported for amphibians, passage time is inversely related to temperature
1735 (e.g. Alexander et al. 2012, LaBonte et al. 2011). Average passage times of 6-7 days have been
1736 reported in different snake species at temperatures above 20°C (McCue 2007, Chu et al. 2009,
1737 Beaupre and Saidan 2012), although Beaupre and Saidan (2012) found in timber rattlesnakes
1738 (*Crotalus horridus*) an average passage time of 12.36 days, independent of temperatures within
1739 a range of 20 to 30°C. With temperatures of 14°C, gut-retention times in vipers were found to

1740 be even longer than 30 days (Chu et al. 2009). This particular feeding regime needs to be
1741 considered when estimating patterns of oral toxicity, as a single dose can result in a continuous
1742 absorption of contaminants over a period of time longer than what is usually considered to
1743 estimate oral acute lethal doses.

1744 The structure of the reptilian integument results in a very efficient protection from evaporative
1745 water loss, which contributes to the full independence of reptiles from the water. Conversely,
1746 the low skin permeability also prevents tegumentary water uptake, which means that reptiles
1747 must obtain water from other sources like food, drinking or metabolic water. The relative
1748 amount of water that is obtained through each route varies among species and populations. In
1749 some desert species under extremely arid and hot conditions, water contents in their prey can
1750 be the only water source. In those cases in which drinking water gains importance, ingestion of
1751 contaminated water could become a relevant exposure route. Bradshaw et al. (1987) estimated
1752 a total water influx rate in green lizards (*Lacerta viridis*) of 12 ml/100g body mass/day. Friday
1753 and Thompson (2009) obtained allometric equations from a compilation of data for 77 reptile
1754 species relating water flux and body mass, and proposed a protocol to estimate food and
1755 metabolic water influxes. Without more detailed data, it is difficult to estimate how much
1756 animals drink, and in turn how big isw the risk of contaminant uptake through drinking water.

1757 Soil particles are also commonly found in reptilian digestive contents, occasionally surpassing
1758 5% of the diet (Beyer et al. 1994). There is some debate about whether soil ingestion is
1759 accidental, therefore affecting species that feed on soil invertebrates, or deliberate to aid
1760 digestion, which would be expected to affect vegetarian species the most (Sokol 1971).

1761 **2.4. Exposure of life stages of amphibians and reptiles to** 1762 **pesticides**

1763 Table 3: and Table 4: compile a summary of information on exposure routes and measurable
1764 effects throughout the different stages of the life cycle of amphibians and reptiles.

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Table 3: Summary of features potentially affecting exposure and effects of pesticides for different life stages of amphibians occurring in agricultural landscapes.

Life stage	Effects (measurable)	Presumed impact on population persistence	Toxicological sensitivity (compared to other life stages)	Exposure route	Likelihood of exposure (exposure media)
Egg / embryo	Mortality Malformation Duration of dev development	Low for most species that produce lots of eggs; for those that produce low numbers of eggs (e.g. midwife toad, whose eggs are terrestrial) it is probably high	Low	Dermal (egg membrane)	High (mainly from water)
Hatchling (newly hatched larvae, still with external gills in the case of anurans)	Mortality Growth Malformation Duration of development Behaviour	Low for most species that produce lots of eggs; for those that produce low numbers of eggs (e.g. midwife toad, whose eggs are terrestrial) it is probably high	High (might be more sensitive than older larvae)	Mostly dermal but possibly also oral	High (from water + food + sediment)
Larvae / Tadpoles	Mortality Growth Malformation Duration of development Behaviour	Low for most species that produce lots of eggs; for those that produce low numbers of eggs (e.g. midwife toad, whose eggs are terrestrial) it is probably high	High (especially for endocrine effects)	Oral Dermal Inhalation (late stages)	High from sediment, water or food Low from air
Metamorphosis (since	Duration	Low-Medium	High (especially for	Dermal	High from sediment

emergence of front limbs to complete tail resorption)	Success rate		endocrine effects)	Inhalation	or water Low from air
Juvenile (since end of metamorphosis until sexual maturity attainment)	Mortality Growth Behaviour Lesions	High	Unknown; maybe more sensitive than adults	Oral Dermal Inhalation	Water, Soil, Food, Plants Air (possibly low) Overspray
Adult	Mortality Reproduction Behaviour Lesions	High	Unknown; maybe less sensitive than juveniles	Oral Dermal Inhalation	Water, Soil, Food, Plants Air (possibly low) Overspray

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1768 **Table 4:** Summary of features potentially affecting exposure and effects of pesticides for different life stages of reptiles occurring in arable habitats.

Life stage	Effects (measurable)	Presumed impact on population persistence	Toxicological sensitivity (compared to other life stages)	Exposure route	Likelihood of Exposure (exposure media)
Egg	Mortality Time to hatch Hatching success	Likely to be high for-short lived species, likely to be low for long-	Unknown	Dermal (egg membrane)	High (soil)

	Weight at hatching Sex ratio (in turtles and some saurian temperature dependent)	lived species (tortoise)			
Juvenile (since hatching or birth) until sexual maturity attainment)	Mortality Growth Behaviour Lesions Metabolic rate	High	Unknown in comparison with eggs. Probably more sensitive than adults (except for reproductive effects) because of the higher surface area:volume ratio	Oral Dermal Inhalation	Oral: high from food and occasionally from drinking water (uptake of water during feeding can be occasionally high) Dermal: high from soil, plants or stone wall at field edges; low from water (except for some water dwelling snakes and terrapins) Inhalation: possibly low (certainly lower than for birds or mammals) Overspray: high
Adult	Mortality Growth Reproduction Behaviour Lesions Metabolic rates	High	Probably less sensitive than juveniles (except for reproductive effects) because of the lower surface to volume ration	Oral Dermal Inhalation	Oral: high from food and occasionally from drinking water (uptake of water during feeding can occasionally be high) Dermal: high from soil, plants or stone wall at field edges; low from water (except for some water dwelling snakes and terrapins) Inhalation: possibly low (certainly lower than for birds or mammals) Overspray: high

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2.5. Identification of potential species groups to be assessed

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2.5.1. Relevant traits for selection of focal species

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An important aspect to consider in future risk-assessment schemes for amphibians and reptiles is how to identify focal species. The selection of focal species requires a comprehensive review of the information on traits determining potential exposure and sensitivity. The recent scientific opinion on the *Coverage of endangered species in environmental risk assessments at EFSA* (EFSA Scientific Committee, 2016) proposed a trait-based approach to identify if an endangered species may not be covered by generic environmental risk assessment, as well as which surrogate species will help to cover the endangered ones in the risk-assessment scheme. This trait-based approach identifies those aspects that contribute to increase risks in order to compare them further between endangered and non-endangered species. This type of trait-based approach can also help to identify which amphibian and reptilian species are more vulnerable to pesticides, and therefore would better play the role of focal species. The traits proposed as important to determine susceptibility are classified into four categories:

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- Traits related to external exposure.

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- Traits related to toxicological sensitivity, which are in turn divided into two groups:

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- o Factors related to internal exposure (toxicokinetics).

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- o Factors related to toxicological sensitivity on the organism level (toxicodynamics).

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- Traits related to recovery.

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- Traits affecting susceptibility to suffer indirect effects of pesticides.

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Within each category, a series of general traits is enumerated. In Table 5: below, the list of these general traits is shown together with their correspondence with specific amphibian and reptile traits.

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Understanding the relative importance that every factor listed in the Table 5: has in determining pesticide risk can be challenging. For example, the presence in-field or in edge-of-the-field ponds undoubtedly has implications for exposure. Factors like shedding skin or producing skin secretions would be relevant if they served to eliminate internally accumulated pollutants; however, it is unknown whether amphibians or reptiles actually use these ways for detoxification. In other cases, like for instance the activity of metabolic enzymes, it is likely that the trait is so conserved across the groups that no differences can be established based on such a trait. Finally, the same trait may have opposite effects; for instance, larger larval sizes may lead to increased bioconcentration of pollutants from the water if the exposure is continuous, but if it is more or less sporadic, higher growth may result in dilution of accumulated substances with the passage of the time. Although the role played by many of the listed traits can be defined, more research will be necessary in the future to understand fully the factors determining the potential risk posed to pesticides on each amphibian and reptile species.

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Table 5: Factors listed by EFSA Scientific Committee (2016) as important to determine susceptibility to a stressor, and possible corresponding specific traits of amphibians and reptiles leading to increased susceptibility to pesticides.

Category / subcategory		General factor	Specific amphibian/reptilian trait leading to increased susceptibility
External exposure		Diversity of routes of exposure	Both terrestrial and aquatic life stages
		Concentration of the stressor in the exposure media	Presence in-field and edge-of-the-field
		Availability of the stressor in the habitat	Presence in-field and edge-of-the-field, larger home range
		Contact duration between the exposure media and the species	Frequency of in-field use, duration of aquatic stages of animals developing in edge-of-the-field ponds, larger home range
Toxicological sensitivity	Toxicokinetics	Surface area to volume ratio	Elongated body shapes
		Intake rate of the exposure media	Higher skin permeability and higher food intake rate
		Potential for the stressor to be released from the exposure media once inside the organism	Gut retention time of food
		Absorption rate	
		Elimination rate	Assimilation efficiency of the food
		Rate of metabolism of the stressor	Metabolic rate
		Excretion rate	
		Presence of specific organs or tissues in which the potential stressor accumulates	Percentage of fat in the body
		Potential for the accumulated stressor to be released or remobilised	Frequency and duration of energy-demanding periods (hibernation, metamorphosis)
		Presence of specific organs through which the stressor can be eliminated	Egg production (maternal transfer), shedded skins, glandular skin secretions
		Growth rate of the species resulting in dilution of the accumulated stressor	Reduced growth rate
Existence of life stages with characteristics potentially leading to high internal concentrations	Attainment of large larval sizes		

	Toxicodynamics	Presence and number of molecular receptors with high affinity for the stressor Potential for the stressor to cause a toxic effect when binding to a receptor in the organism	Hormonal or neurological receptors susceptible for binding xenobiotic molecules
		Capacity to recover from an adverse effect caused by the stressor	Activity of metabolic enzymes
		Existence of life stages particularly sensitive	Small hatchlings, with external gills in amphibians
Recovery		Reproduction rate	Reduced reproduction rate
		Potential to recolonise an affected area by other source populations	Shorter dispersal and migration distances, higher phylogeny, smaller home ranges
		Co-occurrence of adverse effects with other critical stressor events	Frequency of predators, pathogens or other stressors in the exposed habitats
Indirect effects		Position in the food web affected by the stressor	Higher trophic level
		Connection with other components of an ecological network (i.e. conjunction of ecological interactions of an ecosystem) affected by the stressor	Intermediate position in the trophic web, playing roles both as predator and prey
		Dependence on another species that can be directly or indirectly affected by the stressor	Specificity in the diet

1808 2.5.2. Definition of assessment groups

1809 Once the relevant factors are determined, the next step is to identify which species fall into
1810 each trait-defined category to group them further according to their susceptibility to pesticides.
1811 As a first approach, basic taxonomical and broad ecological criteria have been used to define
1812 what can be defined as assessment groups (i.e. groups of species within which is assumed that
1813 a selected focal species would be effectively covering the entire group). The defined
1814 assessment groups could be:

1815 -Anurans (frogs and toads)

1816 -Caudates (newts and salamanders)

1817 -Terrestrial turtles (i.e. tortoises)

1818 -Freshwater turtles (i.e. terrapins)

1819 -Saurians (lizards, skinks and geckos)

1820 -Amphisbaenians (blind snakes)

1821 -Fully terrestrial snakes (colubrids and viperids)

1822 -Water snakes (natricids)

1823 Coverage might be provided by focal species from different assessment groups in some cases.
1824 For example, water snakes are clearly different from fully terrestrial snakes in terms of potential
1825 exposure to pesticides, as they spend a significant part of the time in the water and prey upon
1826 aquatic organisms., Their risks in the aquatic environment, however, would probably be
1827 covered by assessment on amphibians. For the terrestrial environment, their risk assessment
1828 might not be so different from fully terrestrial snakes. The same case argument might be
1829 applied to freshwater turtles, which have been separated from tortoises because of evident
1830 ecological differences.

1831 The next step is to have an inventory of EU amphibians and reptiles and then to identify which
1832 are the species that can appear in arable lands. For the all-species inventory, an updated list
1833 was created using the database of the IUCN red list (<http://www.iucnredlist.org>), completed
1834 with the catalogue of species included in the Atlas of Amphibians and Reptiles of Europe
1835 developed by the European Herpetological Society (Sillero et al. 2014; atlas available at
1836 <http://na2re.ismai.pt/>). For simplification, only species native to the EU territory and excluding
1837 overseas areas (i.e. Macaronesian, Northern African and Trans-Oceanic territories) have been
1838 included.

1839 The next step is to identify which are the species that can appear in arable lands. The
1840 identification of species present on arable lands was not so straightforward; Wagner et al.
1841 (2014) compiled data from the literature, but the list of amphibian species addressed in the
1842 paper is restricted to the taxa listed in Annex II of the of the Habitats Directive (92/43/CEE),
1843 including only 24 species and generally excluding the widely distributed ones. The list of reptiles
1844 examined by Mingo et al. (2016) was quite comprehensive, and the identification of overlaps
1845 with agricultural areas was done superposing with a GIS the known range of species and the
1846 agricultural areas defined by CORINE; this approach, although extremely useful, does not serve
1847 to confirm totally that animals are actually in-field. To create the list of species present on
1848 arable lands, the information included for each species in the IUCN red list was considered. It
1849 was not possible to find an information source good enough to harmonise habitat descriptions
1850 for the species not listed by the IUCN, as well as for one of the species listed therein (*Natrix*
1851 *natrix*) for which the habitat is not described,. We therefore looked directly for papers in
1852 Scopus describing the presence of those species in agricultural lands, and we found positive
1853 results for *Natrix natrix* (Meister et al. 2010) and *Vipera berus* (Leibl and Völkl 2009) in
1854 Germany, although reports for *Vipera berus* from other areas suggest otherwise (Reading et al.
1855 1996). The list of species can be seen in Appendix A, which includes also information on
1856 distribution, namely with the presence in each of the three zones defined for pesticide risk
1857 assessment according to the Regulation 1107/2009, taxonomical classification, and suggested
1858 presence on arable lands.

1859 Having defined the list of species present in agricultural areas, information on the relevant traits
 1860 will provide the basis for classification of the species according to most relevant factors
 1861 determining pesticide risks, and then focal species representing each group can be selected.
 1862 Two recent papers made an attempt to identify focal species of EU amphibians (Wagner et al.
 1863 2014) and reptiles (Mingo et al. 2016), not by grouping them according to different
 1864 susceptibility factors, but ranking them according to an estimation of risk posed by pesticides.
 1865 Both papers followed a similar methodology, which involved the estimation of a pesticide-risk
 1866 factor based on parameters relevant for exposure and sensitivity (**Error! Reference source**
 1867 **not found.**). Three parameters were used in each case, as a result of which an exposure index
 1868 was obtained. The exposure index was then corrected by the probability that species overlap
 1869 spatially with areas of pesticide application (estimated from literature data in the case of
 1870 reptiles and with a GIS analysis in the case of amphibians).

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1872 **Table 6:** Parameters used for estimating pesticide-risk factors in review papers on European
 1873 amphibians and reptiles.

Group	Paper	Risk factor name	Exposure index name	EF1	EF2	EF3
Amphibians	Wagner et al. (2014)	PRF (pesticide risk factor)	HEI (habitat exposure index)	Habitat exposure risk	Migration behaviour	Breeding aggregation in space and time
Reptiles	Mingo et al. (2016)	ERF (exposure risk factor)	ERI (exposure risk index)	Regular occurrence within cultivated landscapes	SVL and body mass (surrogates of physiology)	Clutch size and clutches/year

1874 EF: exposure factors used to calculate the exposure index

1875

1876

1877 An attempt has been made to suggest potential focal species and the outcome of the evaluation
 1878 is presented below. It is important to highlight that the adequacy of focal species will rely on
 1879 the accuracy of biological and ecological information available to define the best candidate
 1880 models to serve as focal species. The conclusions of the next sections should be treated
 1881 cautiously because much information is still required in order to determine actual exposure risks
 1882 and susceptibility to pesticides for most European amphibians and reptiles,.

1883 *Amphibians*

1884 The number of amphibian species reviewed by Wagner et al. (2014) was limited and so
 1885 additional information was compiled from the literature in order to propose potential focal
 1886 species for amphibians. The following criteria were established in principle: i) Species present in
 1887 agricultural areas (according to the IUCN information referred above); and ii) species present in
 1888 at least two of the three assessment zones for risk assessment of PPPs. This was a measure to
 1889 guarantee a wide distribution and ecological amplitude of the focal species.

1890 Eleven anuran and two caudate species met these criteria. Based on the traits defined in the
 1891 previous section, those life-history features potentially relevant in determining the risk that
 1892 pesticides pose to these species were selected from the information compiled by Trochet et al.
 1893 (2014) and from the AmphibiaWeb (<http://amphibiaweb.org/index.html>). For each parameter,
 1894 the worst case was identified in order to make conservative choices and assigned, when

1895 possible, a score of 0 (best case) or 1 (worst case), in an analogous way to Wagner et al.
1896 (2014) in their evaluation of pesticide-exposure risks for European amphibians (Table 7:).
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1898 **Table 7:** Evaluation of candidate amphibian-model species based on relevant biological and ecological traits.

Param.	Sexual maturity (years)		Egg laying mode		Clutch size		Egg laying site		Breeding season duration		Food of juveniles*		Metabolic rate		Home range		Max dispersal distance (m)		Max migration distance		FINAL SCORE
	Worst case	Longer	Other than big cluster		Smaller		Lentic		Explosive		Herbivorous		Higher		Larger		Longer		Longer		
Species	Descr.	Score	Descr.	Score	Descr.	Score	Descr.	Score	Descr.	Score	Descr.	Score	Descr.	Score	Descr.	Score	Descr.	Score	Descr.	Score	•
<i>Lissotriton vulgaris</i>	2.88	1	Single	1	300	1	Lentic	1	Prolong.	0	I	0	1.03	1	N/A	•	N/A	•	866	0	0.63
<i>Triturus cristatus</i>	2.67	1	Single	1	400	1	Lentic	1	Prolong.	0	I	0	0.31	0	N/A	•	860	1	1290	1	0.67
<i>Bombina bombina</i>	1	0	Small	1	300	1	Lentic	1	Prolong.	0	I	0	N/A	•	60	0	N/A	•	170	0	0.38
<i>Pelobates fuscus</i>	1.5	0	String	1	2500	0	Lentic	1	Explosive	1	H	1	N/A	•	N/A	•	N/A	•	500	0	0.57
<i>Hyla arborea</i>	1	0	Small	1	1400	1	Lentic	1	Explosive	1	I	0	1.75	1	10	0	2400	1	12570	1	0.70
<i>Bufo bufo</i>	3	1	String	1	10000	0	Perman.	0	Explosive	1	I-H	1	0.21	0	50	0	N/A	•	4000	1	0.56
<i>Bufo viridis</i>	N/A	•	String	1	3000	0	Lentic or lotic	1	Explosive	1	I	0	N/A	•	N/A	•	N/A	•	5000	1	0.67
<i>Epidalea calamita</i>	3	1	String	1	4000	0	Lentic	1	Explosive	1	H-D	1	0.32	0	1450	1	4411	1	2600	1	0.80
<i>Rana arvalis</i>	3	1	Big	0	3000	0	Lentic	1	Explosive	1	H	1	0.14	0	100	0	N/A	•	1001	1	0.56

<i>Rana temporaria</i>	3	1	Big	0	4000	0	Lentic	1	Explosive	1	H	1	0.04	0	119.5	0	N/A	•	2214	1	0.56
<i>Pelophylax kl. esculentus</i>	3	1	Big	0	2400	0	Perman	0	Explosive	1	H-D	1	0.09	0	N/A	•	78	0	N/A	•	0.38
<i>Pelophylax lessonae</i>	2.5	1	Big	0	2400	0	Perman	0	Explosive	1	I-H-D	1	N/A	•	N/A	•	78	0	N/A	•	0.43
<i>Pelophylax ridibundus</i>	2	0	Big	0	2400	0	Perman	0	Explosive	1	H-D	1	0.18	0	4.25	0	78	0	N/A	•	0.22

1899

*I: Insectivorous, H: Herbivorous, D: Detritivorous

1900 Among the caudates, the crested newt (*Triturus cristatus*) obtained the highest score, whereas
 1901 among anurans the natterjack toad (*Epidalea calamita*) was the species with the highest score.
 1902 It could be suggested that anurans should be divided into two different assessment groups, one
 1903 comprising the most terrestrial species (toads) and another one including the most aquatic
 1904 species (frogs). If that were the case, the European tree frog (*Hyla arborea*) would be the most
 1905 reasonable option for the latter group. Furthermore, *H. arborea* would cover those traits for
 1906 which *E. calamita* does not fall into the worst-case option (i.e. clutch size and metabolic rate).

1907

1908 *Reptiles*

1909 The same two initial criteria used for amphibians were applied for proposing reptilian focal
 1910 species. The exposure risk factors as defined by Mingo et al. (2016) were then used to identify
 1911 the most appropriate models in each case (Table 8:).

1912

1913 **Table 8:** List of reptile species, within each defined assessment group, with highest pesticide
 1914 exposure risk factors (ERF) estimated by Mingo et al. (2016).

Assessment group	Species with highest exposure risk factor
Terrestrial turtles*	<i>Testudo graeca</i> (0.56) <i>Testudo hermanni</i> (0.38)
Freshwater turtles*	<i>Emys orbicularis</i> (0.41) <i>Mauremy leprosa</i> (0.30)
Saurians	<i>Podarcis muralis</i> (0.43) <i>Lacerta agilis</i> (0.39)
Fully terrestrial snakes	<i>Coronella austriaca</i> (0.49)
Water snakes	<i>Natrix natrix</i> (0.23)

1915 *No species identified as present in agricultural areas according to IUCN (but see text for *T. hermanni*)

1916

1917 In Testudines, the ERF from Mingo et al. (2016) suggests *Testudo graeca* and *Emys orbicularis*
 1918 as the tortoise and terrapin with the highest exposure risk, respectively. However, none of
 1919 these species was identified by the IUCN as present on arable lands, and *Testudo hermanni*
 1920 was the turtle most associated with agricultural areas according to Friday and Thompson
 1921 (2009), which has been confirmed from other papers as mentioned above. Although the
 1922 occurrence in agricultural areas is implicitly within the ERF (and so *T. graeca* could be a suitable
 1923 choice as focal tortoise species), the native distribution area of *T. hermanni* in the EU (whole
 1924 Mediterranean basin) is considerably larger than that of *T. graeca* (Balkan / Greek Peninsulas).
 1925 In the case of terrapins, assuming that there is no evidence of their presence in agricultural
 1926 areas (at least in a more or less regular manner), and that, as mentioned above, their aquatic
 1927 exposure could be covered by amphibians, it does not seem necessary to propose focal species.

1928 For Saurians, both the wall lizard (*Podarcis muralis*) and the sand lizard (*Lacerta agilis*) seem
 1929 adequate options to represent the entire taxon, and the same would apply to the smooth snake
 1930 (*Coronella austriaca*) and the grass snake (*Natrix natrix*) as focal species for fully terrestrial and
 1931 water snakes, respectively. Finally, it must be noticed that amphisbaenians are not included in
 1932 this Table 8: because i) their presence in agricultural areas has not been confirmed according
 1933 to the IUCN data, and ii) Mingo et al. (2016) did not calculate an ERF for any of the species of
 1934 this group. However, the fact that these species are not very well known in terms of ecology
 1935 and biology makes it necessary to investigate their potential presence in agricultural areas and
 1936 the consequent risk of any impact of pesticide applications

1937

1938 *Use of species in population models*

1939 This section described the process of definition of assessment groups and the process for
1940 selecting species representative of each group that can be further used as focal species in risk
1941 assessment. As explained in section 4.1.5, model species are also necessary to run population
1942 models to support population-based Specific Protection Goals, and such species must represent
1943 the diversity of amphibians and reptiles susceptible to be exposed and to suffer toxic effects
1944 from pesticides. Therefore, the traits for selecting population model species are the same as
1945 those defined in section 2.5.1 for selecting focal species. The only additional requirement for
1946 the proposed focal species to be also good population-model candidates is that there should be
1947 enough information about them to parameterize the model. Because the six proposed focal
1948 species are among the most widely studied within their corresponding groups, they are also
1949 proposed as appropriate candidates to develop population models.

1950 **2.6. Conclusions and recommendations**

1951 **2.6.1. Conclusions**

1952 Although traditionally studied together under the discipline of herpetology, amphibians and
1953 reptiles present important differences in many of their biological and ecological features. They
1954 share, however, their condition as poikilothermic vertebrates, which differentiates them from
1955 birds or mammals. Sensitivity and chances of exposure to pesticides, which are affected by
1956 poikilothermy through its influence on physiology, growth, development, behaviour or
1957 reproduction, are poorly comparable to those of birds or mammals. Other aspects like
1958 permeable skins (in amphibians) also have a high influence in risk of exposure.

1959 The presence of amphibians and reptiles in agricultural areas is well documented, both in-field
1960 and on the edge of the field. Potential for overspray, dermal exposure by contact with applied
1961 soils or plants, and oral uptake of pesticides through ingestion of contaminated materials exist
1962 for both groups. Amphibians and reptiles have low mobility, and therefore exposure can be
1963 prolonged when they inhabit a treated area, especially in the case of the most territorial reptile
1964 species or of the amphibian aquatic stages.

1965 The potential of surrogate-based risk assessment to cover toxicity of pesticides on amphibians
1966 and reptiles is compromised by some particular biological processes typical of these animals,
1967 including metamorphosis in amphibians or hormonal-dependent sex determination in both
1968 amphibians and reptiles. The peculiarity of the amphibian life cycle compared with other
1969 vertebrate groups also has a major influence on chances of exposure, which is difficult to
1970 predict from data generated from other taxa. Amphibians possess some structures typical of
1971 higher vertebrates that do not occur in fish (e.g the Müllerian ducts that are precursors of
1972 sexual organs), and impacts of pesticides on these structures cannot be identified through fish-
1973 based assessment; pesticide impacts should therefore be assessed at specific, sensitive time
1974 windows within the amphibian aquatic development.

1975 **2.6.2. Recommendations**

- 1976 • Differences in sensitivity among life stages should be considered when determining the
1977 toxicity of pesticides, especially for amphibians, because of the morphological and
1978 physiological differences among them.
- 1979 • Variability in sensitivity throughout the life cycle is also translated in the existence of
1980 key windows in time at which certain effects are more likely to happen. This must be
1981 considered when short-term toxicity is assessed. For instance, maturation of sexual
1982 organs has a very defined time window, and testing reproductive toxicity of pesticides
1983 at a different time could lead to wrong assumptions about effects or lack of effects.
- 1984 • Toxicological endpoints related to certain aspects of biology of amphibians, like
1985 metamorphosis or hormone-dependent sex determination, cannot be predicted from
1986 information generated from surrogate taxa. A specific approach to investigate chronic
1987 toxicity leading to effects on these aspects is required.

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3. Definition of spatial aspects to be considered in the risk assessment

3.1. Spatial boundaries considered at the field scale

The structures considered are defined as follows

In-field: piece of land for cultivation with crops, managed by typically one farmer

Buffer strip: in-field; cropped or non-cropped zone of a defined width at the edge of a field which is influenced by the farmer or contractor's action (e.g. spray drift). The buffer strip normally is enforced by authorities and underlies prescribed actions in order to meet the off-field SPG. In addition buffer strips may provide a recovery potential for the cropped area.

Off-field: area surrounding a field: either (semi-)natural habitats with high ecological value such as hedgerow, grass strip, or simple structures (fence or a bare strip of land); normally no short-term changes in cultivation, in most cases not to be influenced by the farmer. Another off-field category comprises man-made structures, e.g. an adjacent field, roads, etc.

In-crop: the area actually cropped

Off-crop: any uncropped area

The buffer strip is located in-field and has the same protection goals as the in-field area plus the functions to mitigate exposure of the off-field area (drift and run-off reduction) and may serve as a reservoir for recolonisation of the in-field area if there is no suitable off-field habitat. The off-field protection goal is independent from the actual type of off-field habitat of individual fields.

It is necessary to define the temporal and spatial boundaries of the off-field and the way the emission is translated to an exposure in the off-field area. These boundaries relate to the protection goal (where is the community of interest) in relation to the route and distance covered of the emission coming from the in-field. The choice of such a distance will be the result of both scientific (e.g. is there a critical maximum area that can be at risk, without affecting the population of interest) and regulatory decision (is that distance acceptable from a regulatory point of view).

Predicted Environmental Concentrations (PECs) could be provided for different distances from the field boundary and choices need to be made depending on the crop, group of non-target organisms and their specific protection goal. This PEC calculation allows definition of buffer strips and the risk assessment in the off-field area at the same time.

Step 1

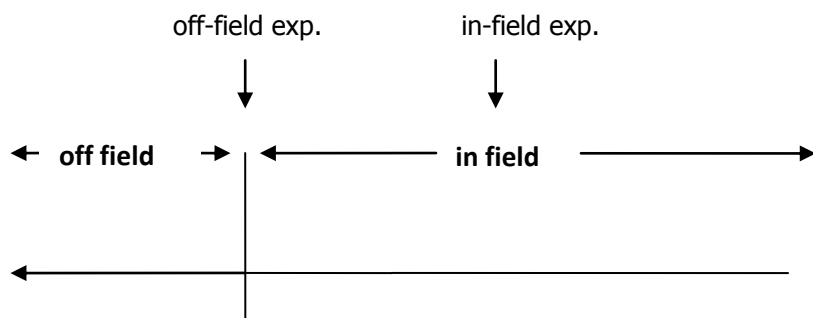
The exposure and risk is assessed for the in-field and off-field area. If the specific protection goals in-field and off-field are met no further risk assessment or risk mitigation are needed (**Error! Reference source not found.**

2025 **Exposure estimate**

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2030 **Figure 4:** Schematic overview on field scale elements

2031 **Step 2**

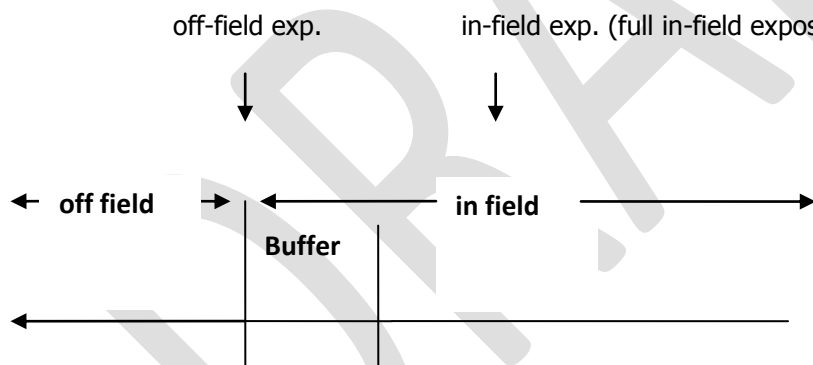
2032 A buffer strip is necessary in case the off-field protection goal is not met in the previous risk
2033 assessment. The buffer strip is in-field. The maximum tolerable exposure to meet the off-field
2034 protection goal is calculated. The width of the buffer strip is calculated on the basis of this maximum
2035 tolerable exposure estimate, the required reduction factor and the reduction potential of the buffer
2036 strip. For example for spray drift, the final width of the buffer strip depends on the combination of the
2037 height of the vegetation in the buffer strip and drift reduction techniques. If for example a wind break
2038 is in the in-field area, then the drift to the off-field is significantly reduced compared with a buffer strip
2039 without vegetation. A table on reduction of spray drift from the combination of spray drift nozzles and
2040 width of the buffer zone can be found in Huijsmans & van de Zande (2011) (Figure 5:).

2041 **Exposure estimates:**

2042 off-field exp. in-field exp. (full in-field exposure, e.g. full application rate)

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2044



2045

2046 **Figure 5:** Schematic overview on field scale elements

2047 **Example of use of boundaries in the risk assessment**

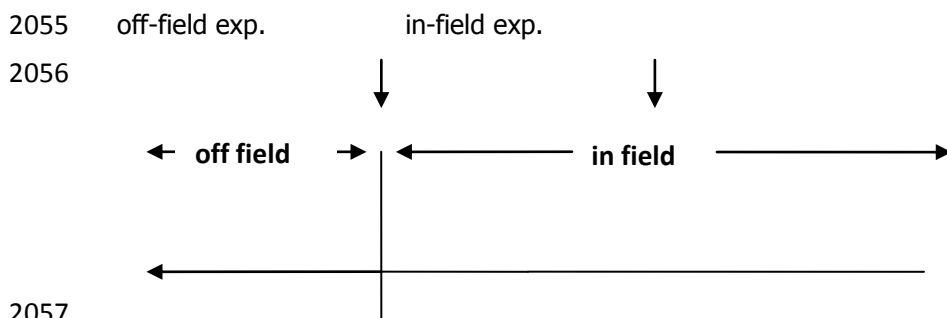
2048

2049 The **initial assessment** should start with calculating the acceptable concentrations in the off-field
2050 area.

2051 The distance from the last row of treated crop at which the off-field protection goal is met can be
2052 back-calculated from this (Figure 6:).

2053

2054 **Exposure estimate**



2058 **Figure 6:** Schematic overview on field scale elements

2059 The risk assessment does not assume a pre-defined distance to the off-field. The exposure
2060 assessment starts at the field edge and calculates at which distance the off-field protection goal is
2061 met. If, for example, the regulatory acceptable concentration (RAC) was equal to the amount of a.s.
2062 at a distance of 5 meters, then the full risk mitigation equivalent to a 5-meter buffer zone would need
2063 to be achieved in the in-field area. Standard options for reducing the width of the in-field buffer strip
2064 could be provided in the risk assessment – e.g. vegetation in the buffer strip of a certain height or
2065 wind breaks or drift-reduction nozzles. The risk manager decides whether the risk is manageable
2066 under the national conditions to achieve the required reduction of exposure in the off-field area (e.g.
2067 considering agricultural practice or national policy on implementation of buffer zones).

2068 With this approach, it is not necessary to assume that the off-field protection goal needs to be met 1m
2069 and 3m away from the last row of the treated crop as was practice previously; instead, the goal needs
2070 to be met at the edge of field or buffer strip as needed. A risk-management decision can therefore be
2071 taken based on knowledge of how much distance there is between treatment and the edge of the
2072 field (it may be different in MSs and crops), based on national policies for implementation of buffer
2073 strips, e.g. obligatory vegetated buffer strips of a certain width.

2074

2075 **3.2. Spatial boundaries at the landscape scale**

2076 Most reptile species that occur in agricultural landscapes show a high site fidelity. They use off-field
2077 and in-field areas for feeding, nesting and hibernation. Amphibian species, in contrast, often have
2078 migratory behaviour and their feeding and spawning sites are often several kilometres away. Most
2079 amphibian species have higher dispersal ability than reptile species that inhabit agricultural
2080 landscapes. It is therefore necessary to evaluate the risk to amphibians, not only at the field scale but
2081 also at the wider landscape context. Landscape structure is thus particularly important in order to
2082 derive a realistic estimate of pesticide exposure to amphibians.

2083 **3.2.1. Spatial aspects in relation to the species to be assessed**

2084 Amphibian and reptile species differ in the importance of sub-structuring within larger populations, as
2085 well as in their mobility and ability to disperse in the landscape. Many amphibians in particular also
2086 exhibit seasonal migrations between breeding and non-breeding habitats. Seasonal migration is of
2087 eminent importance when organisms might be exposed over time to different concentrations, e.g.
2088 moving into (and possibly out of) treated fields. Contrary to e.g. non-target arthropods, few, if any,
2089 can be defined as having populations entirely within a typical field, and the scales over which a
2090 population of amphibians or reptiles might be considered to be contained is larger than a single
2091 cropped area (e.g. field). As a result the traditional definitions of 'in-field' and 'off-field' are not easy to
2092 apply to these organisms and these should be considered similarly to mobile, non-target arthropods
2093 (EFSA PPR 2015). This means that an individual from a population would not be restricted to single
2094 treated or untreated areas and might cover in its range several landscape elements, including in-field
2095 and off-field areas.

2096 Different habitats may be important at different periods during the life cycle or season, and thus the
2097 coincidence between animals and exposure to PPP needs to be considered in time as well as space.
2098 For an individual, this means that it may avoid exposure, or be highly exposed, depending on the
2099 availability of the PPP in the environment and the individual's life-stage.

2100 Amphibians in particular may have complex, sub-structured populations (Annex A) and, because they
2101 breed in discrete, patchily distributed water bodies, metapopulation ideas have frequently been
2102 applied to describe their dynamics (e.g. Gill, 1978; Sjögren, 1991; Sjögren-Gulve, 1994; Hels, 2002;
2103 Hels and Nachman, 2002). However, in its strict form, the metapopulation structure is not all-
2104 encompassing and depends on the phenomena of local extinction and of re-colonisation of all sub-
2105 populations. The population structure will very often form a 'mainland-island' complex, in which the
2106 'island' populations depend on immigration from the 'mainland' for long-term persistence (e.g. Griffiths
2107 et al 2010). Increased mortality or lowered reproductive success can in all cases reduce re-
2108 colonisation rates and therefore reduce long-term viability of the overall population. There is a further
2109 complexity in the case of mainland-island situations, in that stressors affecting the mainland will have
2110 a much larger impact on the long-term population state than if they impacted only island populations.
2111 Other populations may exist as less structured populations, dispersed over a larger area. In this case
2112 source-sink dynamics (Pulliam, 1988) may be important (Annex A). These are spatial dynamics
2113 whereby populations in areas with a negative population growth rate are maintained by dispersal from
2114 source populations, and are a more general form of the mainland-island metapopulation structure not
2115 needing discrete sub-populations.

2116 **3.2.2. Spatial aspects in relation to the landscapes to be assessed**

2117 The consequence of large spatial scale of activity and spatially-structured populations means that the
2118 effects of PPPs on amphibian and reptile populations cannot be considered without considering both
2119 landscape structure and the way the animals interact with it. In addition to toxicological variability, the
2120 effects of PPPs will therefore depend on the species mobility, seasonal behaviour, population size,
2121 meta-population structure and source-sink dynamics.

2122 Complex spatial dynamics at the landscape scale can be difficult to predict as has been demonstrated
2123 for other groups. For example, Dalkvist, Sibly et al. (2013) found that, contrary to expectations,
2124 increasing the area treated with an endocrine disrupter by increasing the area of orchards led to lower
2125 population impacts and faster recovery. Also surprising was that placing source habitats close to
2126 orchards improved recovery and decreased impact due to rescue effects, despite the fact that these
2127 narrow habitats were heavily exposed to the pesticide. In carabids, however, these rescue effects can
2128 become important depleting dynamics under different circumstances (Topping and Lagisz 2012; EFSA
2129 PPR 2015). In dispersive spiders, refuges were shown to be able to buffer considerable agricultural
2130 mortality impacts (Thorbek and Topping 2005). As reported by Topping et al. (2014), experimental
2131 work with Staphylinidae, Linyphiidae and Carabidae indicates that the appropriate scale for assessing
2132 pesticide effects differed between taxa and depended upon the proximity of sources of re-colonisation
2133 as well as dispersal ability. The precise effect of landscape structure interacting with source-sink
2134 dynamics is therefore context dependent and difficult to generalise without more extensive reference
2135 work being available.

2136 **3.2.3. Spatio-temporal effects**

2137 The exposure to shifting resources and shifting stressors in modern agricultural landscapes may cause
2138 declines in species abundance and may also cause non-equilibrium ecological conditions, where
2139 species will suffer conditions of extinction debt (Tilman et al. 1994). Extinction debt means that a
2140 species can still be present, but only because it takes an extended time period for the species to
2141 become extinct. The ultimate cause of this phenomenon is that the ecological conditions for the
2142 species are inappropriate but that, due to spatial and population processes, the extinction time is long,
2143 albeit inexorable. This situation is not strictly relevant to the current assessment criteria for PPPs, but
2144 is an important part of the spatio-temporal dynamics of the system. These dynamics are important
2145 because an understanding of the state of the system prior to application of the stressor is required as
2146 a basis for systems approaches to ERA (EFSA SCER, 2016).

2147 For species with inter-linked, spatially-structured populations there is an inseparable link between
2148 spatial dynamics and temporal dynamics. The metapopulation approach in its simplest form

2149 demonstrates that the whole metapopulation should be exposed simultaneously for effective pest
2150 control (Levins, 1969); the same will therefore be true of negative impacts on non-target organisms.
2151 It also follows from metapopulation theory that the transient time following perturbation of a
2152 population can be long, especially one close to the threshold for persistence, for a species with slow
2153 turnover, and in a habitat-patch network consisting of only a few dynamically important patches
2154 (Ovaskainen & Hanski 2002). Since this considers only a single pulse effect, the implications of
2155 multiple applications spanning multiple seasons may be much more serious.

2156 3.2.4. Conclusion

2157 **Individual-level:** Exposure to PPP can take place differentially in space and time depending upon
2158 the behaviour of the animals coincident with PPP availability in the environment. Therefore realistic
2159 risk assessments should take behaviour within a season into account.

2160 **Population-level:** Population structure and spatio-temporal dynamics can have important
2161 implications for the evaluation of impacts of PPP on amphibian and reptile populations. A systems
2162 approach is therefore recommended by EFSA SCER (2016) in order to include both spatial and
2163 temporal implications of PPP usage and to take the ecological state of the population into account.

2164

2165

2166 4. Population Dynamics and modelling to support the setting of Specific 2167 Protection Goals SPGs

2168

2169 Whatever specific protection goals (SPGs) are defined for amphibians and reptiles, the main features
2170 of interest will be the distribution of animals (where do they occur?), and the abundance of animals
2171 (how many are there in the places where they occur?). We may also be interested in their condition.
2172 The basics of addressing these issues using population modelling are described in Annex A.

2173 Annex A explains that, for amphibians and reptiles, population modelling taking into account highly
2174 detailed environmental and population structure is needed to address adequately the population
2175 processes (especially spatial processes) needed for the risk assessment. This chapter assumes that
2176 this context is taken as read.

2177 4.1. Realism and ecotoxicological questions

2178 What we would ideally like to be able to do is to predict the exposure of individuals, their sensitivity,
2179 and the effect of exposure, and to predict correctly the impact on the population abundance and
2180 dispersion. This is complicated by the fact that complex system properties emerge owing to local
2181 space and time feedback mechanisms linking exposure, animal distribution and behaviour, and
2182 population responses,. In order to cope with this, a model should include the factors assumed to be
2183 important under conditions that might occur in the model's applicable domain. The resulting models
2184 should be able to reflect how the internal organization of populations change and thereby generate
2185 representations of the novel behaviour necessary for complex predictions (Topping *et al.* 2015a).

2186 For spatially structured, long-lived species with complex life cycles and behaviour such as we see in
2187 amphibians and reptiles there is a strong likelihood that simple assumptions about population
2188 dynamics and exposure will fail to predict effects accurately due to feedback between factors, e.g. if
2189 behaviour causes repeated lifetime exposure due to philopatry of a section of the population. The
2190 population will therefore probably need to be modelled as individuals because dispersal behaviour
2191 over long lifespans needs to be taken into account. In addition, the dynamic effects of stressors in
2192 space and time need to be modelled, applying a regulated stressor assuming year-on-year application
2193 according to detailed application schedules (including multiple applications).

2194 Simulating the population state realistically prior to addition of the stressor is also a necessity if
2195 impacts of the stressor are to be correctly determined (EFSA Scientific Committee 2016) and a
2196 systems approach is needed for this. Topping *et al.* (2015b) give an example of the use of a systems
2197 model in the context of environmental risk assessment; this spatially explicit, landscape model

2198 exemplifies the effects of pesticide application on both abundance and distribution of a non-target
2199 species, not only in fields treated with pesticide but in habitats in unsprayed areas ('action at a
2200 distance'). The model also demonstrated how effects might not be seen for 10 or 20 years, a feature
2201 that will not be found in simple demographic models.

2202 This 'systems' model trades off generality for realism, and is the basis of the population modelling
2203 approach proposed for amphibians and reptiles ERA. Taking this system view also negates the need to
2204 consider long-term recovery separately. Population growth rate (PGR) will become <1 if recovery does
2205 not occur with year-on-year application, and population decline towards extinction will occur. It is also
2206 important to note that it is necessary to include species-specific details in models of this type, e.g.
2207 stage-specific density-dependence, or behaviour that might change the vulnerability of the population.
2208 Hence, significant knowledge is required about the species before these models can be constructed.

2209 The proposed general risk-assessment framework (section 7) assumes that models of this type that
2210 could be used to assess risks can be built (or exist) for a set of 'model species'. An example of this
2211 approach is provided in section 7. Selection of species and the criteria used to select exposed
2212 vulnerable species are given in section 2.5.

2213

2214 **4.2. Benefits of population modelling exemplified using a model of** 2215 ***Triturus cristatus* (Great Crested Newt)**

2216 This is an illustrative section, demonstrating how detailed population modelling on landscape scales
2217 can be used to support the ERA for amphibians. It is based on an individual-based model of Great
2218 Crested Newt (Topping et al, in prep), under the ALMaSS system (Topping *et al.* 2003).

2219 There are three main ways in which population modelling can support amphibian and reptile ERA:

- 2220 • Setting Specific Protection Goals
- 2221 • Translation of toxicity data to population-modelling endpoints
- 2222 • As a higher tier assessment (refinement for population-level endpoints)

2223

2224 The model has been run using a rather limited and mixed selection of data inputs to illustrate these
2225 three points. This is deliberate and the results of the scenarios presented here are not indicative of
2226 the results of running a properly defined and agreed great crested newt scenario. For example, all
2227 scenarios were run assuming global optimal pond quality, meaning all ponds would be colonised,
2228 which is not the case in the real world. The important features of the model are described below,
2229 followed by example results and measurement endpoints that could be used to support ERA, including
2230 a section describing what needs to be taken into account in order to use a model like this in a realistic
2231 scenario.

2232 The Great Crested Newt was chosen since it is one of the six species identified for risk assessment
2233 modelling in section 2.5. This species can be categorized as a species that is highly exposed since it is
2234 typically breeding in or around agricultural situations. It is also a low mobility species with seasonal
2235 migration to and from breeding sites. It is sensitive to weather conditions and has density-dependent
2236 processes primarily acting at the larval stage. The other five species, which also need to be modelled,
2237 have differing profiles and would respond differently to PPP exposure.

2238

2239 **4.2.1. Model Overview**

2240 The model is an agent-based simulation model working at landscape scale (here taken as 10 x 10
2241 km). It represents individual newts as eggs, larvae, juveniles and adults. Eggs and larvae are aquatic
2242 in ponds, juveniles are terrestrial, whilst males and females are primarily terrestrial except for breeding
2243 periods. The model itself was written in C++ and is part of the ALMaSS simulation system. The code
2244 and model are documented using ODdox protocol (Topping, Hoyer & Olesen 2010), and the
2245 documentation is available at <https://almassdocs.au.dk/ALMaSSODdox/Newt/index.html>.

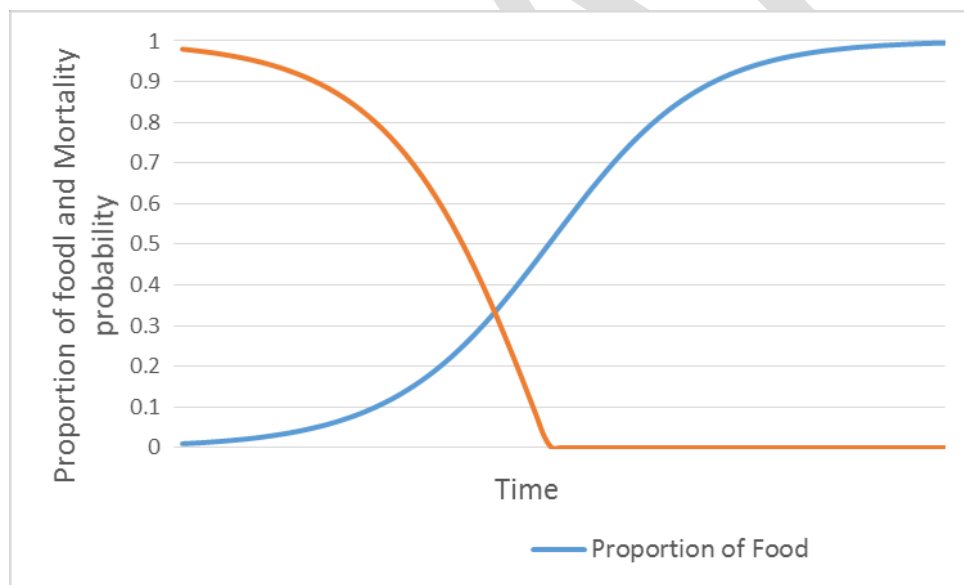
2246 Life-stage model overview

2247 Eggs

2248 The female lays eggs in small daily batches. Eggs develop following a day-degree model until
2249 hatching. Each day there is a fixed probability of death per egg (assumed to be predation and other
2250 causes not explicitly modelled). In addition, direct mortality can occur as the result of specific events
2251 such as acute toxicity of pesticides. In the case of pesticides these occur as a result of the eggs
2252 responding to the concentration of pesticide in the water according to predefined rules (e.g. die when
2253 the concentration exceeds a threshold).

2254 Larvae

2255 When the egg hatches it forms a larva. The larvae are assumed to require aquatic food for growth
2256 and if food is available will grow until metamorphosis into a juvenile. The amount of food needed per
2257 day is calculated as a function of body size (equating to age). Food is modelled using a simple logistic
2258 growth curve ($Food_{t+1} = Food_t + (Food_t * r * (1 - Food_t / K))$) with K and r being specified as input
2259 parameters. K is proportional to the area of pond. Food is removed by larval feeding and 'regrows'
2260 following this curve. There is a probability of daily mortality for unspecified causes similar to the egg,
2261 but in addition, there is a probability of dying when $Food$ from the logistic curve is $< 50\%$ of K . This
2262 probability is inversely proportional to the ratio of $Food/K$ when $Food/K < 0.5$ (Figure 7:). In order to
2263 prevent total elimination of larvae in a pond the food level was never allowed to drop below 1% of K .
2264 This method means that density-dependent larval mortality per individual starts when the food levels
2265 are $< 50\%$ of the maximum food and increases with decreasing food. This is a form of competition
2266 intermediate between complete scramble (where all individuals get resources until no resources are
2267 left) and contest competition where resources are shared unequally so that some individuals get all
2268 the resources they need. The form of competition between individuals can have a large effect on the
2269 population dynamic outcome (Smith and Sibly, 1985).



2270

2271 **Figure 7:** The logistic curve applied to available food for larvae and the related probability of
2272 individual larval mortality.

2273 External events and pesticide concentrations in water can also result in larval death or other
2274 responses, as for eggs.

2275 The larval stage is modelled as a fixed period, which triggers metamorphosis into a juvenile when
2276 reached.

2277 *Juveniles*

2278 The juvenile emerges from the pond and disperses into the surrounding area. It moves with a
2279 preference for cover habitats and wet areas but will otherwise randomly walk. This has the result that
2280 the highest density of newts is near to the pond with decreasing density with distance. The juvenile
2281 may encounter other ponds than the home pond as it moves around the landscape it and these are
2282 remembered.

2283 The juvenile newt can only move around when the humidity is high, which we assume is related to
2284 the rainfall and temperature of the preceding days. Roads have associated mortality risks during
2285 dispersal; these are flagged and probability tests taken to determine whether or not the juvenile dies.

2286 Apart from dispersal mortality, mortality is based on temperature and precipitation following equations
2287 proposed by Griffiths, Sewell and McCrea (2010). In addition, the newt respond to pesticides in the
2288 environment at its location, e.g. by dying or changing developmental time to adult.

2289 Maturation to adulthood occurs when the newt reaches a specified size. This is partly dependent on
2290 the temperature since the newt is assumed not to grow when too cold. On reaching adult size there is
2291 an immediate maturation to either a male or female with equal probability.

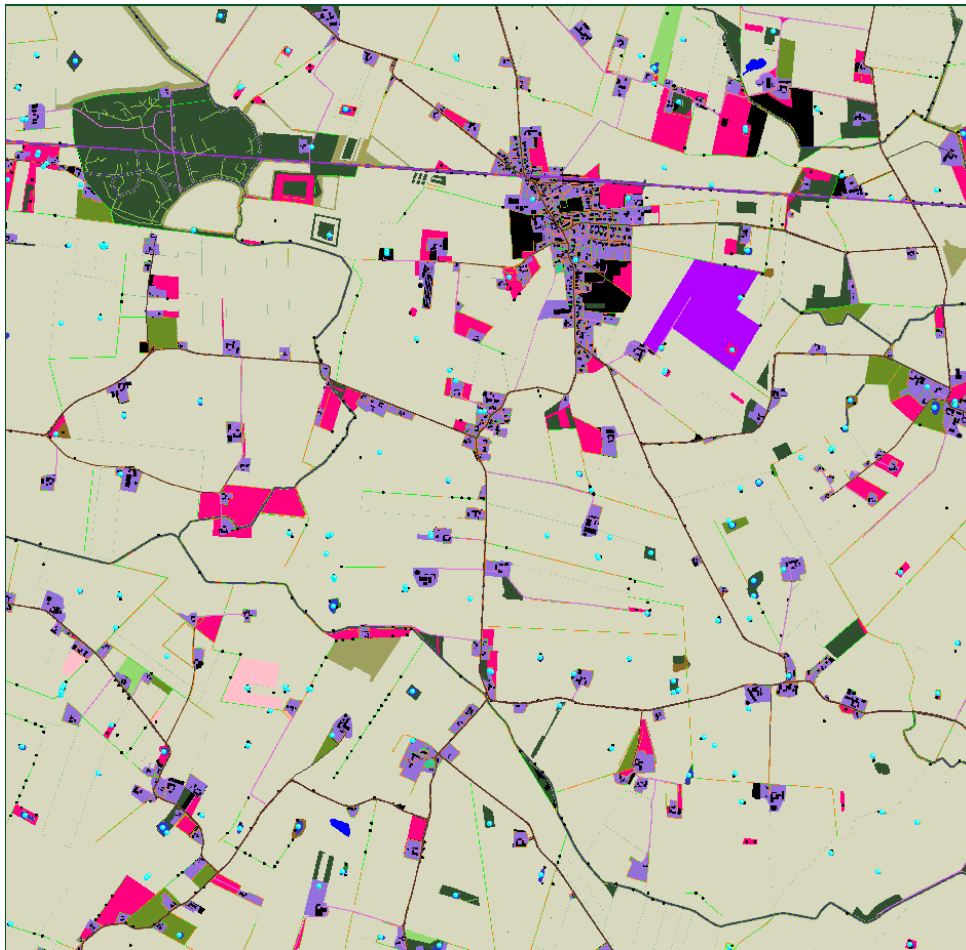
2292 *Adults*

2293 Adults behave in a similar way to juveniles but move relatively less. At the start of the breeding
2294 season, the adults will move in a directed manner towards the nearest pond that they have
2295 experienced. Once they reach the pond, assuming both sexes are present, the females lay a small
2296 number of eggs per day until either the breeding season ends, or the complement of eggs is laid.
2297 When the breeding season is over the newts leave the pond and behave the same way as the
2298 juveniles, except that movement distance is halved compared to juveniles, the majority of newts
2299 staying very close to the pond.

2300 Mortality factors are the same as for the juvenile newt.

2301 **Inputs (Landscapes, farming & pesticides)**

2302 Landscapes used for the model runs were created from combining GIS data with farm subsidy
2303 information for Denmark using the methods developed by Topping, Dalby and Skov (2016). These
2304 landscapes combine highly detailed landscape structure with accurate representation of farming in
2305 terms of crop husbandry and growth. A critical component of the landscape for newts is the pond,
2306 which is also available as a GIS layer in Denmark covering water bodies of 5m² or more. An example
2307 of the distribution of small ponds suitable for *T. cristatus* is shown in Figure 8: . This example shows a
2308 high density of ponds, but is not exceptional in a Danish landscape context.



2309

2310 **Figure 8:** A 5 x 5 km section of the Næstved landscape showing the location of the ponds as light-
 2311 blue dots. Rotational fields are shown as light-brown and it is clear that the vast majority of ponds
 2312 are in or adjacent to a field where pesticides could be used.

2313

2314 Farming was simulated based on the actual farming carried out in the landscape. Polygons
 2315 representing fields in the landscape map were linked with the farm that manages them and then
 2316 management typical of that farm type was applied. The management regime was based on data from
 2317 EU subsidy submission and information on the numbers and types of animals present. The result is a
 2318 very accurate and realistic representation of crop rotation and crop management into which pesticides
 2319 could be added.

2320

2321 Pesticides were simulated in two ways. The first way is as a generic pesticide (insecticide, fungicide or
 2322 herbicide), which had generic properties typical of its type. These pesticides were applied as part of
 2323 the normal crop management with application frequency and timing based on expert judgement by
 2324 agricultural consultants. These pesticides have no direct effect on the newt model. The second type of
 2325 pesticide applied is the focal pesticide for ERA, which is simulated in much more detail. Timing and
 2326 frequency of application were set explicitly for this pesticide. It was applied at a configurable
 2327 application rate to a field polygon, with drift into the surrounding area following a user-defined
 2328 diffusion curve. After application there was therefore a concentration of pesticide per 1m² wherever
 2329 this was sprayed. This concentration was subsequently degraded by removal of a fixed proportion of
 2330 the pesticide per day (expressed as a half-life). Subsequent application to the same area simply adds
 to the environmental concentration that then decayed.

2331

2332 ALMaSS can represent fate of pesticides in more detail and differentiates fate into vegetation and soil
 2333 compartments, although only overspray scenarios were used here as detailed fate was not required.
 The exposure of a newt here was taken to be to 100% of the application rate as overspray (i.e. only

2334 those newts in the field or region of drift were be affected on the day of spraying). The rate of
2335 application was fixed as 1X that required to elicit an LC_x response.

2336 All scenarios were run with weather input from central Jutland, Denmark for 1984-2014 unless noted
2337 otherwise.

2338 **4.3. Linking exposure and effects for long-term landscape-scale** 2339 **population RA**

2340 Linking exposure and effects in a systems model of the type proposed here is rather different from the
2341 traditional approach taken in linking exposure and effects in local short-term ERA. The systems model
2342 enables a dynamic linking of exposure in space and time with the movements of individual animals
2343 and behaviours. This means that there is no need to consider a statistical distribution of exposures to
2344 select, e.g. the 90th percentile; rather the whole distribution of exposures is simulated and the mean
2345 population effect is assessed over whatever spatio-temporal scales are required for the SPG. This,
2346 however, requires that environmental exposure is part of the dynamic simulation. This is easy to do
2347 for some exposure routes; in the examples below we have used overspray as being the easiest route
2348 to simulate. Other exposure route, however, need to be modelled explicitly within the simulation. This
2349 approach was suggested for non-target arthropods for the same considerations of spatio-temporal
2350 effects (EFSA PPR, 2015).

2351 Linking exposure and effects in this way has one critical advantage. It reduces uncertainty resulting
2352 from combining two independent distributions (exposure and effects). This may be particularly
2353 important if there is an interaction between drivers causing environmental exposure and individual
2354 exposure; this could be the case if timing of application coincides with or avoids migratory activity, or
2355 if animals are attracted or repelled by a crop that is treated with pesticide (e.g. due to structural cue
2356 coinciding with the growth stage for application). Integrated simulation also ensures that only
2357 exposure profiles that overlap with the distribution of animals in space and time are represented in
2358 the measure of impact. Thus profiles that rely on environmental factors (e.g. hydrology), that also
2359 determine the distribution of animals, are included only if they are relevant. Exclusion is automatic
2360 since the animals will not be exposed where they do not go (in the model).

2361 The model can use the existing models underlying current environmental exposure estimates. As
2362 noted above, the ALMaSS system used for simulating the Great Crested Newt includes drift,
2363 environmental decay and the facility to model pesticide movements between vegetation and soil
2364 compartments at 1-m resolution for the whole landscape. This is based on simple versions of the
2365 exposure models, for example for vegetation the areic mass of substance on the crop canopy,
2366 expressed as mass per unit area single sided leaf surface is used. The areic mass can be calculated
2367 from the nominal dosage using the fraction intercepted by the crop canopy. Normally, the fraction
2368 intercepted depends on the crop development stage and should be obtained from the improved
2369 FOCUS interception tables that were published by EFSA in 2014;
2370 <http://www.efsa.europa.eu/en/efsajournal/doc/3662.pdf>). Under dynamic simulation, however, the
2371 leaf area index, height and biomass of the crop are simulated daily, and are fed into the exposure
2372 calculations dynamically.

2373 **4.3.1. Individual toxicity**

2374 Effects on the individual are based on the assumption that a given toxicological endpoint is measured
2375 over a test with a time component. For example we may have an LC₈₀ measured over 7 days. The
2376 response to the pesticide is built into the model by assuming a threshold concentration above which
2377 there is a daily probability of mortality. This probability (p) is calculated from $(1-m) = (1-p)^d$, where m
2378 is the proportion assumed to die (e.g. 0.8 for 80% mortality over the test period of 7 days) and d is
2379 the number of days over which the test was carried out. If the newt finds itself in a 1m² grid cell with
2380 an environmental concentration above the trigger, then it is assumed to die with probability p .

2381 This approach is called the stochastic death model in GUTS TK/TD modelling (see Ashauer et al, 2015)
2382 and can be contrasted with the individual threshold approach, which sets an individual threshold
2383 above which death is certain. The implication of this choice is difficult to determine at the system
2384 level, but stochastic death has a larger probability of killing all exposed animals if multiple exposure
2385 occurs whereas at low exposure levels the individual threshold approach leads to higher effects. Both

2386 approaches also make the assumption that an individual that survives exposure does not have any
2387 subsequent change in sensitivity (e.g. if it was weakened by the first exposure it might be more
2388 sensitive to future exposure). There is no obvious reason to choose one or other approach; however,
2389 it is important to make an informed, transparent choice in each case.

2390 4.3.2. TK/TD modelling

2391 Dynamic linking of exposure and effects using simulations of the kind presented here also provide the
2392 opportunity to include further realism in the risk assessment by the inclusion of
2393 toxicokinetics/toxicodynamics (TK/TD) models. Toxicodynamics describes how a chemical affects the
2394 body, while toxicokinetics describes how the body affects a chemical upon exposure. The current newt
2395 model has a simple one compartment TK/TD model that keeps track of internal body-burden and
2396 elimination rates can be specified, exposure patterns in space and time being dynamically simulated
2397 as described above with a daily resolution. This is currently very simple and assumes a constant
2398 elimination proportion (the simplest TK model). A very useful advance would be to link the individual,
2399 temporal exposure profiles with the metabolism of the animals. This would incorporate an element of
2400 risk assessment considered to be specific and important for amphibians and reptiles, i.e. poikilothermy
2401 and its influence on pesticide effects. Inclusion of this linkage would change the rate at which
2402 pesticides were eliminated, dependent upon temperature-driven metabolism, improving the
2403 toxicokinetics. Naturally, further more detailed toxicodynamics would increase the potential realistically
2404 to combine different routes of exposure and even different toxic effects (e.g. direct effects on skin and
2405 subsequent effects on other organs in the body).

2406 Direct integration in the simulation would further reduce uncertainty by integrating exposure with
2407 ecology and behaviour with TK/TD to create population-level effects. Like integration of exposure and
2408 effects, the detailed patterns of individual exposure created by animals moving around in the
2409 simulated environment and being exposed would drive the TK/TD models directly, thus removing the
2410 need to provide statistically generated exposure profiles.

2411 There are no significant technical issues in developing TK/TD models and directly integrating them in
2412 simulations like the great crested newt model. There are currently, however, few data on toxicity for
2413 these groups (see chapter 10), and even less data to enable parameterisation of TK/TD models.
2414 TK/TD models are therefore not feasible currently without further data.

2415 4.4. Endpoints

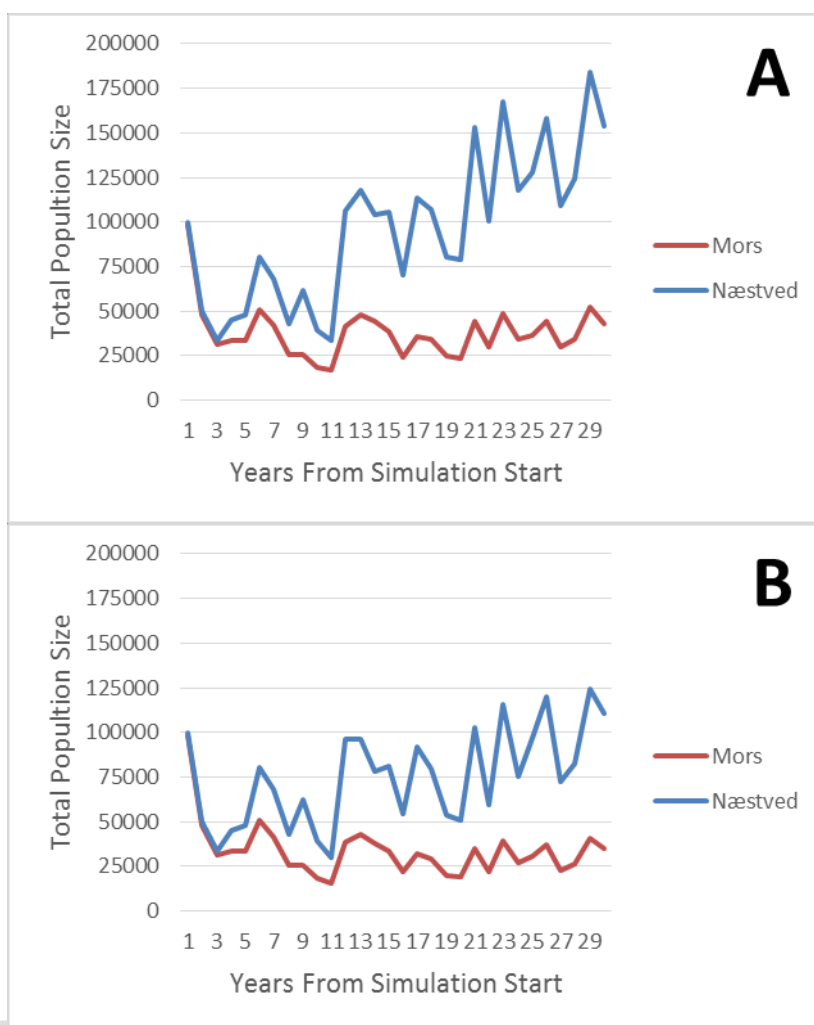
2416 **Impacts**

2417 Measuring population impact requires a no-pesticide situation against which to compare pesticidal
2418 effects. This is termed the baseline and represents a model identical in all respects to the scenario
2419 used to test a pesticide except that the particular pesticide under evaluation is not applied. A baseline
2420 is required for each scenario, and typically a range of baseline scenarios are used to represent the
2421 range of situations a population may be in (for example low resilience population in an intensive
2422 agricultural landscape, and a widespread resilient population in an extensive landscape). This range of
2423 scenarios is needed because it is difficult to identify in advance which baseline will react most strongly
2424 to the combination of pesticide and SPGs. The baseline population size will fluctuate in time, and this
2425 defines the normal operating range of the population for that scenario. To create the baseline the
2426 model needs to be carefully tested to determine whether it performs closely to the real world.
2427 Examples of this can be seen for partridges, hares, voles and skylarks (Topping *et al.* 2010; Topping,
2428 Høye & Olesen 2010; Topping, Dalkvist & Grimm 2012; Topping, Odderskaer & Kahlert 2013). The
2429 newt model used here has not as yet undergone this degree of testing. Although the same strategy of
2430 using pattern-oriented modelling to develop the model was used, the data available for the newt was
2431 not of the same quality or quantity as for the previous species, hence the model developed is simpler
2432 and uncertainty is relatively high.

2433 If the newt model represents the real world well, then we can assume that the population trajectory
2434 described by running the model with a current agricultural scenario represents the current state of the
2435 population. An example of single runs for the Næstved and Mors landscape is shown in Figure 9: .

2436

2437



2438

2439

2440 **Figure 9:** Total adult female population size for two simulation runs on two different landscapes
 2441 (Mors & Næstved). Both simulations were started with 100,000 individuals but clearly have very
 2442 different carrying capacities. A) Without pesticide (baseline); B) The same simulation but with
 2443 addition of a pesticide LC100 from year 11 onwards.

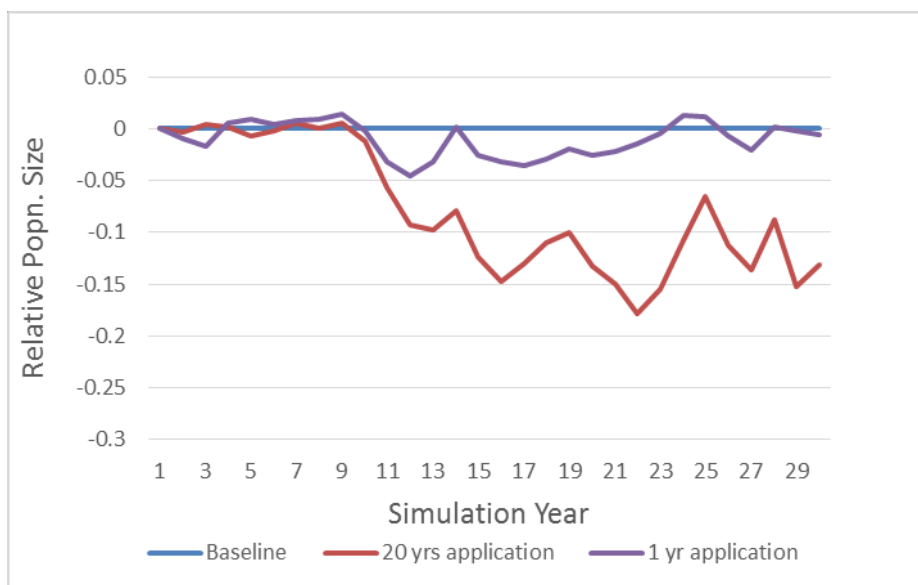
2444

2445 Adding the pesticide to the (otherwise unchanged) scenario alters the population curves (Figure 9: B).
 2446 The heights of the curves are different though the basic shapes are the same as the baseline; this is
 2447 because the other main drivers of population size (farming, landscape and weather) are identical
 2448 between scenarios. Comparison of the raw numbers between runs is, however, difficult and the
 2449 population size relative to the baseline is used to facilitate easy comparison (Dalkvist, Topping &
 2450 Forbes 2009). This allows comparison of the baseline and pesticide scenario directly (Figure 10:).

2451 **Table 9:** The timing of the day of application for each of three pesticide applications for the three
 2452 crops used in ERA scenarios. *OSR applications were dependent upon previous
 2453 applications, i.e. 7% is 30% of the 21% that applied the first application.

Crop	Application 1	Application 2	Application 3
Winter Wheat	50% on 15 th May	50% on 1 st June	50% on 14 th June
Spring Barley	35% on 15 th May	35% on 1 st June	35% on 14 th June
Oil Seed Rape*	21% on 15 th April	7% on 1 st May	5% on 15 th May

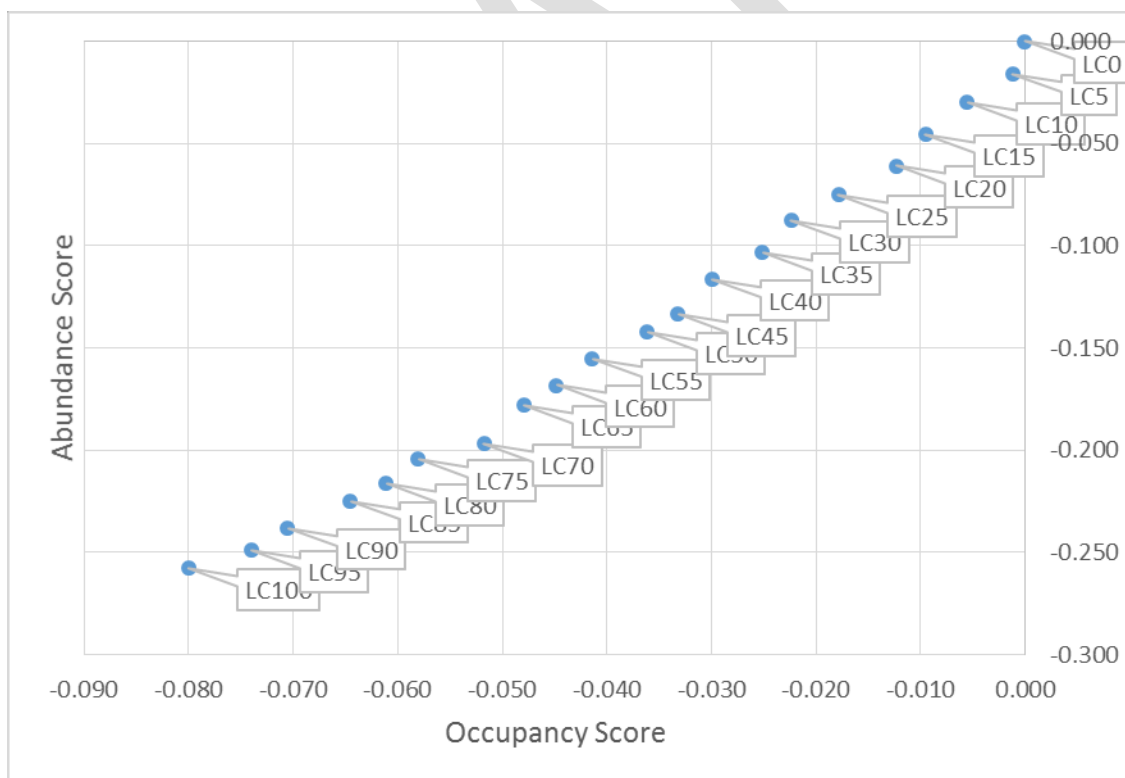
2454



2455

2456 **Figure 10:** LC100 overspray scenario on the Mors landscape assuming application to winter
2457 wheat, spring barley and oilseed rape following the spraying schedule described in Table 9: .

2458



2459

2460 **Figure 11:** Occupancy and abundance scores for LC_x overspray scenarios for the last decade of a
2461 20-year application on the Næstved landscape, assuming application to winter wheat, spring
2462 barley and oilseed rape following the spraying schedule described in Table 9: .

2463

2464 The overall population impact is one endpoint useful to compare changes in population size. Another
2465 endpoint can, however, be considered as introduced by EFSA PPR 2015, for non-target arthropods:
2466 this is the AOR index (Hoye, Skov & Topping 2012). The AOR index describes the change in
2467 abundance (population density where the population occurs) and occupancy (the relative proportion
2468 of the landscape occupied by the population). As with overall population impact, these measures are
2469 relative to a baseline. The AOR index can be plotted easily and provides information on both
2470 abundance and dispersion. Figure 11: shows an example of changes in occupancy and abundance for
2471 increasing LCx overspray scenarios in the Næstved landscape. These scenarios assume application of
2472 the pesticide to be evaluated to winter wheat, spring barley and oilseed rape grown to maturity at
2473 standard rates following the application schedule in Table 9: . The effect of increasing toxicity is
2474 clearly seen both in terms of changing newt population, abundance and newt distribution, which in
2475 this case shows a close to linear response.

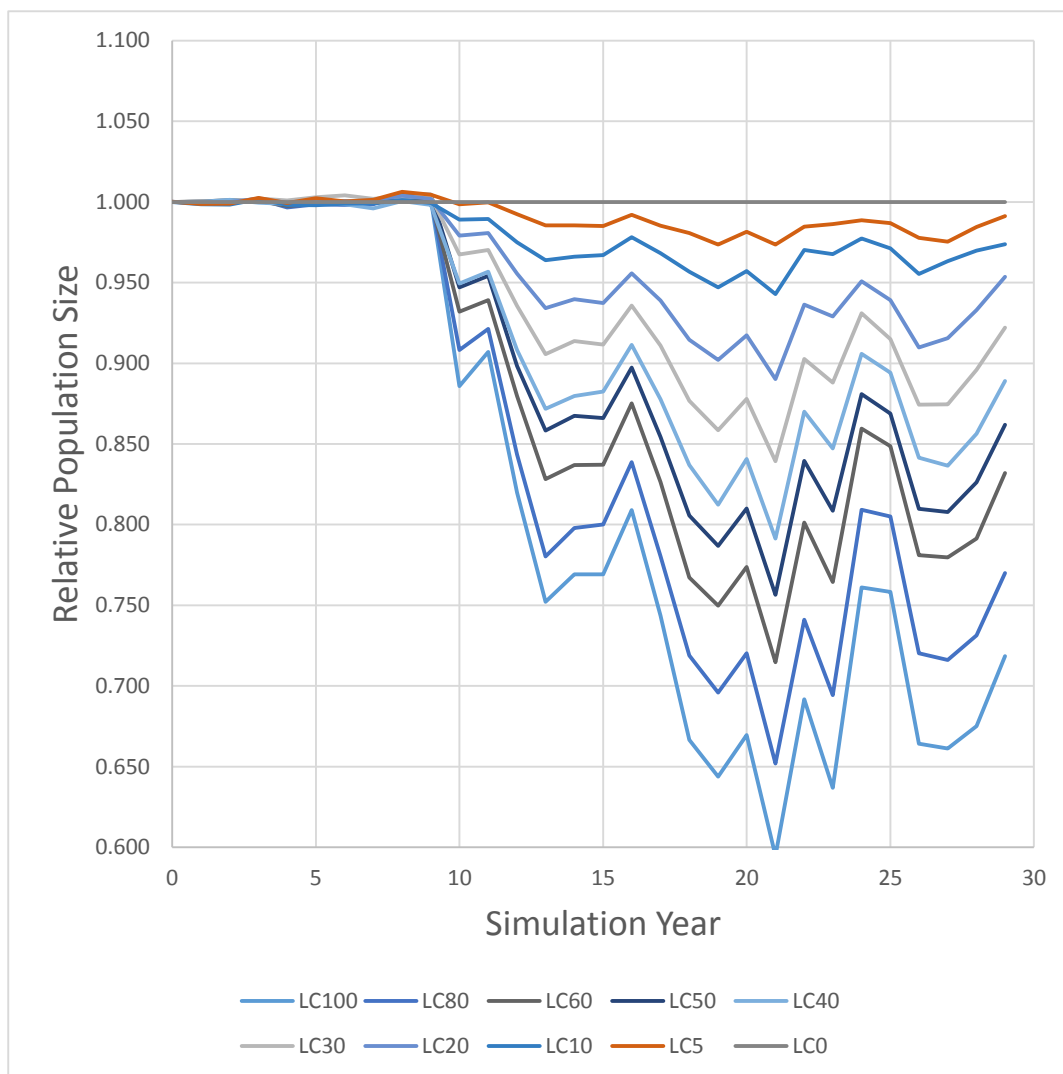
2476

2477 **Year-on-year effects**

2478 Impact can be measured following a single pulse or as the result of year on year application. These
2479 two situations are compared in 4006 for Næstved. In this case, a single year impact of the LC₁₀₀
2480 overspray scenario was a 9% reduction in population size but continuous use led to a 20% population
2481 decline, after 10 years.

2482 Note that, depending upon the size of acceptable impact, it may be difficult to identify a population
2483 response from a single year application. Figure 12: shows that for LC₅ it took three years before the
2484 population impact was observable in the model, but after that period the effect was clear, averaging
2485 2.5% and never returning to the baseline.

2486



2487

2488 **Figure 12:** Change in population size for LC_x overspray scenarios of a 20-year application on the
 2489 Næstved landscape, assuming application to winter wheat, spring barley and oilseed rape
 2490 following the spraying schedule described in Table 9:

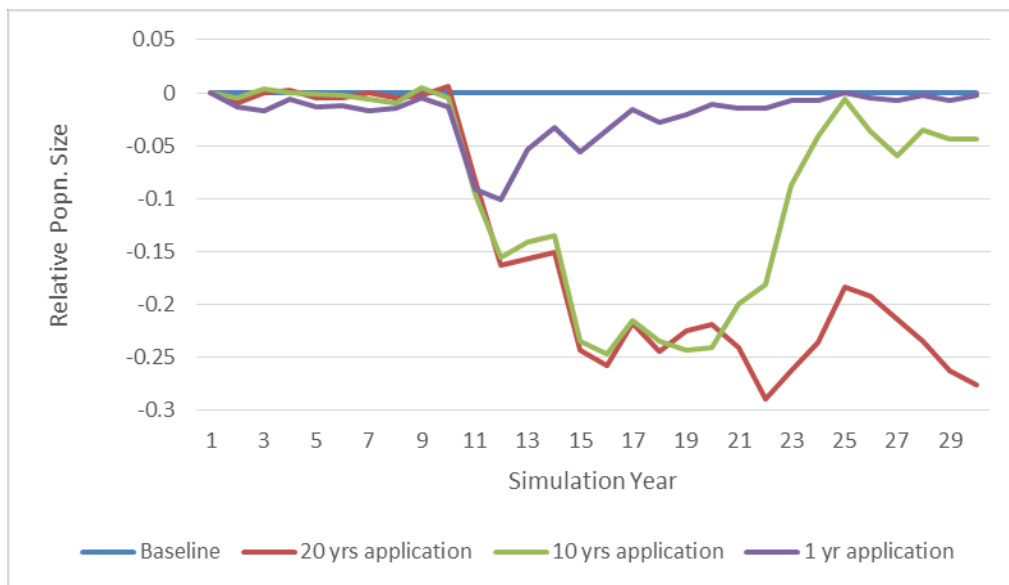
2491

2492 **Recovery**

2493 Recovery might not need to be considered if only negligible effects are permitted, depending on the
 2494 SPG considered. If we are then comparing annual population status as described above, then there is
 2495 no need to consider within season recovery since, if recovery does not occur, there will be population
 2496 impacts. If population impacts are allowed, however, recovery should be possible (unless PGR<1).

2497 Like impact, assessing recovery can be done by comparing changes relative to a baseline condition
 2498 (see Figure 13:). This example is based on a 100% mortality overspray scenario for the Næstved
 2499 landscape following application to all winter wheat, spring barley and winter oilseed rape fields
 2500 according to Table 9: . Each scenario has a 10-year non-application phase followed by a 1, 10 or 20-
 2501 year application of the pesticide during a total of 30 years of simulation. Note that recovery seems to
 2502 occur in the 10-year application scenario by year 25, but full recovery does not actually take place in
 2503 the 10 years following cessation of pesticide application. Even after one year's application, recovery
 2504 takes 15 years in this system. After 10 years of application and after 10 years recovery the population
 2505 is still at 95% of its original size.

2506



2507

2508 **Figure 13:** LC₁₀₀ overspray scenario 1-year, 10-year and 20-years application on the Næstved
 2509 landscape assuming application to winter wheat, spring barley and oilseed rape following the
 2510 spraying schedule described in Table 9: .

2511

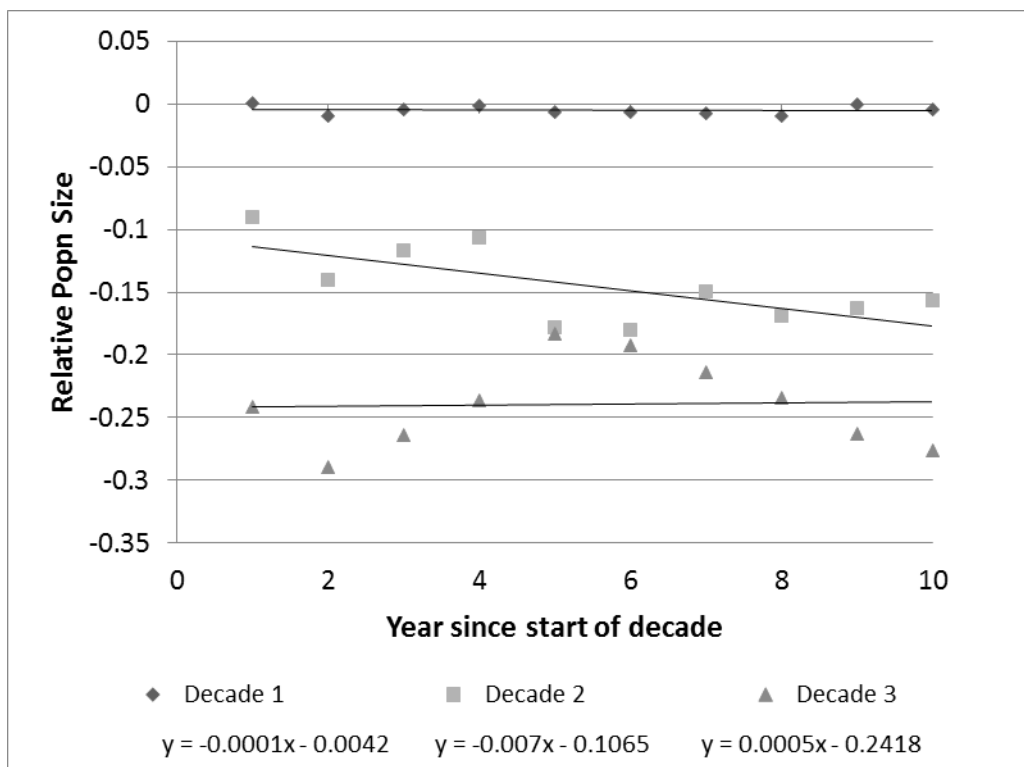
2512

2513 **Population growth rate (PGR) and relative PGR**

2514 As discussed earlier in this chapter and in Annex A, population growth rate is the single, most critical
 2515 metric for population status. If $PGR < 1$ the population will decline. A long-term decline that is a result
 2516 of the regulated stressor is not acceptable for a non-target reptile or amphibian population because
 2517 the SPG cannot be sustained over time.

2518 It may be, however, that $PGR < 1$ *before* the pesticide is applied. Risk managers need to consider how
 2519 to deal with this scenario, which may be a consequence of modern agricultural practice altering
 2520 landscapes or an indirect effect on e.g. prey. Another scenario is where $PGR < 1$ over a short time scale
 2521 but $PGR \geq 1$ over a longer time period. In order to help risk managers to deal with both these
 2522 scenarios, we suggest the use of a *relative* population growth rate in a similar way to measuring the
 2523 relative impact against the relevant baseline. Assuming we have an 'acceptable' population decline
 2524 after a period of pesticide application, what we are interested in knowing is whether the population
 2525 has stopped declining relative to the baseline under continued pesticide use. This is difficult to assess
 2526 by eye from a population-impact graph such as Figure 13: . However, regressing the change in
 2527 population size per year for fixed periods allows a statistical comparison. Figure 14: shows the three
 2528 10-year periods change in relative population size plotted against year from start of each 10-year
 2529 period. It is clear that the first 10 years of pesticide application (Decade 2) cause the decline, after
 2530 which the population seems to stabilize at a level about 75% less than its original size (Decade 1).

2531



2532

2533 **Figure 14:** Three 10-year time series created by splitting the result of the 30 year run with 20
 2534 years application from Figure 13: into three decades then fitting a linear regression of relative
 2535 population size against year within each decade. The equations shown for each decade indicate
 2536 the slope of the relative population growth rate for that decade.

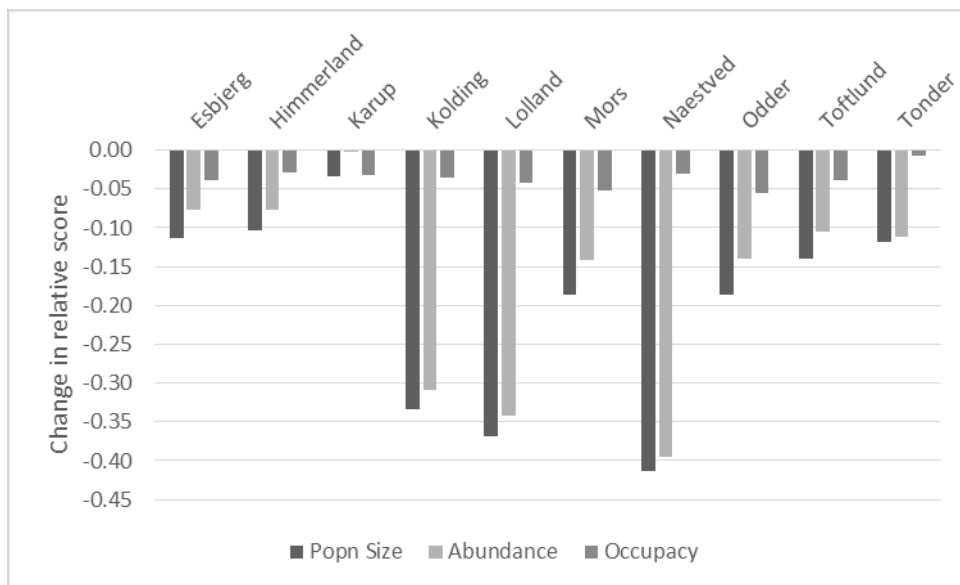
2537

2538 4.5. Translation of toxicity data to population endpoints

2539 The key advantage of population modelling is that it takes existing data as input (which can include
 2540 endpoints of lower tier testing), and translates this into the key, population-level endpoints of
 2541 distribution of animals in space and time and population persistence (i.e. abundance, occupancy and
 2542 population growth rate). Further laboratory tests are not required.

2543

2544



2545

2546 **Figure 15:** Change in relative population size, abundance and occupancy when running the LC₁₀₀
 2547 overspray scenario on 10 different Danish landscapes

2548

2549 To exemplify the way in which a lower-level screening test might be done we have used the newt
 2550 model on the 10 landscapes developed by Topping, Dalby & Skov (2016), assuming the LC₁₀₀
 2551 overspray scenario on winter wheat, spring barley and oil seed rape. Figure 15: shows the population
 2552 size, abundance and occupancy changes for all 10 landscapes. It is clear that the magnitude and
 2553 pattern of responses varies with the landscape context. In some landscapes abundance is very
 2554 sensitive in others occupancy responds most strongly (N.B. with -1 occupancy score, there must also
 2555 be a -1 abundance score and vice versa).

2556 Ideally, many more landscapes should be generated representing the range of landscape/farming
 2557 possibilities from the region being considered, which is now technically easy to achieve with data
 2558 available in Denmark (Topping, Dalby & Skov 2016), and also for the rest of the EU if data-
 2559 accessibility issues can be resolved. When more landscapes are available, cases applying a percentile
 2560 approach to the population-level endpoints would allow selection of one or more representative
 2561 landscapes as realistic worst-case scenarios. If we assume that for our case this landscape is the
 2562 Næstved landscape, then we can read off the impact of the pesticide based on its LC_x from Figure 11:
 2563 , and compare this to the SPG level of concern.

2564 This approach means that a year-on-year effect of pesticide use can be calculated using toxicity
 2565 endpoints, fate, and application schedules from existing testing,. In this case we used LC_x, but
 2566 precisely the same approach can be used for chronic endpoints if chronic toxicity endpoints are
 2567 incorporated into the models. Naturally, the results of different modes of action will differ; therefore,
 2568 the models need to be run for each relevant regulatory scenario.

2569

2570 4.6. Supporting SPG definition using modelling results

2571 One of the problems of landscape and population level ERA is that the SPGs require re-formulating.
 2572 Simulation of populations in space and time allows for an exploration of the population-level impacts
 2573 of stressors and therefore the range of responses to pesticide scenarios. Given this range of response,
 2574 the setting of protection goals can be achieved by considering the acceptability of impacts.

2575 Although the setting of population-level effects is a risk manager decision, it is useful if a science-
 2576 based approach can provide as much information as possible to aid the decision.

2577 Before considering SPGs the relevance of the impacts measured should be determined. In the
2578 Næstved landscape simulations, we saw occupancy changes of up to less than 10% but this should be
2579 considered against the background of what might be a maximum realistic reduction in this landscape.
2580 To demonstrate an extreme overspray situation, the Næstved landscape was also run with all crops
2581 receiving three applications (including farmed grasslands) and LC₁₀₀. This gave an occupancy
2582 reduction of 28% and at the same time a 77% abundance with an overall population size reduction of
2583 83%. At this point, the population was stable, but restricted to breeding in non-agricultural habitats,
2584 having been eradicated as a breeding population in agricultural areas. Some individuals were present
2585 in the agricultural areas each year, but these came from non-agricultural parts of the landscape as
2586 emigrants (action at a distance). This scenario therefore represents the maximum level of impact that
2587 application of overspray to agricultural areas could achieve.

2588 Having scaled the impact, combination of the following endpoints can be used to the thresholds for
2589 the SPG :

2590 1. **Impact on density and distribution.** In our standard scenario assuming LC80, there was a
2591 reduction in occupancy of 6%, which is 6/28 or 21% of the maximal impact. In the rest of the
2592 landscape, the population was still extant, but at mean densities that were 20% lower.

2593 2. **The long-term population impact.** In this case, relative PGR was stable after 10-years of
2594 application, which would not be considered to present a risk of long-term total landscape
2595 extinction (although this might not be the case for other landscapes or model settings).

2596 3. **The potential for recovery at the population level.** Using these results as an example, we
2597 might consider that population impacts could be tolerated if recovery at the population level
2598 is fast. If, however, indications are that recovery after impact is very slow, only negligible
2599 impacts would be likely to be acceptable (as in this example, Figure 13).

2600 In order to define the SPG, these three endpoints need to be combined and translated to impacts on
2601 ecosystem services for the range of toxicities and modes of actions possible, and then tabulated.
2602 These impacts, e.g. a 20% reduction in occupancy and 21% abundance that is long-term stable but
2603 lasts 15 years after cessation of application, need to be translated to the effects the impacts will have
2604 on the services supported by the SPGs (although this is challenging). If an unacceptable level of
2605 impact on service can be identified, the corresponding level of toxicity can be determined from such a
2606 table of combined effects (see section 7.10).

2607

2608 4.7. Refinement of model inputs

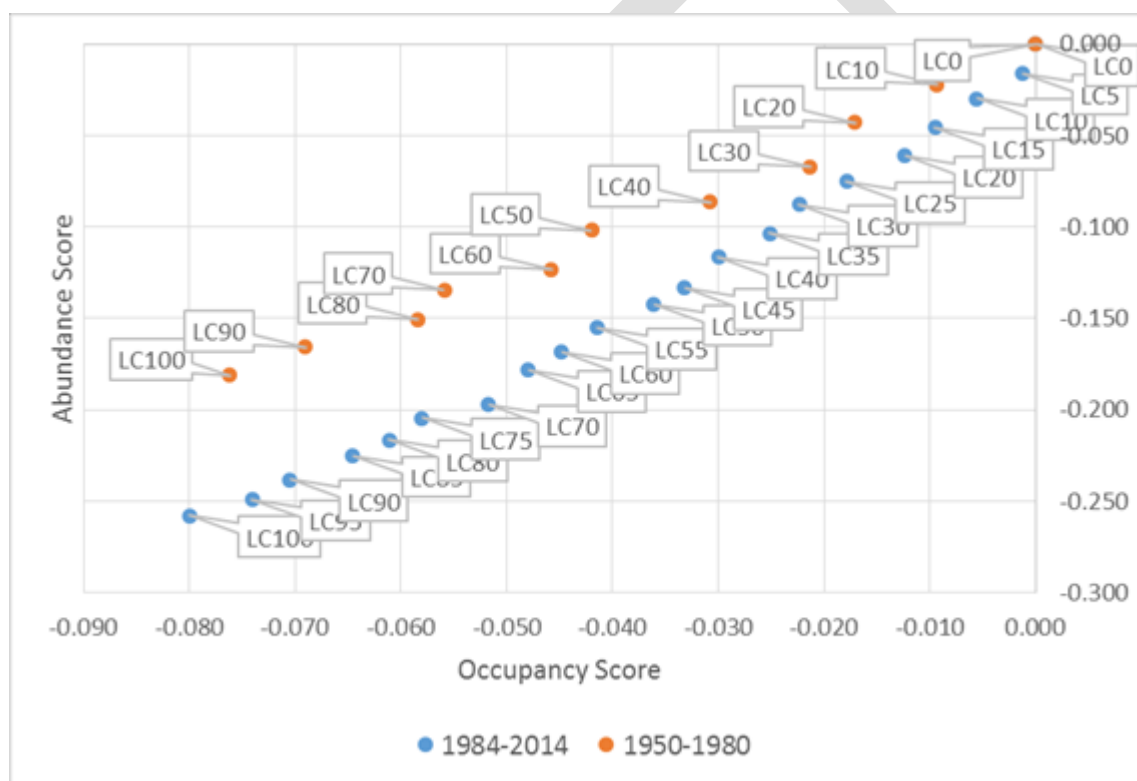
2609 The assumptions in the scenarios above include the typically used proportion of time spent in crop
2610 (PT), i.e. the model calculates the likelihood of a newt being in the crop during application as part of
2611 the normal calculation integrating behaviour and development of the newts. Consequently, refinement
2612 of the species model itself is not desirable, but refinement of exposure can form a part of a higher tier
2613 assessment for the modelling endpoints. Note, however, that in the scenarios used here, exposure
2614 was based on the newt being exposed to the full field rate as overspray, but in these scenarios there
2615 was no toxicity to any other stage, and exposure to environmental residues after spraying and in
2616 ponds was considered to be zero (see below for more realistic assumptions). Whilst refinement of
2617 individual exposures is possible, *for population-level effects it is critical that all stages and exposure*
2618 *routes are taken into account concurrently if realistic effects are to be predicted.*

2619 One key aspect of the scenarios that needs to be considered for long-term population models is the
2620 weather input. The newt model is highly sensitive to weather data. Figure 16: shows the data from
2621 the LCx experiment but with additional points created by running LCx scenarios under weather from
2622 1950-1980 instead of the last 30 years' weather. The weather clearly has a large impact on the
2623 vulnerability of the population to pesticide effects, therefore a careful consideration of weather in
2624 needed in any regulatory scenario.

2625 These new model scenarios did not utilize all possible features of the ALMaSS simulation system. In
2626 particular, assumptions about timing and frequency of application, subsequent exposure and
2627 environmental fate of pesticide use worst-case settings. These assumptions could be refined in higher
2628 tier assessments and this could be achieved in the current model for the terrestrial stages; however,
2629 realistic estimates of pesticide concentration in pond water are not part of the ALMaSS framework and
2630 would need to be developed for pond-living species before this type of model could be used.

2631 It is important to note that population-modelling endpoints should be kept separate from other
2632 endpoints because confounding of the population-modelling results can occur. For example, the LC5
2633 year on year results showed that it is possible to obtain no observable effect (e.g. in a 1-year field
2634 test) but still have a population-level effect after year-on-year use. Figure 17: shows an example
2635 where LC5 produces no observable population impact for three years after application but
2636 subsequently there is a clear impact. These effects would be extremely challenging to observe in the
2637 field due to limits of detection. Refinement of the population model with field testing is therefore not
2638 possible. Similarly, since the model explicitly incorporates the behaviour that changes the time spent
2639 by the newts in crop (often referred to as PT), refinement of toxicity data using PT is also not possible
2640 because it would result in double refinement. Hence, the criss-cross model (section 7.3) cannot be
2641 applied to population-modelling endpoints.

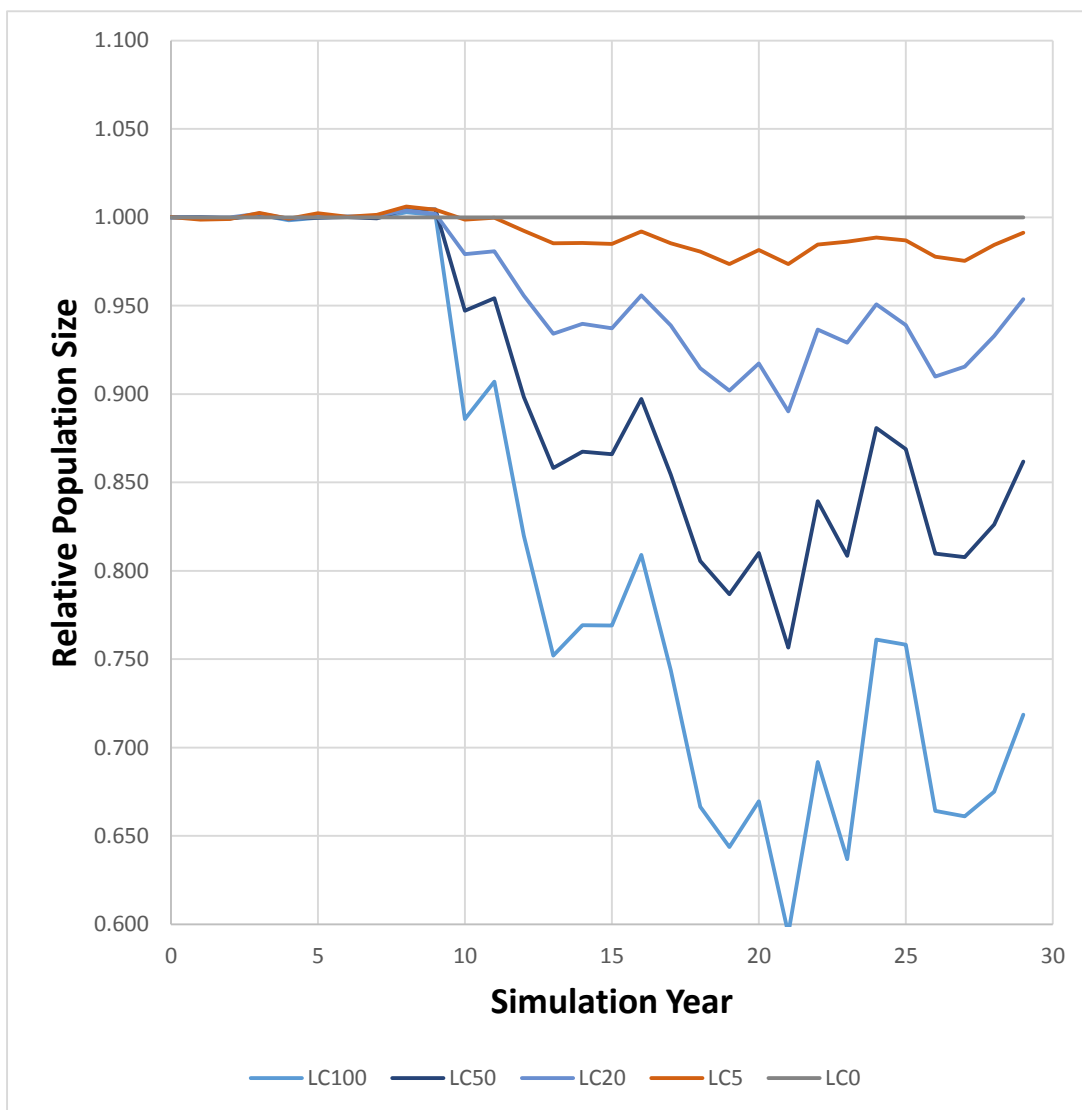
2642



2643

2644 **Figure 16:** AOR scores for the newt overspray scenario using application to wheat, barley and
2645 oilseed rape but different assumptions of overspray LCx under two weather regimes.

2646



2647

2648 **Figure 17:** Time series for four relative population sizes resulting from LCx/LC0 with pesticide
 2649 application starting in year 10.

2650

2651 **4.8. Developing realistic scenarios**

2652 The scenarios and model used here are for illustrative purposes only; no numbers or results should be
 2653 taken as indicative of future trigger values or modelling approaches, or indeed parameter values since
 2654 many were chosen arbitrarily for this exercise. A number of steps and improvements to the model and
 2655 scenario are needed before the model could be used in practice. These are primarily the following:

- 2656
1. More detailed testing and evaluation of the newt model is needed.
 - 2657 2. Pond quality is based entirely on area and should be varied to represent variability of
 2658 resources for larvae, and therefore changing spatial density-dependence patterns.
 - 2659 3. Pesticide-exposure module needs to be extended to include realistic or agreed methods to
 2660 implement pesticide concentrations in ponds.

- 2661 4. Exposure routes linking environmental concentration to the body-burden of pesticide in the
2662 newt need to be defined and included.
- 2663 5. The result of exposure needs to be carefully considered since exposure may be a daily or
2664 regular occurrence in the lifetime of a newt and the combining of probabilities
2665 mathematically may over- or under-estimate effects.
- 2666 6. Landscape, weather and farming scenarios need to be considered carefully to be
2667 representative of the region under consideration.
- 2668 7. Ideally, scenario and model development should be part of an iterative model cycle, fed by
2669 data from the real world, e.g. via monitoring.

2670

2671 **4.9. Conclusions and recommendations**

2672 **4.9.1. Conclusions**

2673 Population structure and spatio-temporal dynamics can have important implications for the evaluation
2674 of impacts of pesticide on amphibian and reptile populations. A systems approach is therefore
2675 recommended, for inclusion of both spatial and temporal implications of pesticide usage and to take
2676 the ecological state of the population before application of pesticides into account, (EFSA SCER,
2677 2016).

2678 Spatially explicit, individual-based modelling at landscape scales is an important part of the ERA
2679 toolbox for amphibians and reptiles. It should be used to help set the tolerable magnitude of effects
2680 for Specific Protection Goals (SPG), to translate toxicity data into population-modelling endpoints, and
2681 as a higher-tier assessment tool.

2682 Precise context for application of the models requires careful consideration. The regulatory scenarios
2683 need to consider all factors; in particular, landscape structure and weather have a large impact on the
2684 outcome of the long-term risk assessment.

2685 The threshold limits of changes to population-level endpoints that correspond to unacceptable impacts
2686 on the SPG need to be identified. This should not be done on an individual endpoint basis but
2687 combining abundance, occupancy and changes in growth rate.

2688 First indications from model development are that recovery in terms of landscape occupancy may be
2689 very slow for the Great Crested Newt, and this is probably true for other species with similar limited
2690 dispersal ability.

2691 **4.9.2. Recommendations**

2692 The suggested risk-assessment scheme (Chapter 7) requires the risks of intended uses of pesticides to
2693 be assessed at landscape scale. In order to obviate the need for direct running of complex models at
2694 lower tiers, it is recommended that these scenarios are developed as a set of pre-run simulations and
2695 made available with an interface for input of standard toxicity and usage data to provide a quick look-
2696 up function for lower tiers.

2697 Landscape-scale, spatially-explicit mechanistic models need to be developed and tested for the six
2698 focal species suggested for the assessment of amphibians and reptiles. These should include:

- 2699 • Mechanistic modelling of dispersal, reproduction and mortality factors for all life-stages;
- 2700 • The potential to introduce a wide range of impacts of PPP in terms of modes of action,
2701 exposure and regulatory scenarios;
- 2702 • Spatial and temporal representation of resource distributions;

- 2703
- 2704
- 2705
- 2706
- 2707
- 2708
- 2709
- 2710
- 2711
- Realistic pesticide-exposure modules including realistic or agreed methods to implement pesticide concentrations in ponds;
 - Exposure routes linking environmental concentration to the body burden of pesticides need to be defined and included, as well as inclusion of a suitable representation of multiple exposure events for an individual;
 - Population-modelling endpoints should include the abundance of the animals, their distribution and relative change in population growth rate as a result of application of the PPP. The latter takes into account long-term impacts, which can be difficult or impossible to see using other approaches.

2712 It is recommended that the use of TK/TD models be considered to represent better the exposure of
2713 amphibians and reptiles when the terrestrial stages move in and out of contaminated areas, as well as
2714 being exposed to multiple applications. Detailed, temporal exposure profiles suitable for TK/TD
2715 modelling can be generated from the individual-based population modelling and incorporated directly
2716 into the landscape-scale models. Collection of data to drive TK/TD modelling must be prioritised as
2717 part of future research.

2718

2719

DRAFT

2720 5. Defining specific protection goals for amphibians and reptiles

2721 5.1. General considerations

2722 Regulation (EC) No 1107/2009 defines General Protection Goals that aim at protecting *inter alia* non-
2723 target organisms, biodiversity and ecosystems. It is thus necessary to define Specific Protection Goals
2724 (SPGs) aiming at implementing this general protection into explicit and viable mandates for risk
2725 assessors, who need to know what to protect, where to protect it and over what time period. Final
2726 decisions on the choice of specific protection goals need to be made in consultation with risk
2727 managers.

2728 The role of EFSA's risk assessment is to propose possible SPG Options based on environmental and
2729 ecological criteria (and related exposure assessment goals), acknowledging existing general protection
2730 goals described in the relevant EU Regulation or Directive and regulatory data requirements. These
2731 SPG options, as well as a description of the possible environmental consequences of each option,
2732 should be proposed and discussed with risk managers. Risk managers should select SPG options, or to
2733 amend SPGs proposed by risk assessors. Agreed SPG Options should form the basis of Environmental
2734 Risk Assessment (ERA) decision schemes, which will be included in subsequent guidance documents.
2735 The choice by risk managers belonging to the European Commission (DG SANTE) and EU Member
2736 States is based on a cost-benefit evaluation - also considering economical and political criteria and
2737 acknowledging consequences for human wellbeing (health and economic benefits) and environmental
2738 costs.

2739 For the purpose of this Opinion on Amphibians and reptiles, the Panel considers it appropriate to
2740 examine which protection goals are already in place regarding the risk of aquatic and terrestrial
2741 vertebrates exposed to intended uses of plant protection products (PPP). Additionally, the procedure
2742 to define specific protection goals as developed by EFSA in consultation with stakeholders (EFSA PPR
2743 Panel, 2010a) and in the Guidance of the EFSA Scientific Committee (EFSA SC, 2016a) is followed.
2744 Understanding the contribution of amphibians and reptiles to ecosystems can help risk managers in
2745 the decision on which SPG option to choose and how to prioritize protection measures. Moreover, it
2746 enables the understanding of interactions and ultimately the prediction of biotic and abiotic changes
2747 associated with the potential loss of species (Hocking and Babbitt, 2014; Sekercioglu et al., 2004).

2748 The aim is to propose SPG Options that

- 2749 I. take current protection goals for non-target vertebrates into account,
- 2750 II. refer to the ecosystem services that amphibians and reptiles provide for mankind, and
- 2751 III. consider especially the endangered status of several amphibians and reptiles species in
2752 European agricultural landscapes

2753 5.2. Legislative framework in place

2754 The Legislative framework currently in place addressing the risk for amphibians and reptiles exposed
2755 to PPPs is reviewed and given below.

2756 The **general protection goals** for non-target organisms exposed to intended uses of PPP are
2757 outlined in the EU Regulation No 1107/2009. The regulation states in Article 4 on the approval criteria
2758 for active substances the following:

2759 *(e) it shall have no unacceptable effects on the environment, having particular regard to the following
2760 considerations where the scientific methods accepted by the Authority to assess such effects are
2761 available:*

2762 *(i) its fate and distribution in the environment, particularly contamination of surface waters,
2763 including estuarine and coastal waters, groundwater, air and soil taking into account locations
2764 distant from its use following long-range environmental transportation;*

2765 *(ii) its impact on non-target species, including on the ongoing behaviour of those species;*

2766 *(iii) its impact on biodiversity and the ecosystem.*

2767

2768 Regarding the **requirements for data** that are to be provided for the assessment of the effect of
2769 active substances and PPP on non-target organisms, Directive No 283/2013 demands i.a., that "*the*
2770 *information on the active substance, taken together with the information concerning one or more*
2771 *plant protection products containing the active substance [...] shall be sufficient to: [...]*

2772 *(d) permit an assessment of the impact on non-target species (flora and fauna), including the*
2773 *impact on their behaviour, which are likely to be exposed to the active substance, its*
2774 *metabolites, breakdown and reaction products, where they are of toxicological or*
2775 *environmental significance. Impact can result from single, prolonged or repeated exposure*
2776 *and can be direct or indirect, reversible or irreversible;*

2777 *(e) evaluate the impact on biodiversity and the ecosystem;*

2778 *(f) identify non-target species and populations for which hazards arise because of potential*
2779 *exposure;*

2780 *(g) permit an evaluation of short and long-term risks for non-target species, populations,*
2781 *communities and processes;*

2782 In submitted ecotoxicological studies (section 8) "*The potential impact of the active substance on*
2783 *biodiversity and the ecosystem, including potential indirect effects via alteration of the food web, shall*
2784 *be considered.*"

2785 While it is stated that effects on terrestrial vertebrates other than birds "*[...] shall be derived from the*
2786 *mammalian toxicological assessment based on the studies referred to in Section 5 (Toxicology)*", a
2787 separate section (8.1.4) addresses "*Terrestrial vertebrate wildlife (birds, mammals, reptiles and*
2788 *amphibians*". Here:

2789 *Available and relevant data, including data from the open literature for the active substance of*
2790 *concern, regarding the potential effects to birds, mammals, reptiles and amphibians (see point*
2791 *8.2.3) shall be presented and taken into account in the risk assessment*

2792 The mentioned section 8.2.3 addresses the question whether "*[...] the active substance is a potential*
2793 *endocrine disruptor in aquatic non- target organisms according to Union or internationally agreed*
2794 *guidelines. [...].*"

2795 In addition to the above, Regulation 284/2013 specifies the data requirement for plant protection
2796 products. For effects of PPP on "*Other terrestrial vertebrate wildlife (reptiles and amphibians)*" it is
2797 stated that "*where it cannot be predicted from the active substance data and, if relevant, the risk to*
2798 *amphibians and reptiles from plant protection products shall be addressed*".

2799

2800 The EU Regulation No 546/2011 implements EU Regulation No 1107/2009 as regards to the **uniform**
2801 **principles for evaluation and authorisation** of plant protection products. The evaluation of PPP
2802 impact on non-target species is presented in section B. 2.5.2. It should be noted that amphibians and
2803 reptiles can be subsumed in 'other terrestrial vertebrates'.

2804 *When calculating toxicity/exposure ratios Member States shall take into consideration toxicity to the*
2805 *most sensitive relevant organism used in the tests.*

2806 *2.5.2.1. Member States shall evaluate the possibility of exposure of birds and other terrestrial*
2807 *vertebrates to the plant protection product under the proposed conditions of use; if this possibility*
2808 *exists they shall evaluate the extent of the short-term and long-term risk to be expected for these*
2809 *organisms, including their reproduction, after use of the plant protection product in accordance with*
2810 *the proposed conditions of use.*

2811 *(a) This evaluation shall take into consideration the following information:*

2812 *(i) the specific information relating to toxicological studies on mammals and to the effects on*
2813 *birds and other non-target terrestrial vertebrates, including effects on reproduction, and other*
2814 *relevant information concerning the active substance as provided for in the Annex to*
2815 *Regulation (EU) No 544/2011 and the results of the evaluation thereof;*

2816 (ii) all relevant information on the plant protection product as provided for in the Annex to
2817 Regulation (EU) No 545/2011, including the information on effects on birds and other non-
2818 target terrestrial vertebrates;

2819 (iii) where relevant, other authorised uses of plant protection products in the area of
2820 envisaged use containing the same active substance or which give rise to the same residues.

2821 (b) This evaluation shall include:

2822 (i) the fate and distribution, including persistence and bioconcentration, of the active
2823 substance and of relevant metabolites, breakdown and reaction products in the various parts
2824 of the environment after application of the plant protection product;

2825 (ii) the estimated exposure of the species likely to be exposed at the time of application or
2826 during the period that residues are present, taking into account all relevant routes of
2827 exposure such as ingestion of the formulated product or treated food, predation on
2828 invertebrates, feeding on vertebrate prey, contact by overspraying or with treated vegetation;

2829 (iii) a calculation of the acute, short-term and, where necessary, long-term toxicity/exposure
2830 ratio. The toxicity/exposure ratios are defined as respectively the quotient of LD50, LC50 or
2831 non-observable effects of concentration (NOEC) expressed on an active substance basis and
2832 the estimated exposure expressed in mg/kg body weight.

2833 Regarding the decision making (section C), the Regulation states that, for non-target species:

2834 2.5.2.1. Where there is a possibility of birds and other non-target terrestrial vertebrates being
2835 exposed, no authorisation shall be granted if:

— the acute and short-term toxicity/exposure ratio for birds and other non-target terrestrial
vertebrates is less than 10 on the basis of LD₅₀ or the long-term toxicity/exposure ratio is less than
5 [...]

2836 unless it is clearly established through an appropriate risk assessment that under field conditions no
2837 unacceptable impact occurs after use of the plant protection product in accordance with the proposed
2838 conditions of use.

2839

2840 Also relevant for amphibians is the indication for decision making regarding aquatic life stages:

2841 2.5.2.2. Where there is a possibility of aquatic organisms being exposed, no authorisation shall be
2842 granted if:

— the toxicity/exposure ratio for fish and Daphnia is less than 100 for acute exposure and less than
10 for long-term exposure, or

— the algal growth inhibition/exposure ratio is less than 10, [...]

2843 unless it is clearly established through an appropriate risk assessment that under field conditions no
2844 unacceptable impact on the viability of exposed species (predators) occurs — directly or indirectly —
2845 after use of the plant protection product in accordance with the proposed conditions of use.”

2846 The so-called ‘unless clause’ gives the opportunity for a refinement of a risk that has been identified
2847 at lower tier assessment steps. Interestingly, for the non-target terrestrial vertebrates, no restriction is
2848 made on the species at risks that need to be addressed. The consideration of the risk for aquatic
2849 organisms points especially to ‘predators’.

2850 5.3. Defining SPGs according to the ecosystem service concept

2851 In the relevant PPR Panel Opinion (EFSA PPR Panel, 2010a) and the Guidance of the Scientific
2852 Committee (EFSA SC, 2016a), several steps are proposed in order to identify and to justify specific
2853 protection goals for aquatic and terrestrial organisms that may be affected as non-target organisms by
2854 use of PPPs. These steps are needed in order to “delineate the environmental components to protect,
2855 the maximum impacts that can be predicted and, in the case of regulated products, tolerated, over

2856 what time period and where.” (EFSA SC, 2016a). The Guidance Document on protection goals (EFSA
2857 SC, 2016a) aims at harmonizing the approach to define SPGs across the different areas of EFSA’s
2858 responsibility.

2859 The approach follows three sequential steps: (1) the identification of relevant ecosystem services; (2)
2860 the identification of service providing units (SPUs) that support relevant ecosystem services and (3)
2861 the specification of the level/parameters of protection of the SPUs, using interrelated dimensions.

2862 This last step involves the specification of the ecological entity and attribute to protect and the
2863 magnitude, temporal scale and spatial scale of the biologically relevant effects for all potential
2864 stressors followed by the definition of what is tolerable after intended uses of PPP.

2865 Specific Protection Goal (SPG) options have to be proposed for each combination of SPU and
2866 ecosystem service.

2867 **5.3.1. Ecosystem services driven by amphibians and reptiles in agricultural** 2868 **landscapes**

2869 The first step in the definition of SPGs is the identification of ecosystem services that are considered
2870 important and are provided by agricultural ecosystems. By means of describing services that mankind
2871 receives from ecosystem performance, the value of abstract ecological entities and processes become
2872 more explicit. Several classification schemes for ecosystem services have been proposed, e.g. MEA,
2873 2005; CICES (<http://cices.eu/>) and TEEB (<http://www.teebweb.org/>). In this Opinion, in accordance
2874 with other Opinions and Guidance of EFSA on the topic (EFSA PPR Panel, 2010a; EFSA Scientific
2875 Committee, 2016a) a list of ecosystem services based on the MEA source has been used since it is
2876 widely recognised and adopted. The Millennium Ecosystem Assessment (MEA, 2005) noted, however,
2877 that ‘modifications of ecosystems to enhance one service generally have come at a cost to other
2878 services due to trade-offs.’ The impacts of these trade-offs should be clearly described also for
2879 ecosystem services in agricultural landscapes, so that risk managers can decide whether and to what
2880 extent costs of trade-offs should be tolerated. In this respect, MEA (2005) claims that ‘many of the
2881 costs of changes in biodiversity have historically not been factored into decision-making’.

2882 Based on the assessment of existing knowledge and published reviews (e.g. Hocking and Babbit
2883 2014a and 2014b; Valencia-Aguilar et al. 2013), nine ecosystem services were identified as being
2884 driven by amphibians and reptiles in the agricultural landscape. These services (and their classification
2885 in brackets) are:

- 2886 a. **Genetic resources, biodiversity (provisioning and supporting).** Amphibians and reptiles
2887 contribute highly to the biodiversity of agricultural landscapes. Several species with (part) of
2888 their habitat in agricultural landscapes have been classified as being endangered in Europe and/
2889 or are protected by law.
- 2890 b. **Education and inspiration, aesthetic values and cultural diversity (provisioning).**
2891 Amphibians and reptiles species are highly valued in human culture. Their aesthetic value is
2892 widely acknowledged and they are used as strong symbols in visual arts and literature.
- 2893 c. **Pharmaceutical resources (provisioning).** Amphibians and reptiles species provide
2894 compounds with specific applications in medicine.
- 2895 d. **Food (provisioning).** Amphibians and reptiles provide food resources to mankind. Froglegs
2896 especially are consumed not only worldwide, but also in parts of Europe.
- 2897 e. **Nutrient cycling (supporting).** The cycling of nutrients in waterbodies and soils is the basis
2898 for life. Amphibians and reptiles contribute especially with digging to the mixing of soil and
2899 sediments, and shift dead organic matter from above- to below-ground and from terrestrial to
2900 aquatic habitats, finally enhancing nutrient mineralization.
- 2901 f. **Soil structure formation (supporting).** Digging activities of amphibians and reptiles in
2902 terrestrial habitats contribute to the formation of soil structure. Tadpoles in ponds affect
2903 sedimentation processes.

- 2904 g. **Pest and disease outbreak control (regulating).** Amphibians and reptiles can contribute to
2905 the reduction of pests in agricultural systems. Preying on e.g. mosquito larvae alters diseases
2906 transmission, especially by amphibians in ephemeral wetlands.
- 2907 h. **Invasion resistance (regulating).** Autochthonous amphibian and reptile species might
2908 provide invasion resistance to alien species.
- 2909 i. **Food provision, food-web support (supporting).** Amphibians and reptiles are important
2910 parts of aquatic and terrestrial food webs. Being themselves predators or herbivores, they
2911 provide secondary production and support biodiversity at higher trophic levels.

2912

2913 **5.4. Special consideration of endangered species**

2914 As noted in chapter 2, a high percentage of amphibian and reptile species is recognized by the
2915 International Union for the Conservation of Nature as endangered (i.e. listed within the categories of
2916 Critically Endangered, Endangered or Vulnerable for their global conservation status), and this
2917 percentage can be locally higher if national or regional red lists are considered. The the Panel and its
2918 Working Group therefore explored whether

- 2919 - endangered amphibian and reptile species occur in agricultural landscapes
- 2920 - separate SPGs are needed for endangered species
- 2921 - separate risk-assessment schemes are needed for endangered species along with specific risk-
2922 mitigation measures

2923 Appendix A of this Opinion lists species included in the Annexes II and IV of the Habitat Directive⁴ and
2924 in the IUCN list of amphibians in agricultural landscapes (taken from Fryday and Thompson, 2012).
2925 There are several species of amphibians and reptiles that were identified as being present in
2926 agricultural landscapes. Some examples of reptiles associated with agricultural landscapes and listed
2927 in Annex IV are: *Testudo hermanni* (Hermann's tortoise); *Emys orbicularis* (European pond terrapin);
2928 *Lacerta viridis* (European green lizard); *Coronella austriaca* (Smooth snake). Regarding amphibians,
2929 we could list by way of example: *Bombina orientalis* (Fire bellied toad); *Bufo viridis* (European green
2930 toad); *Hyla arborea* (European tree frog)

2931 The Panel concludes that there are many amphibian and reptile species listed within the categories of
2932 critically endangered, endangered or vulnerable that are associated with agricultural areas in the EU.

2933 In the Opinion of the EFSA Scientific Committee on endangered species (EFSA SC 2016b), it is
2934 proposed that SPGs should be developed for these species and that such protection goals would need
2935 to be harmonized regarding the environmental risk assessment of substances (e.g. PPP, GMO, Feed
2936 Additives).

2937 In the light of this Opinion, the Panel and its working group came to the conclusion that it is not
2938 appropriate to define SPGs specifically for endangered amphibian and reptile species. This is because
2939 parameters regarding population structure, critical life history traits and behaviour are shared by many
2940 species of amphibians and reptiles, and that these parameters need to be taken into consideration in
2941 the 'standard' risk assessment for these groups.

2942 It is deemed that specific requirements for risk-mitigation options will have to be set regarding
2943 endangered species, since they will depend on the species trait and the environmental context at
2944 Member State or more appropriate, at regional level.

2945

⁴ Council Directive 92/43/EEC of 21 May 1992 on the conservation of natural habitats and of wild fauna and flora. Official Journal of the European Union L 206, 7–50

2946 6. Consolidated SPG Options for amphibians and reptiles

2947 Considering the outcome of the analyses in the chapters above, specific protection goal options for
2948 amphibians and reptiles are proposed by the Panel that integrate I) the legislative requirements
2949 currently in place for vertebrate non-target species; II) the ecosystem services delivered by amphibian
2950 and reptile species and III) the particular conservation status (i.e. poor) of the majority of amphibian
2951 and reptile species in European agricultural landscapes.

2952 These issues determined the choice of the ecological entities to be protected, their attributes and the
2953 magnitude, temporal and spatial scale of tolerable effects. Long-term population modelling outcome
2954 helped underpinning the choices.

2955 6.1. Implications of current legislative requirements

2956 The Panel considers that, as for all other non-target vertebrates living in different habitats in
2957 agricultural landscapes, no lethal repercussion of intended uses of PPP should be elicited. Regarding
2958 long-term population-level effects, Regulation 1107/2009 states that no unacceptable long-term
2959 repercussions on populations should be observed; different protection-goal options need to be
2960 elaborated for consideration by risk managers and the scientific challenges and data needs for the
2961 different options should be identified.

2962 As stated in chapter 5.2, the regulatory framework defines that PPP use should have no unacceptable
2963 effects on non-target species including their behaviour and on biodiversity and ecosystems (EU
2964 1107/2009). The regulation EU 546/2011 states that the possibility of exposure of birds and other
2965 terrestrial vertebrates should be evaluated and that the extent of the short-term and long-term risk to
2966 be expected for these organisms, including their reproduction, should be evaluated. This implies that
2967 amphibians and reptiles should also be considered in the risk assessment. This is also further
2968 developed in the current data requirements for active substance and plant protection products
2969 (Directive 283/2013 and 284/2013), where amphibians and reptiles are specifically listed and not
2970 subsumed within the group 'other terrestrial vertebrates'.

2971 The regulation EU 546/2011 specifies that LC/LD50 and NOEC values should be used as endpoints for
2972 acute effects (mortality) and long-term effects. As decision criteria, toxicity:exposure ratios (TER) of
2973 less than 10 (acute) and 5 (long-term) are given. The regulation also specifies that these ratios may
2974 be exceeded as long as it is clearly established in an appropriate risk assessment that no unacceptable
2975 impact occurs (directly or indirectly) after the use of the plant protection product. It should be noted
2976 that no calibration of these assessment factors has been ever performed for protection goals
2977 regarding amphibians and reptiles. It is, therefore, both not known at present and questionable,
2978 whether the assessment factors that are considered protective for birds and mammals would cover
2979 also the risk to amphibians and reptiles from PPP uses. These concepts are expanded in the chapters
2980 addressing the specific exposure of amphibians and reptiles in agricultural landscapes, their biology
2981 and the current available knowledge to test for effects on these groups (e.g chapter 2).

2982 In the framework of developing the guidance document on birds and mammals risk assessment (EFSA
2983 2009), the following specific protection goals were agreed: *"There was a consultation of Member
2984 States on the level of protection that should be provided. The outcome was that there should be no
2985 visible mortality and no long-term repercussions for abundance and diversity. A high level of certainty
2986 was desired. Because of uncertainties around the methodology on determining visible mortality and to
2987 achieve a high certainty that there are no long-term repercussions for abundance and diversity,
2988 surrogate protection goals were defined. These surrogate protection goals were defined as no acute
2989 mortality and no reproductive effects. The risk assessment scheme was designed in such a way that
2990 acute mortality and reproductive effects from pesticide exposure are unlikely."*

2991 The Panel and its working group considered whether it would be possible to identify tolerable chronic
2992 effects that would not have long-term, population-level impacts. This was however deemed to be an
2993 unrewarding task, giving that data on long-term repercussions of PPP use on amphibians and reptiles
2994 are almost nonexistent and that populations of amphibians and reptiles are in decline – hence their
2995 ability to recover may already be exhausted by the several stressors they are exposed to.

2996

6.2. Evidence based on ecosystem service concept

2997 The definition of Specific Protection Goal options based on the ecosystem service concept envisages
2998 that the relevant drivers are identified, following the identification of relevant ecosystem services
2999 provided in agricultural landscapes. In further steps, their attributes and the magnitude of effects that
3000 can be tolerated without impacting the General Protection Goal are specified – including the relevant
3001 spatial and temporal scales.

6.2.1. Characterization of Service Providing Units (SPUs), ecological entities and their attributes

3002 The second step in the definition of SPGs is the characterization of the main drivers behind the
3003 ecosystem services deemed to be important in agricultural landscape.

3004 In the Guidance of the Scientific Committee on Protection Goals (EFSA Scientific Committee, 2016a),
3005 the definition of 'key driver' applies to 'service providing unit' (SPU). SPUs are defined as the structural
3006 and functional components of ecosystems necessary to deliver a given ecosystem service at the level
3007 required by service beneficiaries (adapted from Luck et al., 2003; Vandewalle et al., 2008).

3008 In a further step, the ecological entities that are drivers of ecosystem services have to be determined
3009 to be considered in respect of the ecosystem services assessed. The PPR Panel (EFSA PPR Panel,
3010 2010a) first proposed a list of ecological entities, which has been amended by the Scientific
3011 Committee (EFSA SC, 2016). It is suggested to differentiate between the entities "individual",
3012 "(meta)population", "functional group", "community" "ecosystem" and "habitat" to be coupled to the
3013 SPU. The concept is based on the assumption that – in principle - addressing organisms at one level
3014 of organization will protect those at a higher level of organization. For example, if the ecological entity
3015 to be protected is the "individual", the entities "population", "functional group" and "ecosystem" will
3016 implicitly be protected. The ecological entity is identified for the definition of every specific protection
3017 goal.

3018 The next step in the definition of SPG is determination of the attribute to be assessed. According to
3019 the Guidance of the Scientific Committee (EFSA SC 2016a), "it is important to consider jointly the
3020 ecological entity and its most ecologically relevant attribute to protect. For each ecological entity
3021 option at least one attribute option must be chosen." The PPR Panel and the Scientific Committee
3022 (EFSA PPR Panel, 2010a;) suggested the following as possible assessment endpoints to be made for
3023 the different drivers considered: changes in behaviour, survival and growth, abundance/biomass, a
3024 process rate or biodiversity. If the individual is selected as the ecological entity, then its attributes
3025 might be survival, growth and reproduction. EFSA (SC 2016a) states that "if the ecological entity to
3026 protect is the (meta)population of a given species, then in most cases the attribute to protect will be
3027 population dynamics in terms of abundance (e.g. numbers of individuals and their fitness) or biomass
3028 (see EFSA PPR Panel, 2014)."

3029 "Amphibians" and "reptiles" are too broad categories to possibly identify single SPUs for every
3030 ecosystem service, since they comprise a wide range of species belonging to different classes
3031 displaying a multitude of traits that affect ecosystem functioning (see chapter 2). Nevertheless, a
3032 rough characterization of the drivers of the different ecosystem services and their associated
3033 ecological entities and attributes to be protected is attempted here, based on available literature.

a. Genetic resources, biodiversity (provisioning and supporting):

3034 Regarding the provision of genetic resources and of biodiversity as regulated goods, the
3035 ecological entity of amphibians and reptiles as SPU is identified on the level of "populations".
3036 Amphibian and reptile species are the most threatened vertebrate groups worldwide. In order
3037 to retain also an option value on the services provided by biodiversity in the future, the long-
3038 term persistence of species should be ensured. Taxonomical diversity is also known to be an
3039 essential support for the provision of all ecosystem services in fluctuating environments like
3040 agricultural fields. Here, the importance of the contribution of single species to different
3041 ecosystem services becomes more critical. The abundance and/or biomass of individuals of a
3042 species are the relevant entities to be considered.

3046 Following the requirements from current legislative framework (see chapter 5), the individual
3047 level has also to be addressed in the case of amphibians and reptiles. As with other
3048 vertebrates potentially exposed in agricultural landscapes to intended uses of PPP, juvenile
3049 and adult mortality due to PPP use is commonly considered unacceptable. According to this,
3050 survival of individuals is the entity to be protected.

3051

3052 b. **Education and inspiration, aesthetic values and cultural diversity (provisioning):**

3053 Cultural services provided by amphibians and reptiles are in some cases bound to species. In
3054 most cases, however, knowledge on taxonomic diversity is not translated into symbolic
3055 visualisation or description of amphibians and reptiles particular species in e.g. arts or religious
3056 documents.

3057 The protection of endangered species has been often described also as a cultural issue, since
3058 in this case species are protected *per se* after a decision taken by society without implicated
3059 direct benefits (EFSA 2016a). However, as stated above, if legislation in place requires the
3060 protection of species diversity, the specific task to be implemented is the protection of
3061 biodiversity as a good that should be provided by ecosystems also after intended uses of PPP
3062 (see also EFSA 2016c). EFSA Scientific Committee has issued an Opinion on how to deal in
3063 environmental risk assessment with the specific protection of endangered species (EFSA SC
3064 2016b).

3065 Cultural services are not absolute since the perception of fulfilled cultural values is very
3066 personal and dependent on the social context. Weinstoerffer and Girardin (2000) see in
3067 humans a general attraction for "diversity, which is source of pleasure, satisfaction or
3068 happiness". The human perception and attraction for nature and biodiversity can also vary
3069 among different species belonging to the same group of organisms. Considering for example
3070 amphibians, different individual aesthetic perceptions may exist towards e.g. small frogs
3071 compared to bigger toads. In order to help achieving the "desirable complementary
3072 relationship between aesthetic pleasure and ecological health" (Van Zanten et al., 2013), it is
3073 suggested in the framework of this Opinion to couple the ecological entity of the SPU for
3074 Amphibians and Reptiles to those driving genetic resources and biodiversity. The ecological
3075 entity to be addressed is, therefore, the populations of different species and their attribute is
3076 the abundance/biomass of individuals belonging to a species.

3077

3078 c. **Pharmaceutical resources (provisioning):**

3079 Amphibian and Reptile SPU provide specific compounds used in traditional and modern
3080 medicine. Compounds isolated from amphibian skin or from reptile poisons include alkaloids,
3081 peptides and amines. Some of these molecules have *inter alia* antimicrobial and neurological
3082 properties and serve as potent antibiotics or analgesics. Even if similar molecules might be
3083 common within genera, up to now specific compounds have been isolated from single species,
3084 indicating the populations of amphibian and reptile species and the biomass /abundance of the
3085 individuals belonging to one species as the ecological entities and their attributes to be
3086 protected in order to provide this ecosystem service.

3087

3088 d. **Food (provisioning):**

3089 Amphibians and Reptiles are protein rich foods that are particularly appreciated in neotropical
3090 countries. In Europe, amphibians are nowadays consumed as delicacies rather than staple
3091 foods, whereby the species identity surely plays a role. Many species are protected and only a
3092 few amphibian species might serve as food in Europe. The SPU and the respective ecological
3093 entity would therefore be the population of particular amphibian species. The provisioning
3094 service is considered minor compared with other services driven by amphibians and reptiles.

3095

- 3096 e. **Nutrient cycling (supporting).**
- 3097 The contribution of activities of amphibians and reptiles to nutrient turnover depends on
3098 behavioural traits during e.g. feeding, breeding or overwintering periods. The role of
3099 amphibians in the transport of energy and matter to and from aquatic and terrestrial
3100 ecosystem compartments is unique. Attempts have been made to quantify net fluxes driven by
3101 amphibian movement across compartments. Even if the rate and direction of such fluxes
3102 depends on the traits of the individual species that have been assessed in particular studies, it
3103 is deemed that such traits are common to species that may be grouped into functional
3104 entities. Therefore, SPU of amphibians and reptiles for the ecosystem nutrient cycling may be
3105 addressed best by context-defined functional groups. The attribute is the abundance and/or
3106 biomass of all the individuals belonging to one functional group that has to be defined
3107 according to its role in nutrient cycling.
- 3108
- 3109 f. **Soil and sediment structure formation (supporting).**
- 3110 Amphibian and reptile activities also affect their physical environment. Sporadic knowledge is
3111 available on the contribution of digging by reptiles and adult amphibians in terrestrial habitats
3112 to the formation and bioturbation of soil structure. Tadpoles in ponds affect sedimentation
3113 processes. Even if some behavioural particularity is known for e.g. specific toad species
3114 digging breeding pools in mud, traits affecting soil- and sediment-structure formation should
3115 be attributable to functional groups of amphibians and reptiles, as ecological entities to be
3116 addressed. The attribute is the abundance and/or biomass of all individuals belonging to a
3117 functional group, defined according to its role in the formation of soil and sediment structure.
- 3118
- 3119 g. **Pest and disease outbreak control (regulating).**
- 3120 Amphibians and reptiles can contribute to the reduction of pests in agricultural systems.
3121 Preying on e.g. mosquito larvae reduces disease transmission, especially by amphibians in
3122 ephemeral wetlands. Please refer also to chapter 2 for the feeding behaviour of amphibians
3123 and reptiles. Species traits influence the degree of pest control by amphibians and reptiles, but
3124 it can be reasonably supposed that functional groups assembling several species with similar
3125 traits are the entity to be protected. The abundance and/or biomass of all individuals
3126 belonging to the different functional groups are the associated relevant attribute.
- 3127
- 3128 h. **Invasion resistance (regulating).**
- 3129 Autochthonous amphibian and reptile species might provide invasion resistance to alien species.
3130 Since invasive species might have deleterious effect on the performance of invaded ecosystem
3131 and are often a cause of the decline of amphibian species (e.g. Ficetola et al. 2007).
3132 Community stability can hamper the probability of successful establishment of alien species.
3133 Given the nature of species-specific ecological niche differentiation, the population of a species
3134 is deemed to be the service providing unit and the abundance/biomass of individuals
3135 belonging to a species the attribute to be protected.
- 3136 i. **Food provision, food-web support (supporting).** Amphibians and reptiles are important
3137 players in aquatic and terrestrial food webs. Being themselves predators or herbivores, they
3138 provide secondary production and support biodiversity at higher trophic levels. Since species-
3139 specific dependencies are not common between amphibians and reptiles and their predators,
3140 the ecological entity to be protected should apply to functional groups. The abundance and/or
3141 biomass of individuals belonging to the different functional groups in terms of food-web
3142 support are the associated relevant attribute
- 3143

3144 6.2.2. Specifying the level and parameters of protection

3145 **The magnitude of effect on the drivers/SPU** that could be tolerated regarding the overall impact
3146 on the respective ecosystem service has also to be determined. In the following, a partitioning of
3147 magnitude of effects is proposed deriving from general effect classes in ecotoxicology. Changes in
3148 effects size are described following a dose-response relationship. It is noted that these classes
3149 describe the magnitude of effects on the drivers' attributes and do not aim at assessing the adversity
3150 of the observed effects (i.e. 'effect' and not 'risk'). Which of these effect classes are considered 'not
3151 adverse' in terms of this Opinion is described in the Specific Protection Goal Options for every
3152 driver/SPU (see section 6.5 and 6.6). From these effect classes, the pertinent one is chosen for final
3153 SPG Option proposal, depending on the organisms' traits that determine e.g. sensitivity, life cycle or
3154 recovery potential.

3155 Scaling of magnitude of effects on individual/population/functional group:

3156 Large effects: pronounced reduction, corresponding to effects above 65%

3157 Medium effects: reduction comparable to median effect size (i.e. corresponding to median
3158 effect class of 50%; effects between 35% and 65%)

3159 Small effects: reduction above No Effect Level and below medium effects (above 10% and
3160 below 35%)

3161 Negligible effects: reduction up to No Effect Level (comparable to 10%)

3162

3163 The definition of 'negligible' especially has often been a matter of debate, also in recent Panel
3164 publications (e.g. Bakker, 2016). This is possibly owing to misunderstandings regarding the target
3165 addressed. We refer here to effects on the 'assessment endpoint': what magnitude of effect might be
3166 tolerable for amphibian and reptile drivers of ecosystem services in order still to meet the proposed
3167 specific protection goal options (e.g. Munns et al. 2016). This target has to be distinguished in
3168 principle from what will be the 'measurement endpoints' (or 'measure of effects', US EPA 1998, 2003),
3169 which are the measurable characteristics related to the chosen assessment endpoints (Suter 1993,
3170 2006). The term 'negligible' is not used in this Opinion in relationship to exposure of non-target
3171 organisms (e.g. Mackay 1988), nor is it related here to effects that are 'not adverse' (i.e. negligible
3172 risk e.g. Duffus et al. 2007; Barnard 1990, Boekelheide and Andersen 2010, Dorato and Engelhardt
3173 2005, Keller et al. 2012, Ricci et al. 1987).

3174 In terms of this Opinion, the **definition of "negligible effects"** on ecological entities reads as
3175 follows: **no increases in the frequency of effects between exposed and unexposed groups.**

3176 This definition relates as closely as possible to the continuum in the dose-response relationship and
3177 does not judge at this point on which effects are acceptable (e.g. Barnard 1990). By contrast, the
3178 Specific Protection Goal Options will mark the points at which the effects on the drivers gain such
3179 magnitude that they can be considered adverse. For example, EFSA PPR (2015) describes that the
3180 magnitude of effects that can be tolerated on non-target arthropods (NTA) might be clearly above
3181 negligible – as long as the NTA abundances are able to recover in a given time frame. It is only above
3182 this threshold or tipping point that the service provision cannot be guaranteed and the magnitude of
3183 effects on the ecological entities becomes clearly adverse.

3184 It should not be a matter of debate that the measurement of negligible effects has to be based in
3185 practice on careful biological and statistical analysis. Every measure of effects in experimental or
3186 modelling approaches will have characteristic explanatory values and care should be taken not to use
3187 underpowered studies to establish no effect levels (e.g. Bross 1985, Millard and Bross 1987, Dixon
3188 and Pechmann 2005, Hoekstra and vanEwijk 1993, Parkhurst 2001).

3189 Regarding the magnitude of effects arising from several years of PPPs exposure in an agricultural
3190 context, relevant measurement endpoints are still to be agreed in the scientific community. If the
3191 assessment of these effects is based on population models that address effects of PPPs on species,
3192 efforts should be made in order to identify those simulation endpoints that can be related to the
3193 magnitude of effects as defined above (see chapter 4). It should be noted that this task is not
3194 straightforward. Chapter 4 highlights the dynamic nature of populations in both time and space. The

3195 standard EFSA definitions of sizes of effect as large, medium, small or negligible (see above) do not
3196 mesh easily with the dynamic characteristics of populations living, moving, feeding, breeding and
3197 dying in a non-uniform landscape.

3198 Depending on the endpoints that will be chosen in future for assessment of PPP effects on population
3199 persistence, negligible, small medium and large effects will therefore have to be re-defined or at least
3200 elaborated. Since modelling endpoints can integrate several years of PPP application ('system
3201 approach', see also section 4.1.5), tolerable effects might be of lower magnitude than those defined
3202 for community assessment at a local or landscape scale.

3203 On the one hand, year on year decline in abundance should not be observed. On the other hand,
3204 negligible effects should also account for population-range restrictions; not only individual abundance
3205 but also range of occupancy should not be reduced by more than a level considered to be negligible.

3206 In terms of this opinion, the definition of possible acceptable magnitude of effects as percentage
3207 reduction compared to a "control" applies to defined contexts, i.e. agricultural systems supporting
3208 high or low amphibian or reptile diversity that can be achieved in managed agricultural systems.

3209 For services supported and provided by amphibian or reptiles, it is difficult to define effect thresholds
3210 marking tipping points for ecosystem functioning and the provision of the service of interest. This is
3211 due to the lack of knowledge on the detailed quantitative relationships between species and functions
3212 in ecosystems. If no absolute threshold can be defined, maximum magnitudes of effects on
3213 drivers/SPUs are suggested marking the acceptable limits, in scientific terms, for the maintenance of
3214 the assessed service at a desired rate and ultimately for the general protection goal (EFSA PPR
3215 Panel, 2010a). This means that, if such limits are breached, severe consequences for the ecosystem
3216 functioning and for stakeholders who rely on certain services can be expected. These limits mark the
3217 upper range of the magnitude of effects in the different SPG options.

3218 From a scientific point of view, the tolerable magnitude of effects should take multiple PPP
3219 applications according to typical PPP spray schedules into account. This could suggest a lower level of
3220 tolerable effects for single PPP applications, if the intended use fits in an application scheme that
3221 includes several other PPPs with potential effects on amphibian or reptiles. Multiple applications of
3222 several PPPs in typical schedules ought also to be taken into account when addressing the recovery of
3223 such organisms in agricultural landscapes (see chapter 4). This approach is currently not supported by
3224 the regulatory framework for approval of active substances/authorization of PPPs. The Panel would
3225 strongly recommend that this aspect (which represents the reality in the field) should be taken into
3226 consideration when setting SPGs.

3227 **One further step is the determination of the temporal scale** to be considered together with the
3228 magnitude of tolerable effects. This step is of particular importance when addressing effects other
3229 than negligible, since it implies that some effects might be tolerable as long as ecological recovery
3230 occurs within a specified period. As stated in the EFSA Guidance on the risk assessment for aquatic
3231 organisms (EFSA PPR Panel, 2013), when including "recovery to identify (un)acceptable effects, all
3232 relevant processes that determine population viability and the propagation of effects to the
3233 community-, ecosystem- and landscape-level are to be considered." In this respect, multiple
3234 application of PPPs might pose a constraint to recovery processes in agricultural landscapes – in
3235 particular the consecutive PPP uses throughout crop-spraying schedules.

3236 Considering the ecosystem services identified as driven by amphibians and reptiles, their timely
3237 provision might be of central importance. For example, as described for the ecosystem service 'food
3238 web support', effects occurring when organisms at a higher trophic level raise their young might have
3239 the highest implications, which cannot be compensated by recovery occurring several months later.

3240 Amphibians and reptiles have life-history strategies spanning over several years (see chapter 2). Full
3241 recovery from chronic effects might only be observed several years after PPP use. Therefore, the
3242 Panel considers time lapses of several years as relevant for the demonstration of e.g. long-term
3243 effects on amphibian and reptile species that may emerge after several years of PPP use or for the
3244 demonstration of recovery of species with a lengthy life cycle. Therefore, the temporal scale of SPGs
3245 as assessment endpoints diverges from the time scale of measurement endpoints, which should cover
3246 also the life cycles of vulnerable species. Measurement endpoints might be experimental set ups or
3247 endpoints derived from simulation of effects over several years.

3248 The temporal scaling of effects on amphibian and reptile species as assessment endpoints may be
3249 classified as follows:

3250 > 6 months: not considered adequate to satisfy protection goals unless effects are negligible.
3251 Negligible effects are considered as no effect level

3252 Months: maximum of 6 months

3253 Weeks: up to 4 weeks

3254 Days: up to 7 days

3255

3256 **Spatial scale of the effects.** According to EFSA (SC 2016b), the spatial scale of the tolerable effects
3257 is also an important parameter determining the level of protection defined in the SPG options. "*The*
3258 *spatial scale of the tolerable effects should consider several ecological characteristics, such as species*
3259 *behaviour and mobility, dispersal ability of relevant life stages, meta-population structure and sink-*
3260 *source dynamics, occupancy, that determine the spatial scale at which the relevant ecological entity*
3261 *operates.*" A comprehensive dedicated section can be found in chapter 3.

3262 6.3. Evidence based on requirements for endangered species

3263 According to the sections above and to the information given in Appendix A to this Opinion, the Panel
3264 concludes that there is a large proportion of amphibian and reptile species listed within the categories
3265 of critically endangered, endangered or vulnerable and that these species are associated with
3266 agricultural areas.

3267 Since parameters regarding population structure, critical life-history traits and behaviour are shared by
3268 many species of amphibians and reptiles (please refer to chapter 2 for details), these parameters need
3269 generally to be taken into consideration when defining SPG options for amphibians and reptiles and
3270 separate SPG options should not be presented for endangered species.

3271 6.4. Attributes and parameters of protection based on population 3272 modelling

3273 In regulatory toxicity testing for environmental risk assessment, dose-response relationships between
3274 chemical and test endpoints relevant to the population level (mortality, growth and reproduction) are
3275 determined. Based on dose-response studies under controlled conditions, threshold-toxicity values
3276 e.g. NOEC (No Effect Concentration) or LC50 (lethal concentration to 50% of the exposed individuals)
3277 are determined for the different test endpoints. The threshold toxicity values are used to determine
3278 the toxicity–exposure ratios in the environmental risk assessment of plant protection products.

3279 A good test endpoint in laboratory studies is not always useful as an endpoint/biomarker in wild
3280 populations. For example, one of the most frequently studied endpoints for endocrine disruption in
3281 amphibians is the frequency of male intersex or ovotestis (presence of ovarian follicles within the
3282 testicle). This can be a very sensitive endpoint in controlled laboratory tests in certain species. In
3283 some species, however, intersex gonads occur normally during the period of gonadal differentiation.
3284 In populations of such species, the intersex frequency is age-specific and therefore a poor endpoint or
3285 biomarker for endocrine toxicity. Therefore, only those laboratory endpoints that can be translated to
3286 meaningful individual impacts should be used in the population-modelling assessments.

3287 Having chosen useful laboratory endpoints for population assessment, the population modelling takes
3288 existing toxicological response data and translates them into key, population-level endpoints of
3289 distribution of animals in space and time and population persistence. Here, the population-modelling
3290 endpoints that might be appropriate to define attributes and parameter of protection for the identified
3291 relevant ecological entities are listed:

- 3292 • Long-term changes in population size with year-on-year use of pesticide
- 3293 • Changes in landscape occupancy
- 3294 • Changes in population density in occupied areas
- 3295 • The pattern of recovery in time and space

3296 • Relative population growth rate, to identify deleterious effects on long-term population viability

3297 In order to define the attributes and the parameters of protection for the identified ecological entities,
3298 these endpoints need to be combined and the relative tolerable magnitude defined when setting the
3299 SPG options. Depending on the endpoints that will be chosen in future for assessment of PPP effects
3300 on population persistence, negligible, small medium and large effects will have to be defined. Since
3301 modelling endpoints are integrated over several years of PPP application ('system approach', see also
3302 section 4.1.5), tolerable effects might be of lower magnitude than those that can be assessed at local
3303 scale.

3304

3305 **6.5. SPG Options and relevant assessment endpoints**

3306 The proposed different options for Specific Protection Goals for amphibians and reptiles in agricultural
3307 landscapes are derived by combining the knowledge on the key drivers (or SPU) and their traits in
3308 terms of their recovery and dispersal potential. These data are integrated to derive i) a magnitude of
3309 effects by intended PPP use that might be acceptable without compromising the delivery of the
3310 ecosystem services of interest and ii) magnitude of effects that might be acceptable considering the
3311 endangered status of several amphibian and reptile species.

3312 In the trade-off between crop production and protection of biodiversity and ecosystem services, the
3313 Panel might propose some effects on amphibians and reptiles to be deemed acceptable. In doing this,
3314 the Panel acknowledges that crop protection might be rated higher in term of provisioning service
3315 than biodiversity and other ecosystem services. It should, however, be stated unambiguously that
3316 amphibians and reptiles are vertebrates with a high conservation status and that they should be given
3317 equal status alongside birds and mammals.

3318 In terms of this Opinion, the definition of possible acceptable magnitude of effects as percentage
3319 reduction compared to a "control" applies to a defined context. For example, in an agricultural system
3320 supporting a high diversity, a given reduction (e.g. 50%) may still retain the function represented by
3321 the SPG. In contrast, in landscapes with very low diversity, the acceptability of effects might be at a
3322 far lower magnitude level, e.g. removing 50% of 2 species may be critical. This context dependency
3323 applies to all proposed Specific Protection Goal options.

3324 The tolerable magnitude of effects should take multiple PPP applications according to typical PPP
3325 'spray schedules' into account. This will possibly implicate a lower level of tolerable effects for single
3326 PPP applications, if the intended use fits in an application scheme that includes several other PPPs
3327 with potential effects on amphibian and reptile populations. Multiple applications of several PPPs in
3328 typical schedules should also be taken into consideration when addressing the recovery of amphibian
3329 and reptile species.

3330 The proposed SPG options are therefore given on the one side as limits of operation of the addressed
3331 SPU in order to be (still) able to deliver the identified ecosystem service. On the other side, legislative
3332 requirements are mirrored by the given options, especially regarding mortality.

3333 If risk managers consider the lower magnitude of effects to be pertinent to the limits of operation (
3334 negligible effects), then no consequences for the service provision are expected. If risk managers
3335 choose a higher magnitude of effects, then consequences regarding the ecosystem service provision
3336 and the long-term persistence of the populations are to be expected. The consequences of choosing
3337 different SPG Options are set out in Appendix B. For reason of simplicity, the proposed SPG Options
3338 are given as "Option: below the limit of operation" and "Option: Limit of operation" for the service
3339 providing units. The consequences of choosing a level of protection "Above limit of operation" are also
3340 given.

3341 *The consequences of choosing SPG options are placed in Appendix B simply in order to increase*
3342 *readability of this chapter. Appendix B is extremely important and the Panel urges all readers to*
3343 *consult it.*

3344

3345 **6.5.1. Amphibians**

3346 **Table 10:** Specific Protection Goal Option as 'limit of operation' for Amphibians

Amphibians			
	ecological entity	attribute	magnitude/ temporal scale
Adults and juveniles	individuals	survival	negligible effects
All life stages	populations	abundance/ distribution/ population growth rate PGR	small effects up to months on species abundance and/or occupancy and/or on PGR changes

3347

3348 **Table 11:** Amphibian SPUs, species examples and relevant assessment endpoints to the proposed
3349 Specific Protection Goals

Organisms	Service Providing Units with (model) species examples	Assessment Endpoint to address the Specific Protection Goal
Amphibians		
Anura	- Toads (e.g. <i>Epidalea calamita</i> , natterjack toad) - Frogs (e.g. <i>Hyla arborea</i> , European tree frog)	- Mortality of adults and juveniles individuals - Species long term abundances and/or spatial occupancy
Caudata	- European newts (e.g. <i>Triturus cristatus</i> , crested newt) - salamanders	- Mortality of adults and juveniles individuals - Species long term abundances and/or spatial occupancy

3350

3351 **6.5.2. Reptiles**

3352 **Table 12:** Specific Protection Goal Option as 'limit of operation' for Reptiles

Reptiles			
	ecological entity	attribute	magnitude/ temporal scale
Adults and juveniles	individuals	survival	negligible effects
All life stages	long term persistence of populations	abundance/ distribution/ population growth rate PGR	small effects up to months on species abundance and/or occupancy and/or on PGR changes

3353

3354
3355

Table 13: Reptile SPUs, species examples and relevant assessment endpoints to the proposed Specific Protection Goals

Organisms	Service Providing Units with (model) species examples	Assessment Endpoint to address the Specific Protection Goal
Reptiles		
Squamata	<ul style="list-style-type: none"> - Lizards (e.g. <i>Lacerta agilis</i>, sand lizard) - skinks - geckos - agamas 	<ul style="list-style-type: none"> - Mortality of adults and juveniles individuals - Species long term abundances and/or spatial occupancy
Ophidia	<ul style="list-style-type: none"> - Snakes (e.g. <i>Zamenis longissimus</i>, Aesculapian snake) - water snakes and vipers, 	<ul style="list-style-type: none"> - Mortality of adults and juveniles individuals - Species long term abundances and/or spatial occupancy
Testudines	<ul style="list-style-type: none"> - Turtles (e.g. <i>Testudo hermanni</i>, Hermann's tortoise) 	<ul style="list-style-type: none"> - Mortality of adults and juveniles individuals - Species long term abundances and/or spatial occupancy

3356
3357

DRAFT

3358 **6.5.3. Overview and consequences of choosing different SPG Options**

3359 **Table 14:** Overview of the proposed protection goal options for Amphibians and Reptiles

Organism group	Ecological entity/ attribute	Magnitude and duration of effects	
		Option: below the limit of operation	Option: limit of operation
amphibians and reptiles			
adults and juveniles	Individual/ mortality		Negligible effects
all stages	Population/ abundance, occupancy, population growth rates changes	Negligible effects Small effects up to weeks	Small effect up to months

3360

3361 - The consequences of the choice of options made by risk managers are complex. Although it is
 3362 a simple fact that no SPG is sustainable if the population of the species concerned is declining over
 3363 time, it is not easy to relate such parameterst to changes in the individual elements of survival and
 3364 reproduction in practice. This is why we propose the development of spatially explicit population
 3365 models (systems models). As shown before, long-term effects may not be evident for many years and
 3366 occupancy of suitable habitat as well as population size may be affected. The Panel has, however,
 3367 been able to predict some of the effects of intended PPP use on the various ecosystem services
 3368 provided by amphibians and reptiles.

3369 - The consequences of choosing different level of protection are described in detail in Appendix
 3370 B. Below -by way of example- the consequences of choosing different SPG options for the the
 3371 provision of the ecosystem services "biodiversity, genetic resources" are given. Following the Option of
 3372 protecting amphibians and reptiles already "below the limits of operation" would insure that:

3373 - The upper level of the normal operating range for amphibians and reptiles in agricultural
 3374 landscapes is sustained. Species-specific interactions, food-web structure and ecosystem
 3375 processes are unaffected by the intended PPP use.

3376 - General protection goal "no unacceptable effect on biodiversity and the ecosystem" set out in
 3377 Regulation (EC) No. 1107/2009 is fully achieved.

3378 - Support of the target "Increase the contribution of agriculture to maintaining and enhancing
 3379 biodiversity" (3a) of the EU 2020 Biodiversity Strategy¹², which has shown no significant
 3380 progress so far.

3381 - This Option contributes to Action 10 of the EU 2020 Biodiversity Strategy¹²: "The Commission
 3382 and Member States will encourage the uptake of agri-environmental measures to support genetic
 3383 diversity in agriculture and explore the scope for developing a strategy for the conservation of
 3384 genetic diversity".

3385 - The aims of Council Directive 92/43/EEC on the conservation of natural habitats and of wild
 3386 fauna and flora are achieved.

3387 - The aims of Council Directive 92/43/EEC on the conservation of natural habitats and of wild
 3388 fauna and flora are achieved, especially regarding species and sub- species listed in Annex IV, for
 3389 which a strict protection regime must be applied across their entire natural range within the EU,
 3390 both within and outside Natura 2000 sites

3391 - UN sustainable development goals (SDG) 5 Sustainable Goals and 2.4 and 12.2 are supported
 3392 These goals are:

- 3393 ○ "By 2030, ensure sustainable food production systems and implement resilient agricultural
3394 practices that increase productivity and production, that help maintain ecosystems, that
3395 strengthen capacity for adaptation to climate change, extreme weather, drought, flooding and
3396 other disasters and that progressively improve land and soil quality" and
- 3397 ○ "By 2030, achieve the sustainable management and efficient use of natural resources
- 3398 - When choosing the 'limit of operation' as the pertinent protection level, one would insure that:
- 3399 - The tipping point for the normal operating range of amphibian and reptile key drivers delivering
3400 genetic resources and cultural services and supporting all ecosystem services is not breached.
- 3401 - Reduction in species diversity reduces the efficiency with which ecological communities capture
3402 biologically essential resources, control pests, produce biomass, decompose and recycle
3403 biologically essential nutrients.
- 3404 - Biodiversity is supported to a degree that insures the long term functioning of agricultural
3405 system, even if sensitive species are affected in the short term and species-specific interactions
3406 might be disrupted.
- 3407 - General protection goal 'no unacceptable effect on biodiversity and the ecosystem' set out in
3408 Regulation (EC) No. 1107/2009 is still achieved if unsprayed areas of pertinent size in a
3409 diversified landscape sustain the upper level of biodiversity normal operating range.
- 3410 - Member States are still supported in the measures they need to take to maintain or restore the
3411 species in Annex II and IV list at a 'favourable conservation status' in the EU (cf Article 2).
- 3412 ○ populations are maintaining themselves over the long term and are no longer showing signs
3413 of continuing decline; their natural range is not being reduced;
- 3414 ○ there is, and will probably continue to be, a sufficiently favourable large habitat to maintain its
3415 populations on a longterm basis.
- 3416 - Consequences of accepting an impact above the limits of operation would indicate that:
- 3417 - Species loss above a tipping point may force ecosystems to move to a different (locally) stable
3418 state or to collapse.
- 3419 - Loss of biodiversity will weaken the ability of agricultural ecosystems to respond to external
3420 changes such as climate change (loss of stability and resilience).
- 3421 - Biodiversity losses will lead to disruption of valuable ecosystem functions thereby reducing
3422 delivered services. Cultural services will be reduced if vulnerable species decline or disappear.
- 3423 - General protection goal 'no unacceptable effect on biodiversity and the ecosystem' set out in
3424 Regulation (EC) No. 1107/2009 is not achieved.
- 3425 - The target "Increase the contribution of agriculture to maintaining and enhancing biodiversity"
3426 (3a) of the EU 2020 Biodiversity Strategy¹² will most probably not be met.
- 3427 - The aim of halting of biodiversity loss by 2020 is not achieved: 'Halting biodiversity loss
3428 constitutes the absolute minimum level of ambition to be realised by 2020' (2009/2108(INI) and
3429 2011/2307(INI) .
- 3430 - UN sustainable development goals (SDG) Sustainable Goals 2.4 and 15.5 are jeopardized. These
3431 goals are:
- 3432 ○ "By 2030, ensure sustainable food production systems and implement resilient agricultural
3433 practices that increase productivity and production, that help maintain ecosystems, that
3434 strengthen capacity for adaptation to climate change, extreme weather, drought, flooding and
3435 other disasters and that progressively improve land and soil quality" and
- 3436 ○ "Take urgent and significant action to reduce the degradation of natural habitats, halt the
3437 loss of biodiversity and, by 2020, protect and prevent the extinction of threatened species"
- 3438 - The aims of Council Directive 92/43/EEC on the conservation of natural habitats and of wild
3439 fauna and flora are not achieved.

- 3440 - Member States are not compliant with obligations arising from the Habitats directive, and do not
3441 take the necessary measures to ensure the conservation of amphibian and reptile species
3442 protected and listed under Annexes II and IV
- 3443 - Member State do not take the requisite measures to establish a system of strict protection for
3444 Annex II and IV species. As consequence of PPP intended uses
- 3445 ○ animal killing / destruction of eggs in the wild
- 3446 ○ deterioration of breeding sites or resting places will take place at a rate considered
3447 unacceptable to maintain their conservation status
- 3448

3449 7. General Framework

3450

3451 7.1. Introduction

3452 Any ecotoxicological risk assessment starts with setting the protection goal, in practice answering the
3453 question 'what has to be protected, to which degree and where?'. In chapter 5 and 6 of this Opinion,
3454 specific protection goal (SPG) options for amphibians and reptiles have been proposed.

3455 Protection goals are operationalized in SPGs for the effect assessment (EFSA, 2010, EFSA SC 2016)
3456 and are expressed first at the level of Service Providing Units that characterize the drivers of
3457 ecosystem services deemed to be important in agricultural landscapes. It should be possible to
3458 address SPG by a practical regulatory risk-assessment procedure, using as much as possible the
3459 current state of the science. SPGs are recognised as having a multi-dimensional nature: (i) ecological
3460 entity, (ii) its attribute(s) or characteristics, (iii) magnitude of effect, (iv) temporal scale of effect, (v)
3461 spatial scale of effect). Please refer to chapters 5.2 and 6 for more details on the SPG option
3462 proposals for amphibians and reptiles.

3463 When defining the several levels and parameters of protection in the SPG options, both the effect and
3464 exposure assessments should be considered (EFSA, 2010). Therefore, when addressing the spatial
3465 dimension of the magnitude of effects deemed to be tolerable in the SPG options, it is important to
3466 consider rationales for both exposure and effects. In this opinion, the dimension of 'spatial scale' for
3467 the SPG options concerns the in-field, the edge-of-field and nearby off-field (local scale) and at
3468 landscape scale of the population ranges. This implies that only amphibian and reptile habitats within
3469 the treated, agricultural fields or at the edge-of-field or at a certain distance from treated fields in the
3470 area of use of pesticides are considered. Amphibians or reptiles not living or not passing a relevant life
3471 stage within agricultural fields or at the edge thereof are *not* considered in the risk-assessment
3472 procedure. Thus, in the spatio-temporal population of exposure values for amphibians living e.g. in
3473 ponds, only ponds within field or situated at the edge-of-fields are included. Ponds in natural areas,
3474 such as coastal or mountainous areas where no agriculture occurs, are excluded from the spatio-
3475 temporal population of relevant ponds. The assumption behind this delimitation is that, when the
3476 amphibians and reptiles key drivers living in agricultural areas are protected from intended uses of
3477 Plan Protection Products (PPP), also those amphibians and reptiles living in non-agricultural areas will
3478 be protected as well. The process of SPG definition needs interactions between environmental fate
3479 and effect experts and between risk assessors and risk managers (decision makers) (EFSA, 2010).

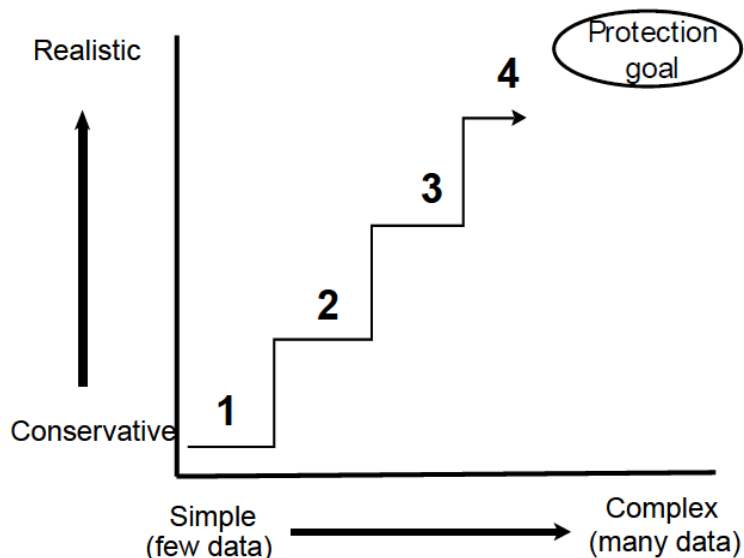
3480 7.2. The principles of a tiered approach

3481 A guidance document of EFSA (EFSA PPR Panel, 2013) provides an overview of the principles of the
3482 tiered approach and the rationale behind adopting them when assessing environmental risks of PPPs.
3483 According to Boesten et al. (2007) and Solomon et al. (2008), the general principles of tiered
3484 approaches are:

- 3485 - lower tiers are more conservative than higher tiers;
- 3486 - higher tiers aim at being more realistic than lower tiers;
- 3487 - lower tiers usually require less effort than higher tiers;

- 3488 - in each tier, all available relevant scientific information is used;
 3489 - all tiers aim to assess the same protection goal.

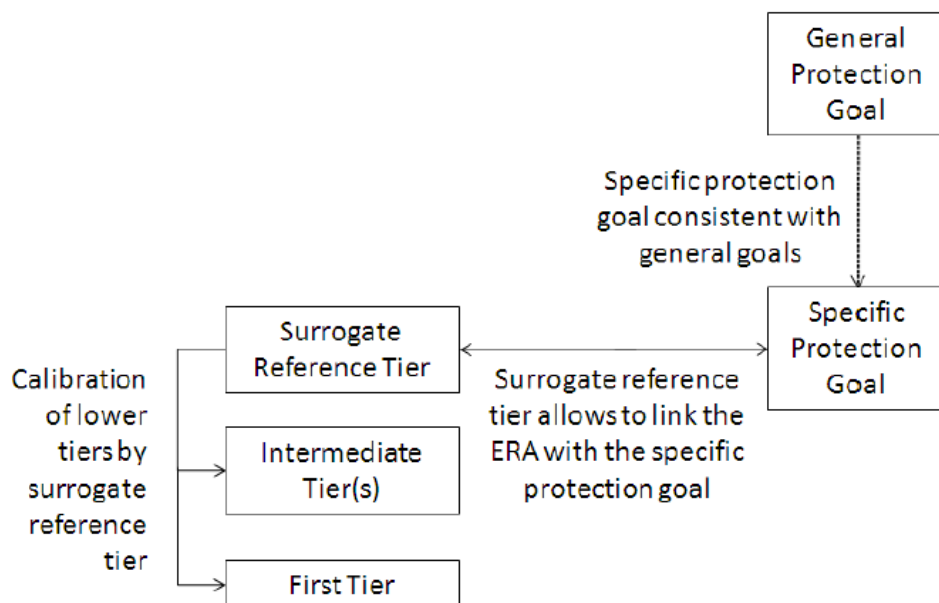
3490 Thus the tiered system needs to be (i) appropriately protective, (ii) internally consistent, (iii) cost-
 3491 effective, and (iv) it needs to address the problem with a higher accuracy and precision when going
 3492 from lower to higher tiers (see Figure 18:).



3493
 3494 **Figure 18:** Tiers in the risk assessment process, showing the refinement of the process through
 3495 the acquisition of additional data (redrafted after Solomon et al., 2008)

3496 7.3. Tiered approach in the risk assessment for amphibians and 3497 reptiles and definition of (surrogate) reference tier

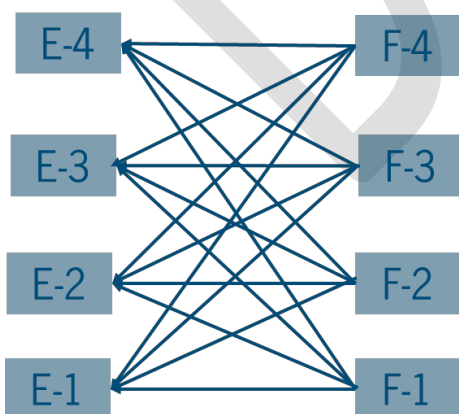
3498 A tiered approach implies the existence of a surrogate reference tier (SRT), which is a representation,
 3499 as accurate as possible, of the real situation in the field (i.e. the reference tier). This SRT should link
 3500 the assessment being performed and the specific protection goals (see Figure 19: below). A SRT is a
 3501 compromise between what would be desirable and what is practical. The SRT should be used to
 3502 calibrate the lower tiers properly in order to make them sufficiently protective, taking into account the
 3503 level of protection defined in the SPGs.



3504

3505 **Figure 19:** Illustration of the relationship between tiers of the risk-assessment process and
 3506 protection goals, in the approach used by the PPR Panel (EFSA PPR Panel, 2010a)

3507 Once the protection goal is clear, the tiered risk-assessment procedure should be designed in order to
 3508 evaluate whether the protection goal will be met after introduction of the active substance or PPP on
 3509 the market. Such a tiered risk-assessment procedure can be represented as a flow chart and consists
 3510 of an effect flow chart coupled to an exposure flow chart. In the effect or exposure flow chart, it is
 3511 always possible to jump to a higher tier, as explained in Figure 20: . Each step of the effect flow chart
 3512 needs an estimate of field-exposure concentrations for the risk assessment. The 'criss-cross' model
 3513 (Figure 20:) shows the recommended and generally accepted way of linking an estimate of the field
 3514 exposure to the effect assessment: all field-exposure tiers may be linked to any effect assessment
 3515 tier, so there are no restrictions. This 'criss-cross' model has the advantage of cost-effectiveness in the
 3516 risk-assessment procedure, because changes in elements of the exposure flow chart have no
 3517 consequences on changes for the effect flow chart. Thus, its modular approach enables the selection
 3518 of the most cost-effective tiers.



3519

3520 **Figure 20:** The 'criss-cross' model: Tiered effect and exposure flow charts for a risk assessment
 3521 addressing a specific protection goal. The boxes E-1 to E-4 are four effect tiers and the boxes F-1
 3522 to F-4 are four tiers for assessment of exposure in the field ('F' for 'field'). Increasing numbers (1

3523 to 4) indicate a higher tier that can be accessed. Arrows from right to left indicate delivery of
3524 field-exposure estimates to the indicated effect tiers.

3525 In the tiered approach, the highest tier represents best the conditions in the field, the SRT of Figure
3526 21: , for the ecotoxicological effect assessment as well as for the exposure assessment.

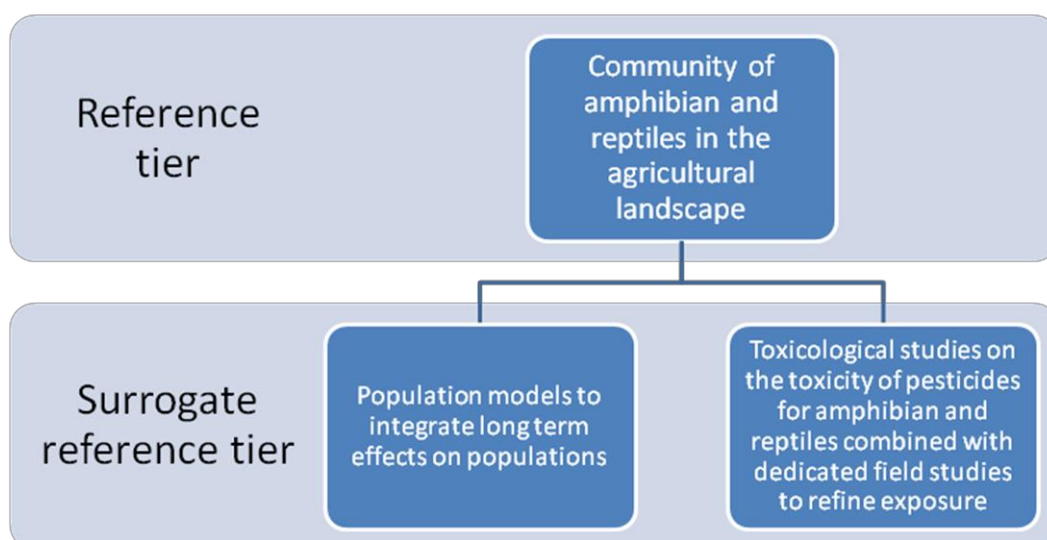
3527 Regarding the highest exposure tier, it represents best all relevant entry routes of pesticides (e.g.
3528 mass deposition, crop interception and wash-off, surface runoff) and pesticide processes (e.g.
3529 volatilisation, degradation, sorption) and generally calculates the exposure as a function of time. It is
3530 also, however, the most complex one, needing much effort. Lower tiers are less realistic, easier to
3531 apply and result in a worst-case exposure. Coupled to an effect assessment, and calibrated to the
3532 higher tier, they allow assessment of risks at the lowest tier without too much effort. In this way lower
3533 tiers function as a filter: if such realistic, worst-case risk assessment indicates already safe uses, the
3534 more realistic, but effort-consuming higher tier risk assessment is not needed. For risk assessments,
3535 the exposure assessment and the effect assessment are equally important. When linking exposure to
3536 effects, the same exposure metric should be used for both field-exposure estimates (expressed in
3537 terms of predicted environmental concentrations, PEC) and effect estimates (expressed as regulatory
3538 acceptable concentrations, RAC, or as ecotoxicological endpoint together with an acceptability
3539 criterion) (EFSA 2010).

3540 The risk assessment of PPPs for amphibians and reptiles should be performed over different spatio-
3541 temporal scales (in-field, boundary-scale, off-field, landscape level). This is in contrast to what is
3542 currently done in the risk assessment for several other non-target organism groups, where only the
3543 in-field – or, separately, the off-field area - is considered. Since an action at a distance is also
3544 expected to occur for amphibians and reptiles on a larger scale within relevant time frames (see
3545 section 4), a landscape-scale assessment covering multiple field scales is considered necessary.

3546 For many amphibian and reptile species and specific for every life stage, recovery will be driven by
3547 small- and large-scale migrations together with internal recovery from reproduction within the
3548 assessed local scales (in-field or off-field areas). The risk to amphibian and reptile key drivers (service
3549 providing units, see chapter 5) should therefore be assessed at a local scale (in-field and off-field
3550 habitat areas), but assessment should also consider processes at the landscape scale (see section 4).
3551 In the latter case, this would be done using spatial population modelling to take spatial dynamics at
3552 this scale into account. The actual reference tier for amphibians and reptiles (including organisms with
3553 either high or low dispersal ability) would therefore be the community of amphibians or reptiles
3554 present at the field scale (in-field and off-field) and influenced by temporal and spatial processes at
3555 the landscape scale.

3556 Since no current risk-assessment scheme is available, it is difficult to define the highest tier for effect
3557 assessment that might be available in future. In aquatic studies, which are relevant e.g. for larval
3558 stages of amphibians, the highest available tier (surrogate reference tier) is represented by mesocosm
3559 studies performed at local scales. In terrestrial effect assessment, dedicated field studies or terrestrial
3560 mesocosms are relevant e.g. for the refinement of some exposure parameters for bird and mammal or
3561 for assessment of the response of in-soil organism communities.

3562



3563

3564 **Figure 21:** Reference tier (RT) versus surrogate reference tier (SRT) in the risk assessment of
 3565 amphibians and reptiles

3566 Amphibians and reptiles may, however, be exposed to multiple stressors due to sequential as well as
 3567 simultaneous use of different pesticides and other agricultural practices, which cannot be exhaustively
 3568 studied in field experiments in the long term. Therefore, a combination of assessing the effects both
 3569 at the local scale - through testing the toxicity of PPPs in effect studies on e.g. aquatic communities -
 3570 and at a larger scale covering field-boundaries and adjacent off-field areas and other field - through
 3571 modelling long-term exposure of single species populations integrating all relevant stressors - is
 3572 proposed as a SRT for amphibian and reptile species to assess population-level effects (Figure 21:
 3573 above). A larger-scale approach (landscape) might be needed for the majority of amphibian and
 3574 reptile species with a range of movement that will be species specific compared with field size.

3575 In this context, it can be assumed that a suitable field study or a mesocosm study (with adequate
 3576 dimensions) can act as a SRT for the assessment of community effects at a field scale for some stages
 3577 of amphibian and reptile species. These studies might well address community composition,
 3578 population dynamics, indirect effects (predation or competition effects), chronic exposure (eventually
 3579 repeated exposure), interactions between and within species and exposure mimicking the actual field
 3580 situation, for example in an aquatic mesocosm.

3581 However, regarding adult stages of amphibian and reptile species, field-effect testing is not considered
 3582 to deliver accurate responses to intended uses of PPP, since i) the test set-ups cannot currently take
 3583 behaviour of mobile species with wide population ranges into account and ii) it is not considered
 3584 valuable and of major use to contaminate whole communities of non-target vertebrates in field
 3585 situations.

3586 Field tests can deliver generic information not depending on the PPP intended to be used, e.g
 3587 regarding presence of species in habitats of interest, their behaviour or on PPP residues on their food
 3588 habits.

3589 Long-term dynamics at a population level over one or more seasons, embracing both population
 3590 growth and spatial dynamics in-field as well as recolonization, should be tackled by modelling
 3591 approaches (combining spatial and temporal population models). The rate parameters of these
 3592 processes may depend on the field size, the spatial configuration of the crop, the PPP application
 3593 scenario (in rows or over the entire area) and the existence and dimension of field boundaries and
 3594 adjacent off-field areas. In order to assess population-level effects, models for different ecological and
 3595 agricultural practice scenarios should be developed for relevant key species, with different
 3596 vulnerability components, and further validated.

3597 In all cases when population modelling is used, the development of suitable baseline scenarios against
 3598 which to evaluate the effect is critical. Depending on the SPG, however, it is not always easy to

3599 determine which baseline will provide the most sensitive outcome (see chapter 4). For this reason, the
3600 Panel recommends that in all cases a representative range with several baselines should be used,
3601 from intensive agricultural systems to extensive sustainable systems, and natural conditions in case
3602 off-field or boundary-scale scenarios are needed. In the case of assessment of effects on a local scale,
3603 especially in order to define normal operating ranges for communities as reference tier, extensive
3604 agricultural systems with high diversity would possibly deliver the communities to be investigated.

3605 **7.4. Surrogate reference tier (SRT) and the systems approach**

3606 Current practice in the prospective assessment of risks from PPP use is to conduct the exposure and
3607 effect assessment for one PPP at a time. An important question is whether the chemical-by-chemical
3608 approach in the current prospective environmental risk assessment (ERA) for PPPs is sufficient also to
3609 prevent cumulative risks from exposure to different PPPs, as well as to predict ecological recovery. It
3610 is therefore important to take into account the impact of multiple stressors on the state of the
3611 population when assessing a particular PPP impact. Thus, a systems approach is considered
3612 appropriate by EFSA (EFSA Scientific Committee, 2016a) owing to the complexity of ecological
3613 systems and the need to evaluate direct and indirect effects and recovery in spatial and temporal
3614 dimensions. In this context, a *systems approach* is defined to mean taking into account the range of
3615 factors considered to potentially interact and affect the result of the risk assessment. This could
3616 include, for example, multiple applications and non-chemical stressors as they might affect the
3617 organisms considered in the assessment. It may also include indirect effects and abiotic factors. The
3618 surrogate reference tier (SRT) for this type of assessment would thus be an implemented ecological
3619 model system including the important factors identified.

3620 In many other risk-assessment schemes (e.g. non-target arthropods, aquatic systems) the *systems*
3621 *approach* has been defined as essential owing to the impacts of both spatial and temporal drivers of
3622 population change. Spatial drivers, in particular action at a distance are relevant for those groups of
3623 organisms (EFSA PPR Panel, 2015a; EFSA Scientific Committee, 2016a). The scales and rates of
3624 movements might be even larger for amphibians and reptiles than for invertebrate species; primary
3625 drivers to be considered are temporal drivers of population change, i.e. the vital rates and migrations
3626 to and from the treated fields. The measurement endpoint in focus of this type of assessment could
3627 be the long-term population growth rate. Please refer to chapter 4.2.2. for more details on relevant
3628 population-model endpoints.

3629 In order to adopt a systems approach and to integrate this into the risk assessment, several steps
3630 have been described as necessary:

3631 Relevant taxa and focal cropping systems need to be identified to create relevant scenarios. These
3632 species need to cover those where population impacts and recovery can be related to the SPGs (e.g.
3633 EFSA 2009b)

3634 The normal operating range of relevant taxa needs to be identified (bearing in mind that this may
3635 vary in time and between different ecosystems). This is used to establish different baselines against
3636 which the system with the addition of the regulated pesticide can be assessed. Such baselines would
3637 need to be established for the range of scenarios needed to represent the range of conditions that the
3638 assessment should cover (e.g. low input and high input agro-ecosystems);

3639 Good mechanistic effect models, which are both manageable and realistic enough, will need to be
3640 developed. Food-web modelling is required in order to assess effects on other species in an ecological
3641 network (De Ruiter et al., 2005). The use of food-web models for assessment would, however, require
3642 that they are predictive and that their predictive quality has been proven in independent experiments.
3643 Hence, although food-web models are conceptually suitable and appropriate, parameterization and
3644 uncertainty of predictions are challenges in their application in risk assessments. For community-level
3645 assessment, recourse must therefore be made to field studies. Note also that the longer time-frame
3646 for field-study assessment provides the potential to detect delayed community or life-history effects
3647 e.g. as a result of reproductive impacts. Food-web models may, however, play an important role in
3648 terms of understanding the case-specific results of field studies. In contrast, population models are
3649 relatively easy to develop and require fewer case-specific data. Hence, for assessment of long-term
3650 impacts, the use of population models is proposed.

3651 The models to be developed do not need to take every possible management scenario into account.
3652 In edge-of-field surface waters, there are typically 2-3 pesticides dominating the mixture in terms of
3653 toxic units (see e.g. Belden et al., 2007; Liess and von der Ohe 2005; Verro et al., 2009).
3654 Consequently, when addressing cumulative stress of pesticides in ERA, it seems cost-effective to focus
3655 on those pesticides that dominate the exposure in terms of toxic units in the relevant medium (e.g. >
3656 90%). It is important, however, that a range of scenarios altering potential vulnerability of populations
3657 is taken into account (e.g. highly stressed populations may be more vulnerable to further stressors).

3658 Information on the distribution of crops in agricultural landscapes and frequently occurring pesticide
3659 combinations may be derived from existing databases (e.g. databases under the EU subsidies scheme
3660 and databases from EU pesticide usage as collected within the frame of the Sustainable Use Directive;
3661 Garthwaite et al., 2015). This information may provide important inputs for population models to
3662 evaluate effect periods and recovery times following pesticide stress in a realistic, agricultural
3663 landscape context (e.g. Focks et al., 2014).

3664 **7.5. Recovery**

3665 Recovery can be assessed at the levels of individuals, populations, communities, or functions. In
3666 broad terms, recovery can be thought of as the return of an ecological entity (e.g. structure such as
3667 abundance, or function such as an ecosystem service) to its normal operating range (sometimes
3668 referred to as baseline properties), having been perturbed outside that range by a stressor (or
3669 multiple stressors). In order to assess recovery, it is first necessary to define what the normal
3670 operating range of the ecological entity and/or process is (EFSA Scientific Committee, 2016a).

3671 Recovery can be classified into two main types, depending upon whether it occurs *in situ* (internal
3672 recovery) or *via* dispersal (external recovery). Both types of recovery may be exhibited by the same
3673 ecological entity (e.g. at different stages in a species' life-history). However, those organisms more
3674 dependent on external recovery will require larger scales (in both time and space) to represent their
3675 systems adequately.

3676 EFSA recommends a systems approach in the cases where recovery is assessed (EFSA Scientific
3677 Committee, 2016a). This is due to the need to consider spatial dynamics resulting in action at a
3678 distance; hence, evaluating recovery at too small a scale may result in erroneous conclusions
3679 (Topping et al., 2014). The systems-level approach takes into account changes in time and space over
3680 a larger scale, thus subsuming recovery under the long-term impacts on the overall system state (e.g.
3681 represented by population size). If initial effects are considered tolerable, recovery can be considered
3682 as an essential and integral dynamic of any system subject to regulated stressors, but may not need
3683 to be taken into account explicitly if long-term system state is used for ERA.

3684 According to EFSA Scientific Committee (2016a), in order to show that there will be actual recovery
3685 under realistic conditions of use, any experimental or modelling approach (or combination of
3686 approaches) needs to consider:

- 3687 - the properties of potential stressors (including the timing of applications relative to life-history
3688 stage, the number and frequency of applications of the same PPP and the cumulative risks of
3689 exposure to multiple PPPs)
- 3690 - direct and indirect effects (species interactions)
- 3691 - the relevant taxa and their traits, e.g. related to demography, dispersal and foraging behaviour
3692 as well as adaptation to potential stressors
- 3693 - the specific features of the landscape, i.e. variations in land use, and the types, spatial
3694 distribution and connectivity of habitats

3695 The tools used to develop the systems approach are mechanistic models for prediction,
3696 experimentation, monitoring, and expert elicitation. Experimentation usually involves semi-field and
3697 field studies, which are primarily used for evaluating community interactions, and experimentation and
3698 monitoring are employed as a reality check and to guard against unexpected effects.

3699 There exists a number of potential modelling approaches to assess recovery (please refer to EFSA
3700 Scientific Committee, 2016a). Employing these approaches to develop systems models, however,

3701 entails a high demand for data and expert skills for both the development and validation of potential
3702 models, especially in cases where external recovery is an important part of the dynamics.

3703 In the case of amphibians and reptiles, recovery may not be considered as an option since any impact
3704 on populations of e.g. endangered species is unlikely to be allowed. Short-term recovery e.g. by local
3705 density-dependent compensation during larval stages may still need to be considered.

3706 **7.6. Ecotoxicologically Relevant Exposure Quantity**

3707 EREQ was developed from the Ecotoxicologically Relevant type of Concentration (ERC, e.g. EFSA
3708 2005, Boesten et al. 2007). The ERC is a description of the best predictor of ecotoxicological effects
3709 that has been further developed in this Opinion (based upon Arts et al, 2015) in order to fit exposure
3710 assessment in e.g. terrestrial risk assessment, where many exposure quantities (e.g. application rates)
3711 are not reported or translated in terms of concentrations. Examples of quantities are mass, length,
3712 surface area, volume etc. (Bureau International des Poids et Mesures, 1998). The more generic
3713 Ecotoxicologically Relevant Exposure Quantity was introduced to include these other quantities,

3714 The Ecotoxicologically Relevant Exposure Quantity (EREQ) provides the link between the exposure
3715 and the effect assessment of PPP. EREQ is not a value but a type of exposure quantity that gives the
3716 best, or appropriate, correlation with the ecotoxicological effects. An example of EREQ for tadpoles is
3717 concentration of dissolved pesticide molecules in the pond water; or for frogs hibernating in the
3718 sediment, concentration in pore water averaged over the top centimetre of sediment.

3719 A clear definition of EREQ is important as it forms the bridge between two fields of expertise:
3720 ecotoxicology and environmental chemistry, each with their own view on exposure in the ecotoxicity
3721 tests and in the field. In order to facilitate clear communication between the two types of experts,
3722 Boesten et al. (2007) explained which aspects of the exposure metrics should be defined. First of all,
3723 the quantity (including its spatial scale) itself plus its temporal scale should be described and next, (i)
3724 its name, (ii) conceptual definition, (iii) mathematical definition and (iv) operational definition (i.e. how
3725 the quantity can be determined experimentally) should be described. The spatial scale of the EREQ is
3726 relatively straightforward, e.g. 'in the pond water', 'in the top 5 cm of sediment'. The temporal scale
3727 should represent the best suited period of time for the effect assessment and is e.g. 'maximum in
3728 time', or 'maximum time-weighted average over five days'. An example of the other aspects is (i)
3729 name: concentration of dissolved pesticide in pond water, (ii) conceptual definition: mass of dissolved
3730 pesticide per volume of pond water, (iii): mathematical definition: $c = c^* - s X$ with $c =$
3731 this quantity (mg/L), $c^* =$ total concentration of pesticide in pond water (mg/L), $s =$ concentration of
3732 suspended solids in water (kg/L) and $X =$ content of pesticide sorbed to suspended solids (mg/kg),
3733 and (iv) operational definition: extract water with organic solvent after filtering water and measure
3734 pesticide mass in solvent. The conceptual and operational definitions are relevant for both the effects
3735 and exposure assessments, while the mathematical definition is especially relevant for the exposure
3736 calculations.

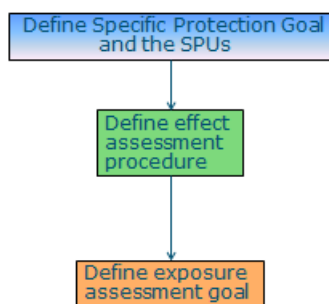
3737 The outcome of the exposure assessment is determined by the EREQs and their temporal scales.
3738 Different EREQs plus temporal scale lead to different selected scenarios. For example, if the EREQ was
3739 the concentration of dissolved pesticide in the pond water, the worst-case pond would have sediment
3740 with a low organic matter content. However, if the EREQ would be the total content of pesticide in the
3741 top 5 cm of sediment, the worst-case scenario would be a pond with high organic matter sediment.
3742 So, the exposure metric may differ for Service Providing Units of the SPGs. Examples are:
3743 mg/individual, mg/kg organic matter in sediment, mg/L, mg/m² soil surface area.

3744 **7.7. Exposure Assessment Goals**

3745 Exposure Assessment Goals concern the estimation of the exposure to pesticides of the Service
3746 Providing Units (SPUs) of the SPG in the environment, in the vicinity of agricultural pesticide-treated
3747 fields (represented by the right-hand boxes marked by a capital F in Figure 20:). So the Exposure
3748 Assessment Goals indicate the spatial unit for which the exposure should be assessed. Selecting
3749 spatial units requires insight into the ecotoxicological traits and behaviour, as well as insight into the
3750 elements determining the probability of exposure. Ecotoxicological experts and environmental fate
3751 experts should therefore cooperate to select the most relevant spatial units.

3752 In order to be able to define the Exposure Assessment Goals, it is necessary first to define the SPGs
 3753 for each of the SPUs and the way the effects are assessed for the selected SPUs (Figure 22: 6 below).
 3754 In the effect assessment the type of concentration (e.g. concentration in the water, peak or time-
 3755 weighted average) that gives the best correlation to the observed ecotoxicological effects needs to be
 3756 established. Only next, it is possible to design the exposure assessment goal schemes that deliver the
 3757 wished Ecotoxicologically Relevant Exposure Quantity (EREQ).

Sequence of activities, needed for exposure assessment goal definition



3758

3759 **Figure 22:** Sequence of activities to be able to define the Exposure Assessment Goal

3760 The next example illustrates this statement. Imagine we would like to estimate the exposure in an
 3761 environmental compartment, e.g. ponds where amphibians live. The exposure is *via* contact with pond
 3762 water receiving spray- drift deposition and pesticide-loaded runoff. However, a contact exposure EREQ
 3763 cannot be delivered, as long as it is not known what is exactly needed: e.g. which type of
 3764 concentration (peak or some time-weighted average?), when (life stage corresponding to a certain
 3765 time of the year, length of exposure), or where exactly (which type or size of ponds?). So, only when
 3766 the effect assessment procedure, including the Service Providing Unit and EREQ, has been defined, it
 3767 is possible to make a full exposure assessment, as described in an Exposure Assessment Goal Table
 3768 15: .

3769 Here we give a schematic description of the elements used in Exposure Assessment Goals in general
 3770 terms based upon the EFSA protection goal opinion and the Guidance by EFSA SC (EFSA, 2010, p. 47
 3771 and EFSA SC 2016). For communication purposes we listed these elements in terms of a table (Table
 3772 15:). More detailed descriptions tailored to, and in terms applicable to, the specific protection goals
 3773 for amphibians and reptiles are presented later in Chapter 11.

3774 **Table 15:** Description of the elements of the exposure-assessment goal linked to a certain specific
 3775 protection goal for a newt species. The abbreviation 'EA' stands for eco(toxico)logical
 3776 aspects of the SPG and RM stands for risk managers.

Element	Explanation	Defined by	Examples
EREQ	Ecotoxicologically Relevant Exposure Quantity, i.e. key for linking with effect assessment	EA	<ul style="list-style-type: none"> - concentration of dissolved pesticide in pond water; - pore-water concentration averaged over top 20 cm of soil surrounding reptile eggs - mass on surface area of newt migrating through agricultural field
Temporal dimension of EREQ	Determined by the requirements set by effect assessment (usually different for acute and chronic effects)	EA	<ul style="list-style-type: none"> - peak concentration in pond water in a single year between May and September; - annual maximum of 21d TWA concentration in pore water of top 20 cm soil during relevant period; - maximum mass gathered on newt during its migration through agricultural field
Spatial unit (SU),	Basis of SPG; link to each	EA	<ul style="list-style-type: none"> - pond with a minimum diameter of e.g. 5m and a

type and size (if relevant)	Service Providing Unit (SPU). Size refers to distance or area over which averaging of EREQ values is considered acceptable in view of SPG	RM	<ul style="list-style-type: none"> – minimum water depth of e.g. 20cm; – 1 m² of agricultural field (in-crop); – agricultural field
Statistical population of SUs	Statistical population of spatial units considered in exposure assessment	RM	<ul style="list-style-type: none"> – ponds within or at a distance of 100 m from agricultural fields treated with this pesticide; – all square metres within agricultural fields treated with this pesticide; – all treated fields in area of use located in possible migratory areas
Multi-year temporal statistical population of EREQ values for one spatial unit	Based on above specifications; time series needs to be long enough to be fit for purpose	EA	<ul style="list-style-type: none"> – all annual maxima in pond water – all annual maxima of 21-d TWA concentration in 1 m² of the agricultural fields treated with this pesticide; – all annual maximum masses on newt in the treated fields
Desired spatio-temporal percentile of the statistical population of EREQ values	Determines which part of the spatio-temporal population is excluded from the effect assessment (and may thus experience effect)	RM EA	<ul style="list-style-type: none"> – 90th overall percentile of all EREQ values (e.g. for a 20-year time series in 500 ponds, this equals 10 000 values); – 90th percentile in space in 50th percentile in time of the EREQ values – 95th percentile in space of all EREQ values

3777

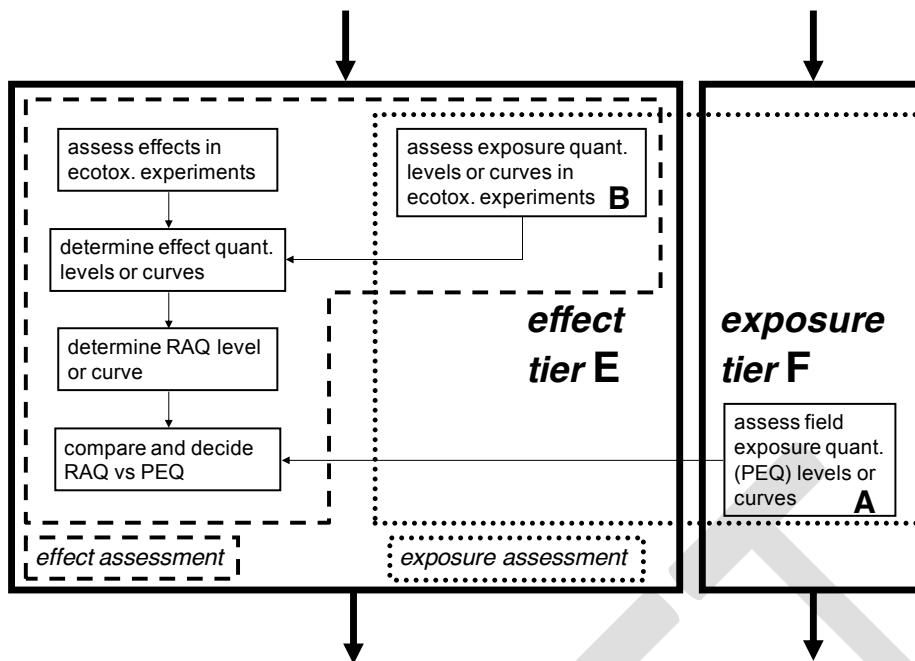
3778

7.8. Linking exposure assessment to effect assessment

3779 The risk assessment consists of two parts: (i) assessment of effects, derived from (eco)toxicological
3780 experiments (= effect assessment) and (ii) assessment of concentration levels in relevant
3781 environmental compartments or on the relevant organisms resulting from pesticide application (=
3782 exposure assessment). The EREQ has been defined as the exposure quantity that gives the best
3783 correlation to ecotoxicological effects and thus forms the interface between the effect and exposure
3784 assessments. The definition of the EREQ has allowed the tiered effect and exposure assessments to
3785 interact in a flexible and efficient way as is shown by the so-called criss-cross model of Boesten et al.
3786 (2007) in Figure 20: .

3787 For the risk assessment, two distinctly different exposure concentration estimates are required: the
3788 concentration related to exposure in the ecotoxicological experiments and the concentration related to
3789 exposure in the field. In the ecotoxicological experiment, assessing the exposure concentration refers
3790 to the selection of the relevant concentration to express the selected endpoint, i.e. the concentration
3791 that correlates best with the selected effect, such as mortality or reduced growth. Examples are the
3792 nominal concentration, or the time-weighted average concentration during the initial 21 days of the
3793 experiment.

3794 The same type of concentration should be used consistently for both types of exposure
3795 concentrations. This is represented in Figure 23: of Boesten et al (2007), which shows the necessary
3796 activities in any combination of tiers of the effect and exposure flow charts. The four blue boxes
3797 drawn vertically at the left-hand side in the effect tier box E concern the effect assessment, while the
3798 two boxes with the red circle around them concern the exposure assessment. So, both in the
3799 exposure tier F as in the effect tier E, the same type of EREQ needs to be estimated as exposure
3800 concentration.



3801

3802 **Figure 23:** Schematic representation of activities in any combination of tiers of the effect and
 3803 exposure flow chart. Note that there are two distinctly different exposure assessments in the risk-
 3804 assessment procedure, one being part of the exposure tier F that delivers field exposure and one
 3805 being part of the effect tier E.

3806 In the risk assessment for regulatory purposes, decisions can be made by comparing the endpoint of
 3807 the effect to the endpoint of the exposure assessment. Adjusting Boesten et al (2007), we might use
 3808 for this purpose the terms of RAQ, Regulatory Acceptable Quantity (instead of RAC, Regulatory
 3809 Acceptable Concentration) and PEQ, Predicted Environmental Quantity (instead of PEC, Predicted
 3810 Environmental Concentration) to encompass all exposure metrics used in both the aquatic and
 3811 terrestrial environments, so not only mass per volume units, but also mass per area units or mass per
 3812 mass units. This new terminology has been used in Figure 23: . Note that Figure 23: illustrates the
 3813 procedure not only for the aquatic environment, but also for the terrestrial environment, even if the
 3814 exposure in the ecotoxicological experiment is assessed in terms of quantities per individual, e.g. mass
 3815 deposited per frog.

3816 Boesten et al (2007) proposed to make regulatory decisions for the aquatic environment according to
 3817 a tiered approach, in which RAQ and PEQ are compared according to (i) in a first step, single RAQ and
 3818 PEQ levels based on conservative assumptions, (ii) in a second step, graphically RAQ and PEQ curves
 3819 (describing the time courses of the RAQ and PEQ), and (iii) in a third step, time-weighted average
 3820 RAQ and PEQ levels. For the terrestrial environment this approach might be expanded to risk
 3821 assessment, if the exposure in the terrestrial ecotoxicological experiment is assessed in terms of
 3822 environmental concentrations or quantities, e.g. mass deposited per m². If the exposure in the
 3823 terrestrial ecotoxicological experiment is assessed in terms of quantities per individual, e.g. mass
 3824 deposited per frog, the suggested tiered risk-assessment approach needs further testing and possible
 3825 development.

3826 7.9. Combination assessment

3827 The likelihood of showing effects depends not only on the type of active substance, the level and
 3828 length of exposure, but also on other substances in the PPP and the route of entry. The later two
 3829 aspects are addressed here by combination assessment.

3830 Mixture toxicity of several substances has been addressed in previous guidance documents and
 3831 scientific opinions from EFSA (EFSA 2013, EFSA 2015, EFSA 2016a). The importance of addressing the
 3832 effects of chemical mixtures in ecological risk assessments has, furthermore, been elaborated in a
 3833 report issued by the European Commission (SCHER, SCCS, SCENIHR, 2012). In this report a

3834 differentiation was made with regards to the relevance of mixture toxicity between the human and
3835 ecological risk assessment. For human health effects, if the intended level of protection is achieved for
3836 each individual substance, the level of concern for mixtures of dissimilarly acting substances should be
3837 assumed to be negligible. For the ecological assessment, however, population/community effects
3838 cannot be excluded based on acceptable no effect concentrations for each component. The current
3839 default assessment factors are not considered adequate to cover effects of mixtures, hence mixtures
3840 also need to be assessed for dissimilarly acting substances.

3841 With regards to assessing the risk to amphibians and reptiles in nature, several aspects need to be
3842 considered:

- 3843 • Pesticidal active substances are combined with co-formulants for optimal efficiency,. It is
3844 therefore not only the active substance but the active substance in combination with the
3845 formulation that affects target and non-target organisms.
- 3846 • Amphibians may be exposed in several environmental compartments (aquatic and terrestrial)
3847 due to their mobility and biphasic life histories, and/or multiple use of different PPP in an area
3848 to mixtures within one compartment.
- 3849 • Amphibians or reptiles may be exposed *via* several routes within one compartment, such as
3850 oral uptake of contaminated food and dermal exposure by contact with contaminated soil.

3851 In order to achieve the protection goals formulated for amphibians and reptiles within the context of
3852 pesticide authorisation, it is therefore relevant to consider the effects of the formulation, the exposure
3853 in different environmental compartments over the life span of an individual and the uptake *via* several
3854 routes of exposure.

3855 **7.9.1. Consideration of PPP formulations in the risk assessment**

3856 Generally speaking, co-formulants (e.g. adjuvants) can increase the toxicity of active ingredient
3857 toxicity relative to formulation toxicity in freshwater species (Mayer and Eilersieck, 1986; Schmuck et
3858 al., 1994). With regards to amphibians, several studies have shown the impact of co-formulants on
3859 the toxicity of active ingredients. Increased toxicity of the formulation to amphibians in comparison to
3860 the technical a.i. were reported for cycloxydim (Wagner et al., 2015), pyraclostrobin (Brühl et al.,
3861 2013; Hooser et al., 2012), azinphosmethyl (Nebeker et al., 1998), paraquat (Linder et al., 1990) and
3862 permethrin (Boone, 2008). The most prominent and well studied example is glyphosate. In order for
3863 the anionic glyphosate to penetrate the cuticle of many plants, it is usually formulated together with
3864 surfactants such as polyethoxylated tallowamines (POEA). The addition of the POEA increases the
3865 acute and sub-lethal toxicity of glyphosate to amphibian larvae (Wagner et al., 2013), which is
3866 determined by the ratio of glyphosate to surfactant (Mann et al., 2009).

3867 Knowledge about the toxicity of the active substance does not *per se* allow a prediction about the
3868 effect of the formulation on the toxicity. This has already been addressed in the data requirement
3869 (Commission Regulation No 284/2013), where studies with PPP are required if the effects cannot be
3870 predicted from the active substance data. Insufficient knowledge is available at present to identify
3871 formulations that increase the effects of the active substance for amphibians and reptiles. One reason
3872 is that scientists outside industry do not know the composition of the formulations.

3873 If the toxicity of all components of the formulation is known, the toxicity can be calculated using the
3874 concentration addition (CA) approach as described in EFSA guidance documents (e.g EFSA 2009, EFSA
3875 2013, see also chapter 7.9.3), although there are slight differences between them (Panizzi et al.,
3876 2017). The underlying concept of CA is that the individual components of the mixture contribute to
3877 the mixture toxicity in proportion to their individual concentration and potency (Kortenkamp et al.,
3878 2009). In contrast to Directive 91/414, the current Regulation 1107/2009 requires that safeners,
3879 adjuvants and synergists should also be assessed. Potential interactions with these are not, however,
3880 routinely assessed (Panizzi et al., 2017). Whether or not the combined toxicity of the whole
3881 formulation can be addressed by the CA approach therefore depends on the available information. An
3882 experiment may often be needed to allow the required assessment of the formulation.

3883 7.9.2. Consideration of mixtures in environmental compartments

3884 Mixture toxicity will also be of significance considering the location of shallow breeding ponds possibly
3885 in the middle of a field, where a mixture of pesticides is expected to be present. Shallow or temporary
3886 ponds may accumulate pollutants without substantial dilution (Mann et al., 2003).

3887 An increase in toxicity to *Rana pipiens* owing to the mixture of nine active substances at ecologically
3888 relevant concentrations (0.1 µg/l) compared with sublethal concentrations of the individual active
3889 substances was observed by Hayes *et al.* (2006); toxic effects included retarded and reduced larval
3890 growth, delayed development (metamorphosis) and increased disease rates, with predictable, adverse
3891 consequences for survival and reproduction. Mixtures of pesticides need to be investigated in order to
3892 assess adverse impacts on amphibian development or to address the role of pesticides in amphibian
3893 declines. Substances (e.g. S-metolachlor) that showed no effect on their own in the study increased
3894 the effect of other substances (e.g. atrazine), but this increased effect could be mitigated by a
3895 surfactant in a commercial mixture. Additive effects have been observed for diazinon, carbaryl,
3896 malathion and glyphosate (Relyea 2004). Synergistic effects were observed for atrazine and
3897 chlorpyrifos in *X. laevis*, but not in *R. calmitans* (Wacksman et al., 2006). Synergistic interactions may
3898 be chemical-specific and not ubiquitously relevant. Mixture-toxicity assessments may be especially
3899 relevant for substances with similar modes of actions but, given the mode of action is rarely known in
3900 non-target organisms, the combination assessment should not be limited to those.

3901 This aspect is relevant with regards to the authorization of products containing several active
3902 substances, but is outside this remit with regards to mixtures originating from the application of
3903 different products over time.

3904 Combined exposure may also occur by moving through different compartments (either from the
3905 aquatic to the terrestrial or from field to field). The carry-over effect from the aquatic to the terrestrial
3906 environment is not expected to occur concurrently. The biphasic life history of amphibians may,
3907 however, lead to an exposure in the terrestrial (by maternal transfer) and aquatic (by dietary of
3908 aqueous exposure) environments. This was investigated in a study with mercury, where a double
3909 jeopardy of exposure in the aquatic and terrestrial phases was identified for the American toad (Todd
3910 et al., 2011). The sequential exposure to active substances was investigated with *Gammarus pulex*,
3911 where the order of exposure was shown to affect the toxicity due to carry-over toxicity (Ashauer et
3912 al., 2017). The assessment of the sequential use of pesticides is not required yet, but the necessity
3913 has been identified (Verbruggen and Van den Brink, 2010). A further challenge with regards to
3914 assessing the spatial-temporal aspects is the assessment of the mixture composition in the
3915 environmental compartments as biodegradation may be affected by the mixture. Data are missing
3916 with regards to exposure of amphibians or reptiles to multiple pesticides in different compartments
3917 over their life history. This combined exposure can therefore only be addressed through experiments
3918 or considered in the uncertainty analysis.

3919 7.9.3. Consideration of toxicity resulting from different routes of exposure

3920 An individual may be exposed by a number of relevant exposure routes as described above. It is
3921 therefore considered necessary to assess the impact of pesticides on amphibians and reptiles resulting
3922 from a combination of exposure routes. A pragmatic worst-case approach for the first tier risk
3923 assessment could be to combine the relevant terrestrial exposure routes by following the approach for
3924 mixture toxicity suggested in the EU guidance documents for different pesticides. The model used to
3925 estimate the toxicity of mixtures in those approaches is the assumption of dose/concentration
3926 additivity of toxicity (Loewe & Muischneck, 1926, frequently referred to as Concentration Addition
3927 (CA)) (e.g. Frische et al.2009, Altenburger et al. 2013).

3928 The following formula is used to derive a surrogate LC50 for the mixture of active substances with
3929 known toxicity assuming dose additivity:

$$LC_{50}(mix) = \left(\sum_i \frac{X(a.s._i)}{LC_{50}(a.s._i)} \right)^{-1} \quad (1)$$

3930

3931 where:

3932 X (a.s. i) = fraction of active substance (i) in the mixture

3933 LC50 (a.s. i) = acute toxicity value for active substance (i)

3934 Because of the direct proportionality of the calculated TER to the LC50 (or any other relevant toxicity
3935 value), it is also possible to calculate a TER(mix) with the following formula, often referred to Finney's
3936 equation:
3937

$$TER(mix) = \left(\sum_i \frac{1}{TER(a.s.i)} \right)^{-1} \quad (2)$$

3938

3939 where:

3940 TER (a.s.i) = calculated TER for the active substance i

3941 TER(mix) = calculated TER for the mixture

3942 A calculated NOEC(mix) does not always constitute a reliable measure of toxicity because of (a) the
3943 dependency of NOEC values from experimental dose-spacing, and (b) the diversity of biological
3944 endpoints in long-term/chronic toxicity tests. Against this background, the calculated TER(mix) for a
3945 long-term/chronic risk is only applied in the assessment in combination with additional considerations
3946 of its possible relevance in terms of actual risk, for example in the risk assessment for bird and
3947 mammals.

3948 The different terrestrial exposure routes for amphibians and reptiles, such as overspray, contact with
3949 soil or plants and uptake of food, might affect the same or possibly different organs of the animal,
3950 leading to the potential accumulation of effects. It cannot be assumed *per se* that the effects occurring
3951 in different organs are not affecting the overall health of the organism more than by exposure to a
3952 single route. A pre-exposure by dermal exposure may make an animal more susceptible to adverse
3953 effects if the same substance is also taken up orally. Dermal uptake by direct overspray or contact
3954 with contaminated soil may lead to local damaging effects on the skin or affect respiration, which may
3955 also be affected by inhalation.

3956 The exposure by different routes is expressed in different units (i.e. kg/ha or mg/kg bw/d). In order to
3957 avoid the conversion of the units to internal doses for the first tier risk assessment, it is suggested to
3958 estimate the risk stemming from different exposure routes in a pragmatic approach using the Finney
3959 equation assuming additive toxicity. By combining the risk ratios for the different exposure routes, the
3960 units are eliminated and the relevant exposure scenarios in conjunction with possible different toxicity
3961 potentials by the different routes of exposure can be considered. An independent action in different
3962 target organs might occur, but is covered by this worst-case approach.

3963 The proposed risk assessment for the combination of exposure routes could be calculated as follows:
3964

$$TER(mix) = \left(\sum_i \frac{1}{TER(e_i)} \right)^{-1} \quad (3)$$

3965

3966 Or (e.g.)

3967 $1/TER(mix) = 1/TERe1 + 1/TERe2 + 1/TERe3$

3968

3969 Where:
3970 TER(mix) = calculated TER for the combined exposure routes
3971 TER (e i) = calculated TER for route of exposure i
3972 e.g. e1 = oral risk quotient
3973 e.g. e2 = dermal overspray risk quotient
3974 e.g. e3 = dermal soil uptake risk quotient

3975 Calculating first the risk separately for every exposure route gives the possibility to use those
3976 parameters that are relevant for the single exposure route (e.g. exposure models) and to use toxicity
3977 values (probability of effect incidence) that are typical of that route. For example, LC50 values from
3978 dermal exposure derived from direct overspray might be lower after spraying the intended rates than
3979 LC50 values for oral exposure after feeding on food items sprayed with the same dose. Each exposure
3980 route may then be refined separately prior to assessing the combined risk. TK/TD-models may be
3981 considered relevant in a higher tier approach. The risk assessment may concentrate on a single
3982 exposure route if it clearly dominates the risk. The risk for the combined exposure is acceptable if the
3983 assessment factor for the first tier is met. If refined assessment factors are used for the different
3984 exposure routes, the approach described in the aquatic guidance document may be considered. This
3985 approach is considered suitable for the acute risk assessment of combined exposure routes as the
3986 same endpoint (mortality) is assessed. For the chronic risk assessment of combined exposure routes,
3987 the systemic effects are expected to be the same, independently of the route of exposure, but may
3988 differ in time of reaction and strength. Local effects, however, depend on the route of uptake. As
3989 described above, effects affecting different organs may decrease the overall health of the organism in
3990 combination. Endpoints may, therefore, also be combined for the chronic risk assessment.

3991

3992 7.10. The risk assessment flow chart

3993 Amphibians and reptiles are vertebrates for which no agreed risk-assessment scheme is available.
3994 There are several tests available that permit the detection of effect concentrations related to acute
3995 and chronic toxicological endpoints. Sensitive and standardized protocols are not, however, available
3996 for all life stages and exposure scenarios identified as relevant for amphibians and reptiles in
3997 agricultural landscapes (see section 8 for further details).

3998 Amphibians and reptiles are not only vertebrates but a group with a high proportion of endangered
3999 species (section 2). The Panel is therefore reluctant in principle to propose dedicated toxicity studies
4000 as a standard requirement for future risk-assessment schemes.

4001 The toxicity data available are, however, scarce and a great proportion of the available information -
4002 apart from studies on amphibian metamorphosis – refers to mortality or to sub-lethal endpoints after
4003 short term exposure as chosen endpoints. It might therefore be necessary, in the short-term, to
4004 perform toxicological studies with amphibian and reptile species in order to increase mechanistic,
4005 toxicological knowledge.

4006 The declared goal of the Panel, though, in the mid- and long-term is to derive initial risk triggers to
4007 discriminate between PPP (or active substances in PPP) with potentially high or low toxicity for
4008 amphibians and reptiles; this is in order that no tests, or only a small number, will be necessary for
4009 addressing the risks for these groups in the future. The strong recommendation of the Panel is to
4010 focus scientific research on the development of combined structure-activity-relationship and *in vitro*
4011 assays to serve in future as first steps in the assessment of risk for amphibians and reptiles. In the
4012 best case, such triggers could be derived in future also from alerts *via* the assessment of toxicological
4013 endpoints available for other non-target organisms or from alternatives to *in vivo* testing.

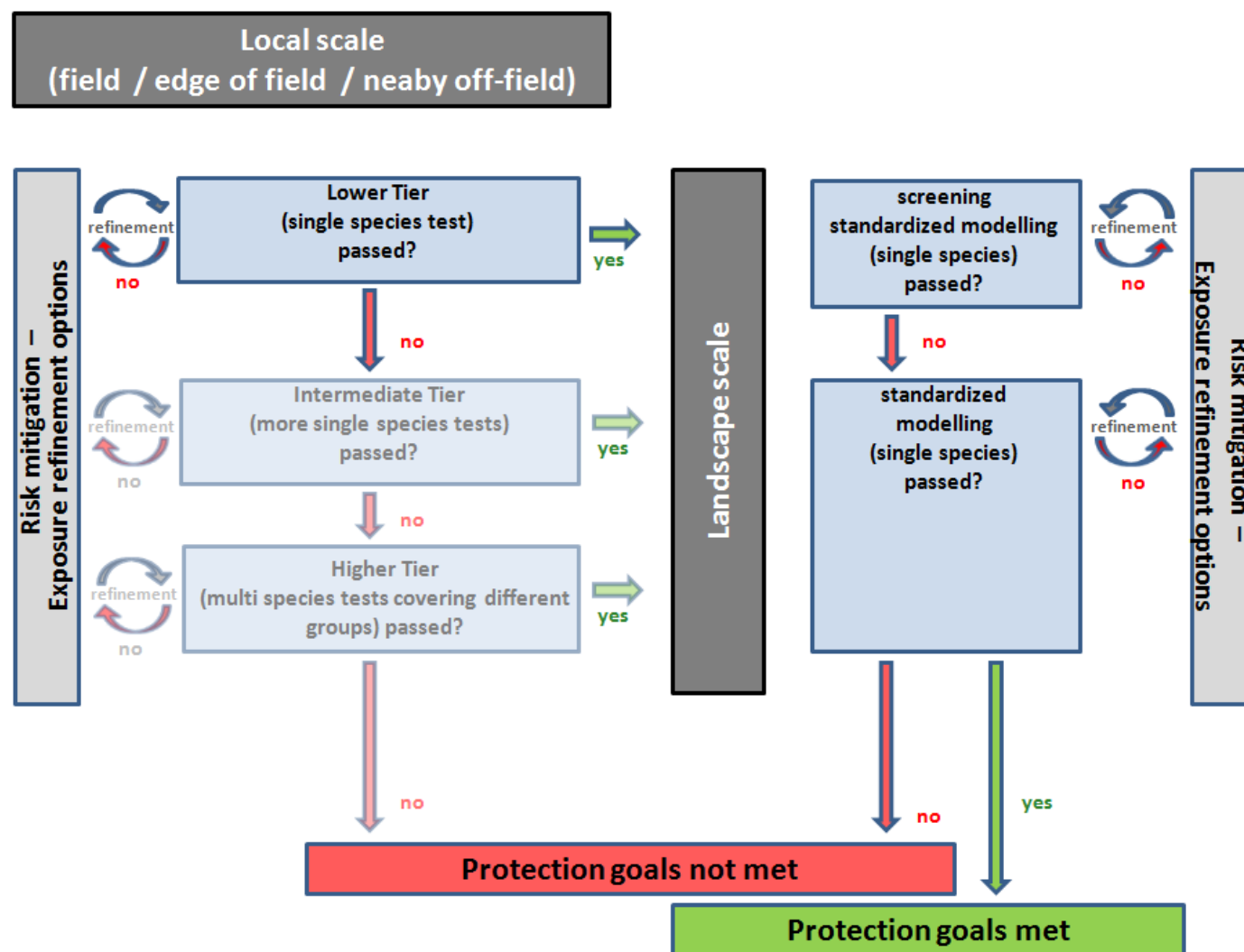
4014 The risk-assessment scheme for amphibians and reptiles would in principle follow similar tiered steps
4015 as for other non-target organisms (see section 7.2). In a proposed risk-assessment flowchart (see

4016 section 7.10), the evaluation of direct and indirect effects should be performed as part of the data
4017 requirements (EU 283/2013⁵ and 284/2013⁶, see also chapter 5.1).
4018

DRAFT

⁵ Commission Regulation (EU) No 283/2013 of 1 March 2013 setting out the data requirements for active substances, in accordance with the Regulation (EC) No 1107/2009 of the European Parliament and of the Council concerning the placing of plant protection products on the market. OJ L 93, 3.4.2013, p. 1–84.

⁶ Commission Regulation (EU) No 284/2013 of 1 March 2013 setting out the data requirements for plant protection products, in accordance with the Regulation (EC) No 1107/2009 of the European Parliament and of the Council concerning the placing of plant protection products on the market. OJ L 93, 3.4.2013, p. 85–152.



4019

4020 **Figure 24:** Illustrative risk-assessment flowchart for amphibians and reptiles exposed to plant-protection products or the active substances in PPP. In
 4021 order to meet the specific protection goals for amphibians and reptiles, the criteria of both the acute and chronic effects assessment at a local scale and
 4022 the long-term population modelling at landscape-scale components have to be passed.

4023 The proposed risk-assessment scheme, as shown in the flowchart (Figure 24:), has in principle
4024 subsequent components: (i) determination of acute and chronic toxicological responses for amphibian
4025 and reptile species at a local scale of assessment (ii) assessment of long-term effects of year-on-year
4026 pesticide application using population modelling at a landscape scale. The principle of the scheme is
4027 that the active substance or PPP must meet acceptability criteria with respect to both components in
4028 order to decide that the protection goal for amphibians or reptiles are met.

4029

4030 **7.10.1. Assessment of risk at the local scale**

4031 The first step, as indicated in the scheme, is to investigate the effects of active substances or PPPs on
4032 amphibians and reptiles in simple laboratory tests. No tests have to be submitted at present according
4033 to the current data requirements (EU 283/2013 and EU 284/2013), though all available information
4034 should be delivered to authorities by the applicants.

4035 Specific exposure scenarios and morphological characteristics of amphibians (and to a lesser extent
4036 reptiles) are not covered by the current assessment. The Panel therefore advises performing studies
4037 with a specific focus. It is proposed to characterize the toxicity of PPP (or the active substance) on
4038 developmental endpoints in chronic studies, since these might not be covered by other available data
4039 (see chapter 10). It is also proposed to identify the toxicity of applied PPP on terrestrial life stages of
4040 amphibians and reptiles *via* dermal exposure routes, since dermal toxicity seems not to be predictable
4041 from endpoints available for other non-target organisms.

4042 Please refer to Table 17: ,Table 18: and Table 19: below for a summary of the life stages considered
4043 relevant in amphibians, the acute and chronic endpoints, the coverage by available endpoints for
4044 other non-target organisms and the conclusions for the risk assessment.

4045 Summarizing the evidence for amphibians, tests in which exposure starts at the juvenile stage and is
4046 prolonged to detect the reproductive performance of amphibians after metamorphosis seem necessary
4047 in the near future (e.g. extended AMA or LAGDA Test, see chapter 8). It should be noted that such
4048 toxicity data also deliver essential toxicity-input parameters for long-term modelling of populations
4049 after year on year of intended uses of PPP. Tests would be needed in worst-case overspray scenarios,
4050 in which the exact exposure quantities can also be determined, in order to characterize the toxicity *via*
4051 dermal exposure for amphibians.

4052 Neither data nor test methods are available for chronic toxicity effects on reproduction of amphibians
4053 and reptiles where adult animals are exposed as in studies with bird and mammals. It is proposed to
4054 extrapolate from available test designs that assess developmental endpoints for amphibians in aquatic
4055 systems to the development of terrestrial stages of amphibians after they have left the water. The
4056 Panel is aware that long-term exposure in both environments may not be comparable. Malformations
4057 derived from exposure in aquatic life stages (e.g. larvae) with far-reaching consequences on adult
4058 performance could however be identified by such tests (e.g. extended LAGDA studies, see chapter 8).

4059 Knowledge gaps should ideally be addressed directly after being identified by this Opinion, so that
4060 possibly no standard additional data will be required for performing a risk assessment according to
4061 future Guidance. If, however, knowledge at time of Guidance development is still lacking, then an
4062 interim guidance for risk assessment with specific requirements for amphibians and reptiles might be
4063 necessary. Guidance could be amended later, as understanding of toxicity mechanisms will be
4064 improved by dedicated studies. Please see the recommendation of the Panel regarding research
4065 priorities for the development of initial triggers for selective testing and alternatives to *in vivo* testing.

4066 The Panel proposes for the time being that some *in vivo* toxicity studies with amphibians are required
4067 for upcoming assessment of active substances for approval. The basis for testing requirements is the
4068 available evidence of the impact on amphibians by intended uses of PPP that have passed the
4069 standard risk-assessment procedures (see chapter 1.4) and the legislative requirement in place (see
4070 chapter 6.1).

4071 Regarding reptiles, however, for the time being the knowledge is so scarce that the Panel cannot
4072 propose specific toxicity studies to close the data gaps. Please refer to section 8.4 for further details
4073 and to Table 16: below.

4074 Regarding the lowest tier of the proposed risk-assessment scheme, test endpoints on effect of PPP on
4075 amphibians and reptiles are compared with the predicted environmental exposure quantities and then
4076 related to acceptability criteria (trigger values). If the acceptability criteria are met for the assessment
4077 at a local scale, then a screening of possible risks at the landscape scale is performed. The
4078 assessment can only conclude that the protection goals are met when both components of the risk
4079 assessment pass the acceptability criteria.

4080 If the relevant trigger values are not met at the lowest tier, the risk may be refined by (i) refinement
4081 of exposure calculation (see chapter 9) or/and (ii) further ecotoxicological testing (section 8), which
4082 might improve the description of the risk for amphibians and reptiles and address specific
4083 uncertainties present at the lowest assessment steps and/or (iii) incorporation of effective risk-
4084 mitigation options.

4085 Intermediate effect assessment steps might address e.g. toxicity testing on more amphibian or reptile
4086 species, in order to reduce uncertainties in species sensitivity distribution. For amphibian species with
4087 life stages in aquatic and terrestrial environment, assessment has to be performed both for the
4088 aquatic and for the terrestrial stages. So-called microcosms or mesocosms (artificial species
4089 assemblages or field tests with amphibians and reptiles) are possible in theory but not useful in
4090 practice, for amphibians in particular; no current field set-up is available that might satisfactorily cover
4091 movements of these animals. The Panel does not, therefore, recommend further testing of
4092 amphibians and reptiles at higher tier assessment steps. Exposure-refinement scenarios, with the
4093 support of risk-mitigation options, should be used.

4094 To indicate the possible lower predictive value for the field situation in addition to difficulties in the
4095 calibration of such test set-ups with lower tiers, all intermediate and higher tier assessment step are
4096 depicted in light grey in the assessment scheme. The Panel is aware that the uncertainties present in
4097 the different assessment steps have to be investigated and/or quantified in order to perform a proper
4098 calibration of a risk-assessment scheme.

4099

4100 **7.10.2. Assessment of risks at the landscape level**

4101 The component of the scheme addressing possible risks at the landscape level includes the
4102 descriptions of the effects of year-on-year application of PPPs in a so-called 'systems approach' with
4103 appropriate population models (see section 4). The assessment of long-term effects on amphibian and
4104 reptile species is needed, since it tackles uncertainties in the risk assessment that already have to be
4105 addressed at lower tiers. Experience is still lacking in the implementation of population modelling in
4106 the risk assessment of PPP. The Panel therefore, recommends further research activities in order to
4107 develop relevant population models for amphibian and reptile species and to relate model outcomes to
4108 other measurement endpoints in the risk assessment (see section 4.2).

4109 As defined in chapter 4, one advantage of population modelling is that it takes existing toxicological
4110 response data and translates them into key, population-level endpoints of distribution of animals in
4111 space and time and population persistence. The overall population impact is one endpoint useful for
4112 comparing changes in population size. Other endpoints addressing occupancy in addition to
4113 abundance might also prove helpful. Moreover, species abundance and occupancy can be modelled
4114 over years. The pattern of recovery of a population after PPP impact can be observed if required in
4115 population models, allowing prediction of short or long potential recovery periods after stopping PPP
4116 uses. Population growth rate (PGR) is a critical metric for population status. If $PGR < 1$, the population
4117 will decline and the protection goal is not sustainable.

4118 Appropriate trigger value(s) for model output need to take into account uncertainties relating to the
4119 model, including extrapolation of population modelling results to effects at community level, as well as
4120 further uncertainties. If the trigger value(s) for model output are not met at the lowest tier, it may be
4121 possible to refine the exposure assumptions used in modelling. The results of modelling approaches
4122 assessing the effects of year-on-year application of PPP on amphibians and reptiles species can only
4123 be refined to a very limited extent with further ecotoxicological testing at higher tier (e.g. toxicity data
4124 for other species). Accordingly, information from higher tier tests with amphibians and reptiles (which
4125 are not recommended) will be of limited use in refining the risk indicated by population models, since
4126 these approaches are affected by different uncertainties in the risk assessment. By contrast, a

4127 monitoring programme following registration of PPP would allow for the assessment of effect
4128 predictions on population of amphibians and reptiles.

4129 **7.10.3. Mitigation of identified risks**

4130 Although not in the remit of this Opinion, the Panel acknowledges that risk mitigation measures exist
4131 that have been implemented in practice to mitigate the risk for amphibians and reptiles after intended
4132 uses of PPP (e.g. Brühl et al 2015; Berger et al. 2015).

4133 Measures specifically dedicated to avoid exposure of amphibians to PPP might include i) the improved
4134 management of terrestrial hot spots of amphibian presence, ii) measures for reducing migration
4135 demands of amphibians on crop fields, (iii)) controlling PPP application on fields by time shift of
4136 application dates to reduce exposure of non-target species.

4137 The use of PPPs should clearly follow the principles of Integrated Pest Management, being the last
4138 option after exhaustion of alternative measures. Significant numbers of amphibians and reptiles are
4139 endangered, therefore specific measures are to be taken (Art. 12 EU 2009/128) where "Member States
4140 shall, having due regard for the necessary hygiene and public health requirements and biodiversity, or
4141 the results of relevant risk assessments, ensure that the use of pesticides is minimised or prohibited in
4142 certain specific areas. Appropriate risk management measures shall be taken and the use of low-risk
4143 plant protection products as defined in Regulation (EC) No 1107/2009 and biological control measures
4144 shall be considered in the first place..."

4145 Such risk-mitigation measures will be most effective if applied at a local level rather than at a wider
4146 regulatory level (e.g. Member State Level), since their applicability and implementation will depend on
4147 the species that are at risk in the particular environmental context as well as on the intended PPP use.

4148 As amphibian and reptile populations will be affected differently by PPP application in differently
4149 structured landscapes, effective management options at landscape scale will have to address the
4150 structure and the proportion of off-field and/or unsprayed habitat in landscapes flagged as showing
4151 unacceptable risk. Absence of knowledge of the minimum requirements for the proportion and
4152 distribution of such features is an important data gap. It must be filled to keep exposures below
4153 critical values for intensively used and pond rich arable landscapes. (Berger et al. 2015).

4154 Apart from the above, proven effective measures that could be considered for mitigation of identified
4155 risk at local and landscape level for targeted species are:

- 4156 - Enforcing unsprayed buffer strips around (as well as proper management of) breeding ponds
4157 and other suitable wet areas,
- 4158 - provision of suitable terrestrial habitats next to breeding ponds
- 4159 - establishing flowering strips and areas in fields to reduce migration distances and to provide
4160 terrestrial habitats
- 4161 - time shifting of PPP application

4162

4163 **7.11. Addressing uncertainty in the risk assessment**

4164 Two areas where uncertainty needs to be generally addressed in the risk assessment of amphibians
4165 and reptiles are the calibration of a risk-assessment scheme and the treatment of additional
4166 uncertainties in the assessment. Calibration of a risk-assessment scheme is the problem (when only
4167 lower tier effects measurement data is available) of addressing uncertainty about what the outcome of
4168 the effects-measurement component (field or mesocosm study) of the surrogate reference tier (SRT)
4169 would be. There are likely to be additional uncertainties that need to be addressed even when highest
4170 tier effects data are available for an assessment, for example sampling variability for a field
4171 study/mesocosm or uncertainties affecting the population modelling.

4172 The EFSA Scientific Committee (2015) draft "Guidance on Uncertainty in EFSA Scientific Assessment"
4173 provides specific guidance on the treatment of uncertainty when standardised assessment procedures
4174 are being developed. It is necessary in particular to identify and to describe all the uncertainties that
4175 affect assessments for which a standardised procedure is being developed. Methods are provided to
4176 assist with this task. The standardised procedure should include allowance for as many sources of

4177 uncertainty as is feasible. This reduces the burden for subsequent applications of the procedure as
 4178 those applications need only consider uncertainties that were not already taken into account.

4179 Uncertainties regarding the coverage by the assessment of risks for other surrogate non-target
 4180 organisms in respect to the risk for amphibians and reptiles have been identified in the following.

4181 The outcome of a risk assessment is affected by uncertainties stemming from measurements,
 4182 assumptions, extrapolations or models relied upon in the risk assessment. A certain number of
 4183 uncertainties are addressed by setting an assessment factor, but further uncertainties may exist.
 4184 Uncertainties may be knowledge based and can thus be quantified, reduced and potentially removed,
 4185 or they may reflect the randomness of natural processes and can only be quantified (Skinner et al,
 4186 2016). The uncertainties are potentially greater for amphibians and reptiles than for surrogate species
 4187 owing to the shortage of data. The question is therefore whether increasing the assessment factor can
 4188 cover the uncertainties or whether new data need to be generated prior to being able to conduct a
 4189 risk assessment for amphibians and reptiles. The degree of precaution decision-makers are prepared
 4190 to tolerate depends on where uncertainties reside in the risk assessment, how large they are and
 4191 whether they are resolvable or not (Skinner et al., 2016). For this the uncertainties need to be
 4192 located, and the sources of uncertainty and their impact on the final assessment outcome need to be
 4193 identified (Draft EFSA guidance on uncertainty) in order to decide about how to proceed.

4194 In the following Table 16: , sources of uncertainties in the current risk assessment for surrogate
 4195 species are identified and evaluated for their potential impact on the risk assessment for amphibians
 4196 and reptiles.

4197 For the calibration of the risk-assessment scheme for amphibians and reptiles these sources of
 4198 uncertainties need to be quantified. A number of uncertainties cannot be quantified at present. It is
 4199 suggested that unquantified uncertainties could be combined in an increased assessment factor (draft
 4200 EFSA guidance on uncertainty). It is not, however, possible to suggest an adjusted assessment factor
 4201 for amphibians and reptiles to cover all uncertainties based on the currently available data and
 4202 models. The Panel suggests adjusting the exposure models and gathering more toxicological data for
 4203 comparison prior to deriving assessment factors.

4204

4205 **Table 16:** Sources of uncertainty and their effects on the risk assessment for amphibians and
 4206 Reptiles

4207

Source of uncertainty in the current risk assessment	Potential to be protective	Potential to be underprotective	Impact on the risk assessment for amphibians and reptiles
Variability in toxicological sensitivity between species within one group of organisms	Variability between species is very narrow	Variability between species is very large.	Variability in species unknown → further data needed
Representativeness in toxicological sensitivity of surrogate species for other species within one group of organisms	Surrogate species is a sensitive species	Surrogate species is not a sensitive species	Sensitivity of tested species (e.g. <i>X. laevis</i>) unknown → further data needed
Toxicological sensitivity of tested life stage	Most sensitive life stage is tested	Tested life stage does not cover sensitivity of other life stages	Sensitivity of different life stages (esp. adults) unknown, possibly compound specific (e.g. effects on eggs) → further data needed
Ecological relevance of observed effects in the toxicological studies	Critical effects have been addressed directly	Critical (e.g. endocrine) effects may remain unnoticed	Not all effects are adequately addressed. Sublethal studies needed to address e.g. metamorphosis and immunosuppression.
Study length to observe effects	Study duration long enough to observe critical and relevant	Study duration too short to observe latency of effects	Short-term exposure of juveniles in the aquatic may lead to long-term effects in terrestrial adults

	effects		
Route of exposure addressed in the study design	Relevant route of exposure adequately addressed in the study design	Relevant route of exposure not adequately addressed in the study design	Dermal exposure currently not adequately addressed
Representativeness of laboratory studies and exposure models for the field	Laboratory studies and exposure models are representative for the field	Indirect effects occurring in the field are not adequately addressed in the studies. Exposure models are not representative.	Extrapolation needs to be checked against field studies
Interaction with other non regulated stressors	No interactions occur	Interactions are relevant	Not addressed e.g. Pesticide exposure may increase susceptibility to diseases
Multiple regulated stressors in a temporal scale (e.g. multiple applications of different products on one field)	Other products in spray schedules have no increased adverse effect	Additive or synergistic adverse effects due to treatments with several products one after the other	Not addressed. Particularly relevant for species living within the field (e.g. reptiles) or moving across the fields (e.g. amphibians)
Multiple regulated stressors on a spatial scale (e.g. multiple inputs in a catchment)	Habitat lies solely in field and adjacent off-crop areas	Habitat is larger than one field, resp. receives input from several sources	Spatial scale relevant for aquatic species. Habitat range needed for terrestrial species is not addressed → further data needed
Location/proximity of surfacewater body to the field	Distance from field to water body is equal or greater than assessed.	Pond may be situated in the middle of a field.	Distribution of aquatic amphibian habitats needed and exposure models need to be adjusted
Size of standard water body (30 resp. 100 cm deep)	Depth of natural water bodies is equal or greater (90 th percentile)	Habitats are very shallow temporary water bodies of a few cm depth	Description of aquatic amphibian habitats needed and exposure models need to be adjusted
Distribution of the test substance in test vessel to determine relevant exposure concentration	Substance is distributed in the field as in the laboratory study	Patches with increased concentration due to poor circulation in standing or slow flowing waters.	Relevant exposure concentration needs to be modelled
Assessment of different routes of exposure separately	Exposure models are worst-case enough so that different routes of exposure do not need to be combined	Exposure of an individual may be orally, dermally and by inhalation.	Exposure models need to be adjusted to account for combined exposure routes of an individual
Assessment of exposure in different systems (aquatic and terrestrial) separately	Species have distinct, separate habitats	Exposure of an individual in the aquatic and terrestrial system concurrently.	Exposure models need to be adjusted to account for combined exposure in water and on land
Health status of laboratory animals in comparison to animals in the field	Test Animal is equally healthy in the laboratory and the field	Pre-exposure in the field increases sensitivity of the animal	Effect currently poorly understood (possibly development of resistance or increase in sensitivity) → further data needed
Population spatial structuring	The population exists as a spatially undifferentiated population not relying on fragile spatial dynamics for long-term survival	The population exists as an unstable metapopulation or source-sink population that can easily be disrupted.	Not addressed. Most amphibians and many reptiles exist in spatially structured populations potentially subject to disruption.
Long-term year on year effects	There is no effect of previous year's impacts, i.e. full recovery within a season	There are carry-over effects of impacts from previous years increasing the vulnerability in the following years.	Not addressed. Amphibians and reptiles are long-lived, increasing the chance of cumulative effects over a number of years building up.

4208

4209 **Table 17:** Amphibians, aquatic stages. Relevant life stages, exposure routes, endpoints timespan, possible coverage by endpoints available for other non-
 4210 target organisms and conclusions for the risk assessment. Please refer to the specific chapter in this Opinion for further details.

Life stage	Exposure route		timespan	Covered by	conclusion	
Aquatic stages	Egg, hatchling, larvae, metamorphic, juvenile, adult	contact	Water	acute	Fish acute(OECD 203) with the addition of an extrapolation factor to cover a defined percentage of amphibian sensitivity distribution	Can be addressed with tests delivered under current data requirement
				chronic	Studies with surrogate species do not cover toxicity to amphibians as no correlation could be found	new study required: extended AMA or LAGDA
	Hatchling, larvae, metamorphic, juvenile, adult		Sediment	acute	No study required for sediment dwelling organisms according to current data requirement	
				chronic	Route of exposure covered by spiked sediment study with <i>Chironomus</i> sp. (OECD 218)	Coverage of the sensitivity has not been evaluated yet
	larvae, juvenile, adult	oral	Food	acute chronic	Not covered by fish studies as uptake of food in the aquatic phase is considered to be more relevant for amphibians than for fish	Sufficiently addressed by uptake via the water phase
	larvae		sediment	acute	No study required for sediment dwelling organisms according to current data requirement	
				chronic	Route of exposure covered by spiked sediment study with <i>Lumbriculus variegatus</i> (OECD 225)	Coverage of the ecotoxicological sensitivity has not been evaluated yet

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4212

4213 **Table 18:** Amphibians, terrestrial stages. Relevant life stages, exposure routes, endpoints timespan, possible coverage by endpoints available for other non-
4214 target organisms and conclusions for the risk assessment. Please refer to the specific chapter in this Opinion for further details.

Life stage	Exposure route	timespan	Covered by	conclusion	
Terrestrial stages	contact	overspray	acute	Not covered by dermal study with birds or mammals as skin is not covered by feathers or fur and a larger part of the skin is exposed and has specific functions	New study required: overspray study (no standardized study available)
			chronic	Not covered by dermal study with birds or mammals as skin is not covered and a larger part of the skin is exposed and has specific functions	Not currently addressed, some conclusions can be drawn from the exposure of tadpoles followed till adult stages in the extended AMA or LAGDA study
		Soil	acute	Not covered by dermal study with birds or mammals as skin is not covered and a larger part of the skin is exposed and has specific functions	New study required if based on the overspray study the trigger is not met: exposure of adult on sprayed soil
			chronic	Not covered by dermal study with birds or mammals as skin is not covered and a larger part of the skin is exposed and has specific functions	Not currently addressed, some conclusions can be drawn from the exposure of tadpoles in the extended AMA or LAGDA study
		plants	Acute/chronic	Not covered by dermal study with birds or mammals as skin is not covered and a larger part of the skin is exposed and has specific functions	Sufficiently addressed by overspray scenario
		Water puddle	acute/chronic		Sufficiently addressed by dermal exposure route via overspray or soil
		oral	food	acute	Not covered by acute oral study (gavage?) with birds or mammals as no correlation between toxicity could be established
	chronic			No data available for a comparison	Not currently addressed, no reproducible method available, considered relevant
	inhalation		acute/chronic		Sufficiently addressed by dermal exposure route

4215

4216 **Table 19:** Reptiles. Relevant life stages, exposure routes, endpoints timespan, possible coverage by endpoints available for other non-target organisms and
 4217 conclusions for the risk assessment.

Life stage	Exposure route		timespan	Covered by	conclusion	
Terrestrial stages	embryo	contact	Soil	acute/chronic	No data available for a comparison	Not currently addressed, no reproducible method available, considered relevant
	Juvenile, adult		water	acute/chronic	No data available for a comparison	Not currently addressed, no reproducible method available, considered relevant
			plants	acute/chronic	No data available for a comparison	Not currently addressed, no reproducible method available, considered relevant
			Overspray (incl. stone walls, drift deposition)	acute/chronic	No data available for a comparison	Not currently addressed, no reproducible method available, considered relevant
			soil	acute/chronic	No data available for a comparison	Not currently addressed, no reproducible method available, considered relevant
		oral	food	acute/chronic	No data available for a comparison	Not currently addressed, no reproducible method available, considered relevant
			Drinking water	acute/chronic	No data available for a comparison	Sufficiently addressed by oral uptake of food

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4222 8. Toxicological endpoints and standard tests relevant for amphibians and 4223 reptiles

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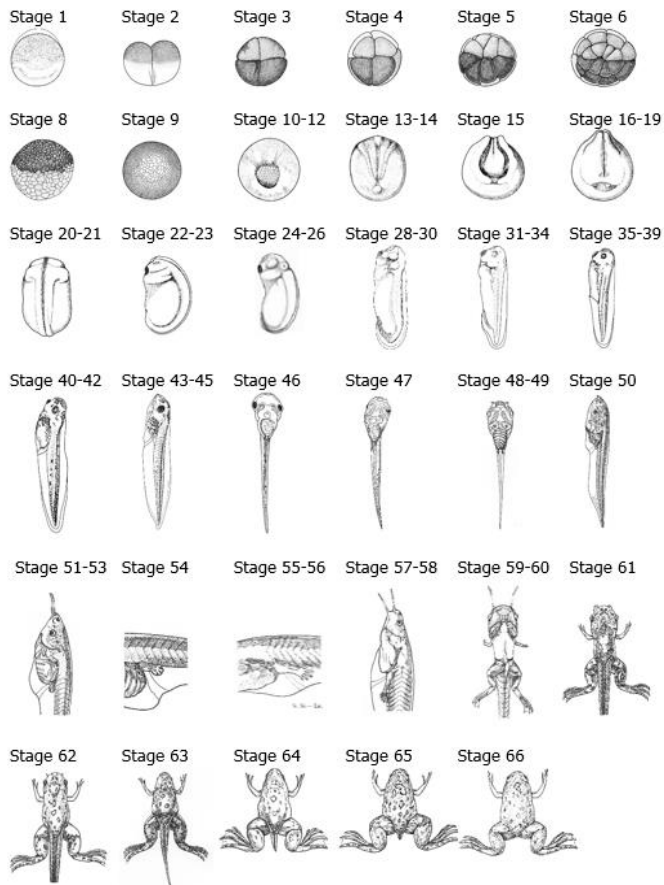
4225 8.1. Introduction

4226 Many features in the life cycles of amphibians and reptiles have been shown in laboratory experiments
4227 to be targeted by chemical exposure including embryo/larval survival, developmental rate, gonadal
4228 differentiation, spermatogenesis, oogenesis, fertility rate, and behaviour. This chapter is focused on
4229 test endpoints that are relevant to effects at the population level, i.e. those related to impaired
4230 survival, growth and reproduction, including standardized endpoints (sections 8.1 - 8.4). In addition,
4231 endpoints reflective of changes in behaviour and the immune status are discussed (section 8.5).
4232 Amphibian and reptilian model species used in toxicity studies are discussed in section 8.6.

4233 Lethal effects of pollutants on amphibians have been analysed in every stage of the life cycle.
4234 Information on terrestrial stages is rather limited compared with the relatively large list of papers
4235 recording embryonic or larval mortality. The exposure route for aquatic stages is usually dermal, with
4236 pollutants added to the water, although in some cases contaminated food has been used as the
4237 exposure vehicle in larvae (e.g. Cary et al 2014). In terrestrial stages, studies recording mortality
4238 usually expose juvenile or adult individuals dermally, either through contact with contaminated
4239 surfaces (e.g. Oldham et al 1997) or by overspray (Brühl et al. 2013). Recent compilations of some
4240 acute mortality data from aquatic and terrestrial amphibian stages can be found in Weltje et al (2013)
4241 and Crane et al (2016).

4242 With regards to sublethal toxicity in amphibians, growth and development are the most commonly
4243 measured effects, to the point that the few standard tests available for amphibians are based on the
4244 monitoring of these endpoints (see subsection below for details of standard tests). Growth has been
4245 addressed as a response to pollutant exposure in all amphibian stages except adults. The endpoints
4246 that have been used as growth indicators are mainly body length (either total or excluding tails, if
4247 present) and body mass, although body condition has also been used as a more biologically relevant
4248 variable (e.g. Edge et al 2011, Smith and Dibble 2012). Growth indicators are commonly not
4249 measured at a pre-defined time point (e.g after X days of exposure), but at developmental milestones
4250 such as hatching or completion of metamorphosis. There is much evidence in the amphibian biological
4251 literature that these variables at developmental milestones are important predictors of future
4252 performance of individuals (e.g. Semlitsch et al 1988). Development is analysed in pre-juvenile
4253 stages; staging systems of amphibian embryos and larvae (e.g. Gosner 1960, Harrison 1969,
4254 Nieuwkoop and Faber 1994) facilitate the exposure and monitoring of developmental rates and are
4255 used systematically in studies of developmental toxicity with amphibians (Figure 25: and Figure 26:).
4256 Endpoints for other types of sublethal toxicity are presented in sections 8.4 and 8.5.

4257 The list of toxicological endpoints analysed in reptiles is much shorter than that of amphibians. As
4258 highlighted by Sparling et al (2010), whereas amphibian ecotoxicological literature has experienced a
4259 moderate growth since the beginning of the century, reptiles continue to be an understudied group in
4260 this context and the availability of tested endpoints is extremely limited. The first consequence of the
4261 lack of ecotoxicological knowledge in reptiles is that they are the only vertebrates for which no
4262 standard tests exist.



4263

4264

4265 **Figure 25:** Developmental stages of *Xenopus* as described by Nieuwkoop & Faber (NF, 1994).
 4266 The embryonic period ranges from NF stage 1 to 44, and the larval period from NF stage 45 to 65.
 4267 Figure modified from Nieuwkoop & Faber (1994).

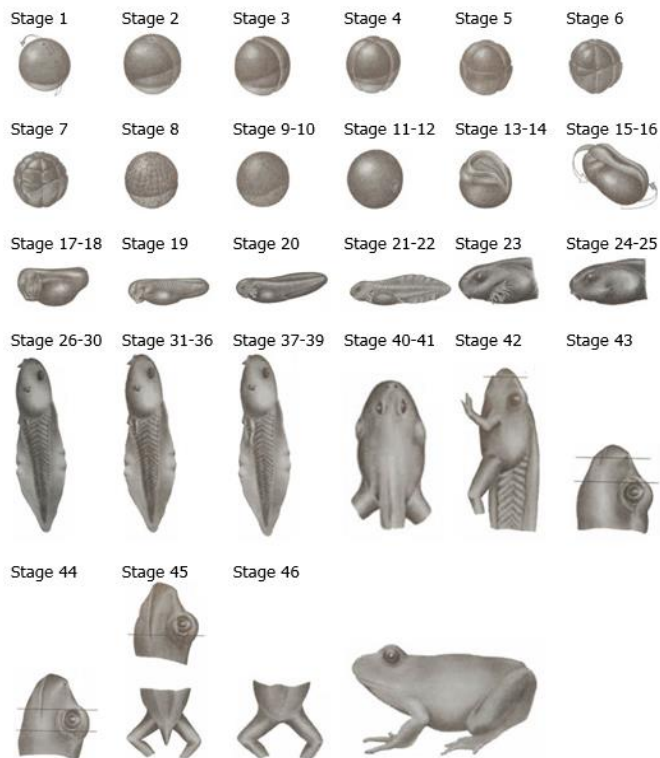
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4275 **Figure 26:** Developmental stages of *Anura* species according to Gosner (1960). The embryonic
4276 period ranges from stage 1 to 19, and the larval period encompasses stage 20 to 41. At stages
4277 42-45 anurans are referred to as metamorphs. Figure modified from Gosner (1960).

4278

4279 8.2. Available standardized toxicity tests for amphibians

4280

4281 Two standardized amphibian test guidelines are currently available within the OECD framework: there
4282 are the Amphibian Metamorphosis Assay (AMA, OECD Test Guideline 231) and the Larval Amphibian
4283 Growth and Development Assay (LAGDA, OECD Test Guideline 241), both of which focus on the
4284 analysis of growth and developmental effects of chemicals on amphibians. In addition there is the
4285 FETAX, the Frog Embryo Teratogenesis Assay – *Xenopus* (Bantle et al 1990) standardized by the
4286 American Society of Testing and Materials (ASTM test no. E1439-12). The Organization for Economic
4287 Co-operation and Development (OECD) and American Society for Testing and Materials (ASTM) are
4288 international, standardization organizations that publish standard guidelines for toxicity tests. The
4289 development of an OECD Test Guideline from a proposed new test method or from an existing
4290 standard or guideline (e.g. ASTM) involves a critical evaluation regarding its validation and regulatory
4291 acceptance. The process of test-guideline development involves a detailed assessment of existing
4292 information; additional testing to generate new data is often needed. An inter-laboratory comparative
4293 study is required when relevant (OECD, 2005).

4294

4295 8.2.1. The LAGDA assay

4296 The LAGDA test design involves exposure of early life stages (embryo, larva, juvenile) of *Xenopus*
4297 *laevis* to the test substance via the water (OECD 241). It is recommended to use a flow-through
4298 exposure system and evidence should be presented to demonstrate that the concentrations of the test
4299 chemical were maintained within $\pm 20\%$ of the mean measured values. The exposure is initiated at
4300 embryo stages NF 8-10 (before hatching, see Table 25: for staging table) and proceeds until 10

4301 weeks after the median time to reach NF stage 62 in the control group (about 4 months). The
 4302 exposure period encompasses the sensitive windows of sex determination, gonadal differentiation and
 4303 metamorphosis in *Xenopus laevis*. In addition to the sampling at the end of exposure, interim sub-
 4304 samples are taken at NF stage 62. Endpoints in LAGDA are listed in Table 18 and include those
 4305 measuring general toxicity (i.e. mortality and growth (length and weight)). In addition, endpoints
 4306 indicative of specific endocrine-toxicity modes of action (targeting oestrogen-, androgen-, or thyroid-
 4307 mediated physiological processes) are evaluated including histopathology of the gonads, gonad ducts,
 4308 and thyroid gland (OECD 241). Hence, the LAGDA test involves an exposure period that permits
 4309 measurement of endpoints both for disrupted sex differentiation and altered metamorphosis and is
 4310 therefore a more complete test than AMA (described Table 20:).

4311 **Table 20:** Endpoints, observation- and sampling time points in the Larval Amphibian Growth and
 4312 Development Assay (LAGDA). The exposure period is initiated at embryo stages NF 8-10
 4313 and ends at 10 weeks after the median time to reach NF stage 62 in the control group.

Endpoint	Daily	Interim sampling Larvae NF62	Test termination Juveniles 10 weeks after NF62
Mortality and abnormalities	X		
Time to NF stage 62		X	
Histo(patho)logy (thyroid gland)		X	
Morphometrics (growth in weight and length)		X	X
Liver-somatic index (LSI)			?
Genetic/phenotypic sex ratios			X
Histopathology (gonads, reproductive ducts, kidney and liver)			X
Vitellogenin (VTG) (optional)			X

4314

4315

4316 8.2.2. The AMA assay

4317 Metamorphosis is a particular feature of amphibian development. Many studies have tested disruption
 4318 of metamorphosis in animals exposed to pollutants at some point during the larval period (e.g.
 4319 Sparling et al 2006a). AMA is a screening assay that was designed to identify substances that may
 4320 interfere with the normal function of the hypothalamic-pituitary-thyroid (HPT) axis (OECD 231). As the
 4321 components and functions of the HPT axis are highly conserved among vertebrates, AMA was
 4322 suggested to represent a generalized vertebrate model. The amphibian metamorphosis is a thyroid
 4323 hormone dependent process that provides the possibility to investigate whether substances interfere
 4324 with the HPT axis. The general experimental design involves exposing *Xenopus laevis* tadpoles for 21
 4325 days, starting at developmental NF stage 51 (see Figure 25: for staging table). The endpoints in AMA
 4326 are listed in Table 21: and include hind limb length, snout-to-vent length (from the tip of the nose to
 4327 the opening of the cloaca at the tail base), developmental stage, wet weight, thyroid
 4328 histomorphometrical variables, and mortality (OECD 231). As for exposure system, a flow-through
 4329 system is preferred but in certain cases a static-renewal system may be suitable. The concentrations
 4330 of the test compound should be maintained at $\leq 20\%$ variability of measured test concentration over
 4331 the 21 day exposure period (OECD 231).

4332

4333 **Table 21:** Endpoints and observation time points in the Amphibian Metamorphosis Assay (AMA).

Apical Endpoints	Daily	Day 7	Day 21
Mortality	X		
Developmental stage (NF)		X	X
Hind limb length		X	X
Snout-vent length		X	X
Body weight (wet)		X	X
Thyroid gland histology			X

4334

4335 8.2.3. FETAX- The Frog Embryo Teratogenesis Assay-*Xenopus*, ASTM, 4336 E1439-12

4337 FETAX is a rapid (96-h) test with *Xenopus laevis* to screen for acute embryo toxicity (mortality
4338 malformation, and growth inhibition). FETAX was developed to provide data on developmental toxicity
4339 for the hazard evaluation in the human risk assessment of chemicals (Bantle 1990). In brief, dejellied
4340 eggs (egg from which the protective jelly layer surrounding the egg is removed) are exposed to the
4341 test compound via the surrounding water for 96 hours. Mortality is recorded at the end of each 24
4342 hour period and malformations are recorded at the end of the 96 hour period. The type and degree of
4343 malformations should be evaluated against an atlas of malformations (Bantle et al. 1991). Inhibition of
4344 embryonic growth, which is the most sensitive endpoint in FETAX (Hoke and Ankley 2005), is
4345 determined at the end of the 96 hour test period by measuring head to tail length of the embryos.

4346 8.3. Other test guidelines and methods used for amphibians and 4347 reptiles

4348 Apart from the two standard OECD test guidelines (LAGDA and AMA) and the widely used FETAX,
4349 some other tests have been proposed or developed by different agencies or research institutions for
4350 testing toxicity of chemicals on amphibians. The ones more commonly appearing in the scientific
4351 literature are briefly described below.

4352 8.3.1 Standard Guide for Conducting Acute Toxicity Tests

4353 *Standard Guide for Conducting Acute Toxicity Tests on Test Materials with Fishes, Macroinvertebrates,*
4354 *and Amphibians, ASTM E729 - 96(2014)*

4355 This standard Guide for Conducting Acute Toxicity Tests on Test Materials with Fishes,
4356 Macroinvertebrates, and Amphibians, ASTM E729 - 96(2014) describes general procedures for acute
4357 toxicity (for example, lethality and immobility) testing of a test material added to dilution water, but
4358 not to food, on certain species of freshwater and saltwater fishes, macroinvertebrates, and
4359 amphibians during 2- to 8-day exposures, depending on the species. The guide describes three basic
4360 exposure techniques: static, renewal, and flow-through and other aspects of aquatic acute toxicity
4361 testing.

4362 8.3.2 Guidelines to conduct tests with exposure via sediment

4363 *Standard Guide for Conducting Whole Sediment Toxicity Tests with Amphibians, ASTM E2591-*
4364 *07(2013)*

4365 This guide covers procedures for obtaining laboratory data on the toxicity of test material (e.g.,
4366 sediment or soil) to amphibians. Test duration is 10 d and the overlying water may be continuously
4367 replaced or static replacement is done. The test procedure describes the use of larvae of the northern
4368 leopard frog (*Lithobates pipiens*). Other anuran species (for example, the green frog (*Lithobates*
4369 *clamitans*), the wood frog (*Lithobates sylvaticus*), the American toad (*Anaxyrus americanus*)) may be
4370 used if sufficient data on handling, feeding, and sensitivity are available. Test material may be

4371 sediments or hydric soil collected from the field or spiked with compounds in the laboratory. Sediment
4372 toxicity testing with *Xenopus laevis* has focused on evaluating the developmental effects of sediment
4373 extracts, as opposed to whole sediments, on frog embryos using the FETAX, ASTM, E1439-12.

4374 *EPA Tadpole/sediment subchronic toxicity test OPPTS 850.1800 (1996)*

4375 This guideline is used to develop data on the subchronic toxicity of chemicals sorbed to natural
4376 sediments to bullfrog tadpoles (*Rana catesbeiana*). Test duration is 30d and is performed under flow-
4377 through conditions. Tadpoles are exposed via spiked sediment of three different natural sediments.
4378 Exposure is by ingestion, either by direct dosage of spiked slurry into their buccal cavity at the
4379 beginning of the test in test chambers with only clean dilution water or by allowing tadpoles to ingest
4380 contaminated sediment ad libitum. Survival, growth and in addition abnormal behaviour are recorded
4381 and evaluated and LC50, EC50, LOEC and NOEC values are calculated at days 10, 20 and 30.

4382 8.3.3 Other proposed test methods

4383 Apart from the tests described above, some other tests have been proposed or developed by different
4384 agencies or research institutions for testing toxicity of chemicals on amphibians. The ones more
4385 commonly appearing in the scientific literature are briefly described below.

4386

4387 *AMPHITOX: A Customized Set of Toxicity Tests Employing Amphibian Embryos*

4388 AMPHITOX is a set of acute toxicity tests on amphibian embryos reported at an ASTM Symposium by
4389 Herkovits and Pérez-Coll (2003). To our knowledge the AMPHITOX protocol(s) has not been published
4390 as an ASTM Standard Guide. AMPHITOX can be customized to acute (AMPHIACUT), short-term
4391 chronic (AMPHISHORT), and chronic (AMPHICHRO) exposure periods. The main endpoint is mortality
4392 but malformations can also be recorded and the exposure periods range from 24 hours up to 14 days.

4393 *The *Xenopus tropicalis* test system for developmental and reproductive toxicity*

4394 Chemical exposure during the development of the reproductive system in amphibians may lead to
4395 permanently impaired fertility (Gyllenhammar et al 2009). Adverse developmental effects on
4396 reproductive function may not be detectable in the early life stages when the reproductive organs are
4397 immature (Kvarnryd et al, 2011). It is therefore important to evaluate long-term consequences of
4398 early life-stage exposure to chemicals. The African clawed frog *Xenopus tropicalis* has several
4399 characteristics that facilitate such studies, including a short generation time (4-6 months) compared
4400 with *Xenopus laevis* (12-24 months) (Hirsch et al. 2002; Olmstead et al. 2009). *Xenopus tropicalis* has
4401 therefore proven useful when investigating developmental reproductive toxicity, which requires life-
4402 cycle studies (Pettersson et al 2006; Porter et al 2011). A *Xenopus tropicalis* life-cycle assay has been
4403 proposed, including study design, exposure regime, and endpoints for chemical disruption of sex
4404 differentiation, reproductive organ development, the thyroxin-regulated metamorphosis, and fertility
4405 (Berg 2012).

4406 *Laboratory tests to address dermal toxicity of chemicals in the terrestrial environment*

4407 All the test methods described above refer to amphibian aquatic stages, which is because the majority
4408 of ecotoxicological studies conducted on amphibians have been performed with aquatic stages. The
4409 number of assays carried out on the terrestrial phase is comparatively scarce, as is the number of
4410 studies focused on reptiles. Belden et al. (2010) used a methodology to test acute toxicity of
4411 chemicals on terrestrial juvenile amphibians after overspray, a methodology that was further repeated
4412 with different chemical substances and amphibian species (Brühl et al. 2013, Cusaac et al. 2016), and
4413 that has also been used by industry in assessment dossiers (unpublished data). The test consists of
4414 housing animals in a terrarium and applying the chemical onto the terrarium at a realistic rate with a
4415 device simulating a professional pesticide application. The approach simulates a real scenario if it is
4416 assumed that animals are in field at the same at pesticide applications; this scenario can be refined
4417 when the chances of animals being active at the same time of pesticide application are low (e.g.
4418 animals with nocturnal activity). In those cases, dermal exposure by contact with the applied soil may
4419 be considered, using a methodology similar to that for overspray but adjusting the application rate to
4420 expected degradation happening from real application to passage of animals over the treated soil, and
4421 introducing the animals *in terraria* after soil application. Likewise, crop interception can be considered

4422 by adjusting the application rate, or by including the vegetation in the terrestrial enclosure to simulate
4423 a real interception by plants (Carpenter et al. 2016).

4424 This type of experiment on terrestrial dermal exposure can also be adapted to reptiles. The procedure
4425 is similar to the one used with amphibians and consists of the application of the chemical substance to
4426 the terrarium soil before including the animals the contaminated enclosure (Buono *et al.* 2007, De
4427 Falco et al. 2007). Percutaneous exposure in reptiles has been conducted most commonly by dipping
4428 parts of the animal or by pipetting the solution onto some of the animal's surfaces (e.g. Talent 2005,
4429 Weir et al 2015). Reptilian habits (independence from water, common use of loose soils for nesting, or
4430 frequent diurnal activity), however, make them likely to be oversprayed. Such a scenario of direct
4431 application to individuals has also been applied experimentally with similar methods to the ones
4432 described for amphibians (Carpenter et al. 2016). Although all these tests on terrestrial dermal
4433 exposure have been used mostly to test acute toxicity, the same type of experimental enclosures
4434 could be made valid for testing chronic toxicity simply by adapting exposure concentrations and
4435 experimental times. This would allow for combining dermal with oral exposure by treating food before
4436 giving it to animals, testing repeated application effects, and even analysing the effects that pollution
4437 can cause on reptilian eggs being incubated in treated soils (Rey et al. 2009).

4438 *Mesocosm test to evaluate effects of chemicals on amphibians and reptiles*

4439 Mesocosm experiments have been conducted on amphibians and reptiles and could serve as higher-
4440 tier studies to evaluate the effects of pesticides or other chemicals on these animals. Studies for
4441 regulatory purposes on amphibians and reptiles have, however, not been required to date, which
4442 means that mesocosm studies conducted on these animals have not necessarily had the same
4443 purpose and design as mesocosm studies conducted for regulatory purposes on other taxa.
4444 Mesocosms for ecotoxicological studies have been used much more frequently in amphibians than in
4445 reptiles, especially for amphibian aquatic stages. Most of the mesocosm studies focused on the effects
4446 of environmental pollutants on amphibians are designed with the purpose of evaluating such effects in
4447 an ecological context, which makes it impossible to differentiate direct toxicity caused by the chemical
4448 from indirect effects. These mesocosms often include, besides the chemical exposure, the presence of
4449 competitors, predators or pathogens; the response variables can be simply individual abundance after
4450 a given time, without the possibility of elucidating whether casualties are because of direct
4451 intoxication, predation, lack of food, disease or, most likely, a combination of several of these factors
4452 (e.g. Boone and James 2003, Relyea 2006). The simulation of more or less complex ecological
4453 communities sometimes results in positive, indirect effects of pollutants on amphibians in the short
4454 term by means of, for example, removing predators faster than the amphibians themselves (e.g.
4455 Relyea 2005); in other cases, the addition of stressors other than the pesticides results in high
4456 mortality rates of tadpoles at concentrations that are sublethal in the laboratory (e.g. Relyea and
4457 Diecks 2008). For reptiles, Amaral et al. (2012a) also designed a mesocosm simulating a complex
4458 community scenario to evaluate pesticide effects on lizards, but the usefulness of their design still
4459 needs to be confirmed as the attempt resulted in high control mortality. The complexity of the
4460 mesocosm designs available in the scientific literature renders them unsuitable for testing direct
4461 toxicity and standardizing their use for regulatory purposes. These mesocosm designs could serve as a
4462 starting point to set up higher-tier studies adapted to environmental risk assessment of pesticides for
4463 amphibians or reptiles in case this became necessary, although this is not recommended by the Panel
4464 at present.

4465

4466 **8.4 Endpoints for reproductive and endocrine toxicity in amphibians and** 4467 **reptiles**

4468 Reproductive toxicity is defined as impaired sexual function or fertility in adult individuals, and
4469 includes developmental toxicity in the offspring. Endpoints in reproductive toxicity tests include
4470 impaired fertility and reproductive organ changes in the parents as well as effects on viability, sex
4471 ratio and growth in the offspring. Reproductive toxicity such as impaired egg/sperm production can
4472 result from exposure of the adult individual as well as from exposure at early life, pre-juvenile stages.
4473 The final maturation of the egg and sperm occurs in adults but the development of sperm/egg starts
4474 very early in life (during the larval stages for amphibians, *in ovo* for reptiles) and may consequently be
4475 damaged by chemical exposure in early life stages. Given that the critical period of sex differentiation

4476 coincides with the aquatic larval phase in most amphibians, disruption of sex-organ development is an
4477 important endpoint for reproductive toxicity following larval exposure to water-borne pollutants.
4478 Amphibian test endpoints for developmental and reproductive as well as for endocrine disruption are
4479 listed in Table 22: .

4480 **8.4.2 Sex ratio change and ovotestis frequency**

4481 **Amphibians**

4482 Alteration of the sex ratio (implying complete or partial sex reversal) relative to the control group is a
4483 commonly used endpoint for endocrine disruption in laboratory studies in *Xenopus laevis*, *Xenopus*
4484 *tropicalis* and several species of ranids including *Lithobates pipiens*, *Lithobates sylvaticus* and *Rana*
4485 *temporaria* (Kloas et al 1999; Hayes et al 2002; Mackenzie et al 2003; Pettersson & Berg 2007).
4486 Exposure of *Xenopus laevis* larvae to the herbicide atrazine (2.5 µg/L) caused demasculinization of the
4487 testes (Hayes et al 2002). Mackenzie et al (2003) investigated effects of larval exposure to estrogenic
4488 and antiestrogenic compounds (µg/L-concentrations) on gonadal differentiation in leopard frogs
4489 (*Lithobates pipiens*) and wood frogs (*Lithobates sylvaticus*). Exposure to the test substances induced
4490 alterations of gonadal differentiation in both species. Comparisons between the two species indicated
4491 that *L. pipiens* is more susceptible to sex reversal and development of intersex gonads.

4492 Estrogen-induced sex-reversal during early-life stages has been shown to persist long after exposure
4493 discontinued in *Xenopus tropicalis*, *Rana temporaria*, and *Lithobates pipiens* (Pettersson et al 2006;
4494 Pettersson & Berg 2007; Hogan et al., 2008; Gyllenhammar et al 2009). Exposure of *Xenopus*
4495 *tropicalis* larvae to environmentally realistic estrogen (ethynylestradiol, EE2) concentrations induced
4496 female-biased sex ratios that persisted in the adult animals, 9 months after the exposure period was
4497 ended (Pettersson et al 2006; Gyllenhammar et al 2009). Exposure of *Rana temporaria* and *Lithobates*
4498 *pipiens* larvae to EE2 induced female-biased sex ratios that could be observed a few months after the
4499 exposure was discontinued (Pettersson & Berg 2007; Hogan et al 2008).

4500 Disrupted gonadal differentiation is also an effect of anti-thyroid substances. Exposure of *Xenopus*
4501 *laevis* larvae to thiourea, a thyroid hormone synthesis inhibitor, completely prevented testes
4502 formation, producing 100% females (Hayes 1998). Exposure of *Xenopus laevis* tadpoles to another
4503 anti-thyroid substance, ammonium perchlorate (59 µg/L), resulted in a skewed sex ratio (female-
4504 biased) compared with the control group, suggesting that testicular development was inhibited
4505 (Goleman et al, 2002). However, in other frog species (*Hyperolius viridiflavus*), inhibition of thyroid
4506 hormone synthesis prevented ovary development.

4507 The most frequently studied endpoint for endocrine disruption in wild amphibians is male intersex or
4508 ovotestis, i.e. the presence of ovarian follicles within the testicle. High incidences of ovotestis in male
4509 amphibians inhabiting agricultural areas have been reported (Hayes et al. 2003; McCoy et al, 2008).
4510 In some species, however, intersex gonads occur normally during the period of gonadal
4511 differentiation. In such species, the intersex frequency is age- specific and therefore a poor indicator
4512 of endocrine disruption. Moreover, the timing of estrogen exposure determines the extent of sex-
4513 reversal of the testis in *Xenopus laevis* tadpoles (Chang & Witschi, 1956; Villapando & Merchant-Larios
4514 1990). When exposure initiates at stage 44-50 all tadpoles develop ovaries whereas if exposure starts
4515 at stage 51-54, 50% of the tadpoles have ovaries and 50% have ovotestes. When estradiol exposure
4516 started later, at stage 55-56, the gonadal sex ratio was not affected compared with the control group
4517 (Villapando & Merchant-Larios 1990).

4518 **Reptiles**

4519 Studies on oviparous reptiles, e.g. alligators, have been important in advancing knowledge of reptilian
4520 ecotoxicology in the field. Guillette and colleagues investigated the effect of estradiol treatment on
4521 gonadal differentiation in the alligator (*Alligator mississippiensis*), which has temperature-dependent
4522 sex differentiation (Crain et al 1999). Exposure of alligator eggs to estradiol induces development of
4523 females at a male-producing temperature. Histological analysis of the gonads of female hatchlings
4524 showed that estradiol exposure increased the ovarian medullary regression (Crain et al 1999). Another
4525 study in alligators showed that *in ovo* exposure to the anti-androgenic pesticide metabolite p,p'-DDE
4526 (1,1-dichloro-2,2-bis (p-chlorophenyl) ethylene) caused a female-biased sex ratio among hatchlings
4527 (Milnes et al 2005).

4528 A study on another crocodilian reptile, the broad-snouted caiman (*Caiman latirostris*) investigated the
4529 effects of in ovo exposure to bisphenol A or 17 β -estradiol on sex determination and gonadal histology
4530 (Stoker et al 2003). The study concluded that BPA causes estrogen-like developmental effects by
4531 reversing gonadal sex and altering gonadal histoarchitecture (Stoker et al 2003).

4532 Effects of 17 β -estradiol and the estrogenic chemical bisphenol A on sex ratio and gonadal histology
4533 were investigated in the painted turtle (*Chrysemys picta*), which has a temperature-dependent sex
4534 determination (Jandegian et al, 2015). Farm-raised turtle eggs assigned to the different exposure
4535 groups were incubated at a male-producing temperature (26 °C). Estradiol exposure induced female
4536 gonads in 89% of the exposed 'males', but in none of the control males. Bisphenol A exposure
4537 resulted in the development of ovarian-like tissue and seminiferous tubule disorganization in the
4538 testes of hatchlings (Jandegian et al, 2015). These gonadal alterations are similar to the effects of in
4539 ovo exposure to estrogenic chemicals in birds (Berg et al 1999; 2001a), suggesting that endocrine
4540 disrupting chemicals can induce similar effects on gonadal differentiation in birds and reptiles.

4541 8.4.3 Reproductive organ development and fertility

4542 Amphibians

4543 Gonadal histomorphometry including proportions of germ cell stages to determine degree of gonad
4544 maturity in juvenile *Xenopus tropicalis* have been described as potential endpoints to measure effects
4545 of toxicants on gonadal development and maturation (Säfholm et al 2016). Endpoints for
4546 developmental toxicity in the adult amphibian testis including several histomorphometrical variables
4547 have been developed for *Xenopus tropicalis* and *Xenopus laevis* (Gyllenhammar et al 2009; Kvarnryd
4548 et al 2011; Hayes et al 2010). The endpoints include seminiferous tubule morphometry and
4549 proportions of male germ cell stages, analysed in histological sections (Säfholm et al, 2012). A
4550 reduced amount of mature spermatozoa in the seminiferous tubule lumen and reduced fertility rate
4551 (measured as percentage fertilized eggs in mating trials) in adult male *Xenopus tropicalis* was induced
4552 by exposure of larvae to environmentally realistic EE2 concentrations (Pettersson et al 2006;
4553 Gyllenhammar et al 2009). It has also been shown that larval exposure to the herbicide atrazine (2.5
4554 μ g/L) decreased the frequency of seminiferous tubules with mature spermatozoa in adult male
4555 *Xenopus laevis* (Hayes et al 2010). By determining the proportions of various oocyte stages in
4556 histological sections from ovaries of adult female *Xenopus tropicalis* it was shown that several
4557 progestogens (levonorgestrel, norethindrone, and progesterone) inhibit oogenesis in adult *Xenopus*
4558 *tropicalis* by interrupting formation of vitellogenic oocytes, after adult exposure to environmentally
4559 relevant ng/L-concentrations (Säfholm et al, 2012; 2014). Ovary histomorphometrical endpoints were
4560 also used to show that oogenesis was severely impaired after larval exposure to the progestagen
4561 levonorgestrel (Kvarnryd et al 2011).

4562 Impaired differentiation of the Müllerian duct or of oviduct development are effects of larval exposure
4563 to EE2 or the progestin levonorgestrel observed in *Xenopus tropicalis* (Pettersson et al 2006;
4564 Gyllenhammar et al 2009; Kvarnryd et al 2011). Hence, the Müllerian ducts are targeted by several
4565 kinds of endocrine-disrupting chemicals in amphibians. Histomorphometrical measurements of the
4566 Müllerian ducts (including size and developmental stage frequencies) that may be useful as
4567 toxicological endpoints have been developed for *Xenopus tropicalis* juveniles (Jansson et al 2016;
4568 Säfholm et al 2016).

4569 Reptiles

4570 Various histomorphometrical measurements in the gonads of reptiles have been used as endpoints for
4571 disrupted gonadal development. In viviparous (live-bearing) reptilian species (comprising about 30%
4572 of the reptilian species), exposure to environmental pollutants during embryonic development may
4573 occur via the mother, via the yolk and the placenta. A study on the viviparous lizard (*Niveoscincus*
4574 *metallicus*) showed that maternal exposure to the synthetic estrogen diethylstilbestrol (DES) disrupted
4575 gonadal development in both male and female offspring. The male offspring of DES-exposed mothers
4576 showed seminiferous-tubule disorganisation and a reduction of germ cells in the testes compared with
4577 those from control groups. The female offspring to DES-exposed mothers exhibited abnormalities of
4578 ovarian structure, oocytes and follicles compared with controls (Parseley et al 2015).

4579 The Müllerian ducts are also targeted by estrogenic compounds in reptiles. Increased Müllerian duct
4580 epithelial cell height in female alligator hatchlings was determined after *in ovo* exposure to 17 β -
4581 estradiol (Crain et al 1999). This is similar to the effects of *in ovo* exposure to estrogen in birds (Berg
4582 et al 2001b), suggesting that endocrine-disrupting chemicals can induce similar effects on Müllerian
4583 duct development in birds and reptiles.

4584 8.4.4 Vitellogenin

4585 Elevated concentration of the egg-yolk precursor protein vitellogenin in plasma is probably the most
4586 commonly used biomarker for estrogenic action of chemicals in oviparous vertebrates (Sumpter &
4587 Jobling, 1995; Selcer & Verbanic, 2014). The synthesis and incorporation of vitellogenin into the
4588 growing oocyte are stimulated by estrogen but the regulation of vitellogenesis involves multiple
4589 hormones. Suppressed vitellogenesis is associated with reduced egg production and therefore
4590 reproductive success, which has been demonstrated in fish (Thorpe et al 2007). Plasma vitellogenin
4591 concentration in juvenile *Xenopus laevis* is one of the endpoints in the LAGDA test (OECD 241). A
4592 method for measuring plasma vitellogenin concentration in juvenile *Xenopus tropicalis* has also been
4593 developed (Brande-Lavridsen *et al* manuscript).

4594 8.4.5 Secondary sex characters

4595 The secondary expression of sex characters depends on sex-hormone levels and is potentially useful
4596 as a non-invasive endpoint for endocrine disruption. The male secondary sex characters, size of
4597 forelimb and nuptial pad, are dependent on androgen and thereby properly functioning testes
4598 (Emerson et al. 1999). Both these characters are sexually dimorphic during the reproductive phase in
4599 a wide range of frog species including the aquatic tropical species *Xenopus tropicalis* and terrestrial
4600 temperate species such as *Rana temporaria*. Exposure to anti-androgens was shown to decrease
4601 nuptial pad size in adult *Xenopus* (Wyk et al 2003). The nuptial pad size in adult male *Xenopus laevis*
4602 was reduced after larval exposure to the herbicide atrazine (2.5 μ g/L) (Hayes et al 2010). This implies
4603 that both adult and larval exposure to endocrine disruptors can affect nuptial pad display. The
4604 development of nuptial pads in adult female *Xenopus tropicalis* exposed to the synthetic progestogen
4605 levonorgestrel (1.2 μ g/L) for four weeks indicates that this may also be used as an endpoint for
4606 endocrine disruption in female amphibians (Säfholm et al 2012).

4607 Cloacal enlargement is a female secondary sex character that develops at sexual maturity. Cloacal
4608 length in adult female *Xenopus tropicalis* was reduced after exposure to levonorgestrel (1.2 μ g/L),
4609 indicating that it might be useful as a non-invasive endpoint for endocrine disruption in amphibians
4610 (Säfholm et al 2012).

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4612

4613 8.4.6 Calling/sexual behaviour

4614 The mating process can be altered if mating behaviours are affected. In this context, pollutant effects
4615 on calling behaviour have been studied through the analysis of intensity and frequency of digitally
4616 recorded calls emitted by *Xenopus laevis* males (Hoffmann and Kloas 2012a;b). This endpoint has
4617 been proposed as a non-invasive method for assessment of anti-androgenic endocrine-disrupting
4618 chemicals (Behrends et al 2010).

4619 Competitive breeding trials have been used to measure effects of toxicant exposure on mating
4620 behaviour in *Xenopus laevis* and *Xenopus tropicalis*. One control male and one exposed male are put
4621 together to compete for one female and frequency of successful copulations is scored. Using such
4622 competitive breeding trials, it was shown that larval exposure to the herbicide atrazine (2.5 μ g/L)
4623 suppressed mating behavior in adult male *Xenopus laevis* (the atrazine-exposed males were out-
4624 competed by control males) (Hayes et al 2010).

4625

4626 **Table 22:** Overview on amphibian test endpoints at different life stages

Life stage sampled	Endpoint measured	Endpoint for:	Exposure period	Age/larval stage sampled	Test guideline	Reference
Embryo	Mortality rate	Developmental toxicity	4 d early embryo	n.a.	FETAX	
	Malformation rate	Developmental toxicity	4 d early embryo	n.a.	FETAX	
Larvae	Mortality and malformation rates	Developmental toxicity	From NF51 - 21 d in <i>X. laevis</i> .	7 and 21 d after NF51 in <i>X. laevis</i> ,	AMA	
			- 14 d in <i>X. tropicalis</i>	5 and 14 d after NF51 in <i>X. tropicalis</i>		Carlsson & Norrgren, 2007.
	Growth (weight and length)	Developmental toxicity	From NF51 21 d in <i>X. laevis</i> ,	7 and 21 d after NF51 in <i>X. laevis</i>	AMA	
			14 d in <i>X. tropicalis</i>	5 and 14 d after NF51 in <i>X. tropicalis</i>		Carlsson & Norrgren, 2007.
			From embryo stage NF 8	Interim sampling at NF62	LAGDA	
	Developmental rate, developmental stage reached	Developmental toxicity	From NF51 21 d in <i>X. laevis</i> ,	7 and 21 d after NF51 in <i>X. laevis</i> , 5 and 14 d after NF51 in <i>X. tropicalis</i>	AMA	Carlsson & Norrgren, 2007.
			From embryo stage NF 8	Interim sampling at NF62	LAGDA	
	Hind-limb length	ED mode of action; thyroid system disruption	From NF51 21 d in <i>X. laevis</i> , 14 d in <i>X. tropicalis</i>	7 and 21 d after NF51, in <i>X. laevis</i> , 5 and 14 d after NF51 in <i>X. tropicalis</i>	AMA	Carlsson & Norrgren, 2007.
	Thyroid gland histomorphometrical variables e.g. follicle epithelium height	ED mode of action; thyroid system disruption	From NF51 21 d in <i>X. laevis</i> , 14 d in <i>X. tropicalis</i>	21 d after NF51 in <i>X. laevis</i> , 14 d after NF51 in <i>X. tropicalis</i>	AMA	Carlsson & Norrgren, 2007.
			From embryo stage NF 8	Interim sampling at NF62	LAGDA	

Juvenile	Phenotypic sex ratio	ED modes of action targeting estrogen-, androgen-signalling pathways	From embryo stage NF 8	About 2 months post-metamorphosis in <i>X. laevis</i> .	LAGDA	
	Phenotypic sex ratio (gonadal histology)	ED mode of action; targeting estrogen-, androgen signalling pathways	Larvae	At completed metamorphosis, NF66, in <i>Xenopus</i>		<i>X. laevis</i> : Kloas et al 1999; <i>X. tropicalis</i> : Pettersson & Berg, 2007
	Plasma vitellogenin concentration	ED modes of action targeting estrogen-, androgen-signalling pathways	From embryo stage NF 8	About 2 months post-metamorphosis in <i>X. laevis</i>	LAGDA	
			Larvae	At completed metamorphosis, NF66, in <i>X. tropicalis</i>		Brande-Lavridsen et al in prep
	Müllerian and Wolffian duct histomorphometrical variables including size and developmental stage frequencies	Potential reproductive toxicity	Larvae or juveniles	1 month post-metamorphosis in <i>X. tropicalis</i>		Jansson et al 2016 Säfholm et al 2016
	Gonad histomorphometry including proportions of germ cell stages to determine degree of gonad maturity	Potential reproductive toxicity	Larvae or juveniles	1 month post-metamorphosis in <i>X. tropicalis</i>		Säfholm et al 2016
Adult	Intensity and frequency of digitally recorded calls emitted by <i>Xenopus laevis</i> males	ED mode of action; targeting androgen signalling pathway	4 d	1 -2 years in <i>X. laevis</i>	OECD TG under development	

	Larynx histomorphometrical variables including size	ED mode of action; targeting androgen signalling	Larvae	1 -2 years in <i>X. laevis</i> , 4 -6 months in <i>X. tropicalis</i>		Sassoon, Segil & Kelley 1986; Tobias et al, 1993; Hayes et al 2010
	Males: testis histomorphometrical variables including proportions of germ cell stages	Reproductive toxicity	Larvae or adults	1 -2 years in <i>X. laevis</i> , 4 -6 months in <i>X. tropicalis</i>		Cevasco et al, 2008; Gyllenhammar et al, 2009; Kvarnryd et al 2011
	Females: ovary histomorphometrical variables (e.g. proportions of oocyte stages) and frequencies of oviduct malformations	Reproductive toxicity	Larvae or adults	1 -2 years in <i>X. laevis</i> , 6 months in <i>X. tropicalis</i>		Cevasco et al 2008; Säfholm et al 2012
	Fertility rate (proportion fertilized eggs)	Reproductive toxicity	Larvae or adults	1 -2 years in <i>X. laevis</i> , 4 -6 months in <i>X. tropicalis</i>		Gyllenhammar et al 2009; Kvarnryd et al 2011
	Expression of secondary sex characters (e.g. nuptial pads size, cloacal size)	ED mode of action; targeting sex hormone signalling pathways	Larvae or adults	1 -2 years in <i>X. laevis</i> , 4 -6 months in <i>X. tropicalis</i>		Wyk et al 2003; Säfholm et al 2012

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4629 8.5 Other potential endpoints for toxicity in amphibians and reptiles

4630 8.5.1 Amphibians

4631 A group of sublethal endpoints that could indicate potential threats at the population level are those
 4632 related to the impairment of the immune function. The amphibian immune system, especially that of
 4633 *Xenopus*, has been described in detail, and it shares most of the components of the immune system
 4634 of mammals. Because the immune function is formed by a number of elements often interacting
 4635 among them, testing immunocompetence of the whole organism can be very complex. The most
 4636 commonly used structural and functional tests in wildlife immunotoxicological studies have also been
 4637 applied to amphibians. These include measurements of both constitutive (e.g. leukocyte counts or
 4638 phagocytic activity) and induced (e.g. antibody synthesis or inflammatory responses) immunity (e.g.
 4639 Froese et al 2009, Cary et al 2014). Amphibian immune function has a particular component, the skin
 4640 antimicrobial peptides, that are not present in other vertebrate groups, at least with the same degree
 4641 of importance (Rollins-Smith et al 2005). These peptides seem to be the main barrier of defence
 4642 against a number of pathogens, including the deadly fungi of the genus *Batrachochytrium* that are

4643 behind the decline and extinction of many amphibian populations worldwide (Rollins-Smith 2009).
4644 Both the amount and composition of skin antimicrobial secretions have been studied in amphibians
4645 after exposure to environmental chemicals; most importantly, their efficiency in inhibiting fungal
4646 growth in vitro can be used as a direct indicator of the immunocompetence of organisms when
4647 dealing with these pathogenic fungi (Pask et al 2013). The outcome of potential immune suppression
4648 associated with pollutant exposure has also been investigated through the direct analysis of parasitic
4649 or pathogenic loads in exposed animals (Rohr et al 2008, Paetow et al 2012), and even by challenging
4650 exposed animals with pathogens in laboratory studies as a measure of final effects of immune
4651 depression (Davidson et al 2007).

4652 Developmental abnormalities can be quantified through the direct analysis of malformed embryos or
4653 larvae (as done in FETAX and AMA, respectively), but also by means of indicators of developmental
4654 stress like fluctuating asymmetry, which in amphibians is usually recorded for paired biometrical
4655 variables (e.g. femur length, tarsus length, Zhelev et al. 2015) rather than for patterns of asymmetry
4656 in colouration or design.

4657 There are many other ways, besides the responses derived from hormonal disruption, by which
4658 pollutants can affect the reproductive process. In species with external fertilisation, both male and
4659 female gametes are directly in contact with the environmental pollutants in the aquatic environment
4660 right before fertilisation; the variation of the fertilisation rate of ova as a function of the concentration
4661 of chemicals in the environment can therefore be analysed in laboratory studies (Ortiz 2008). In the
4662 majority of anuran species, mating and egg-laying happen in the same sequence, which complicates
4663 the establishment of tests for addressing each of these features as specific endpoints. In caudates,
4664 however, mating and egg-laying are separate processes, which has allowed the design of studies in
4665 salamanders using the alteration of these specific features as toxicological endpoints. The courtship
4666 process is subject to modified patterns if at least one of the mates suffer from toxic effects of
4667 pollution; then, the entire courtship can be screened through video recordings and altered patterns
4668 can be observed by comparing exposed and non-exposed individuals (Secondi et al 2013). Egg-laying
4669 behaviour is very elaborate in some newt species that protect embryos by wrapping eggs with plant
4670 leaves; this allows for easily observing alterations in contaminated environments, as the proportion of
4671 wrapped eggs can be quantified with little effort (Ortiz-Santaliestra et al 2007). All these alterations
4672 related to the mating, fertilisation and egg-laying processes are not the only way by which
4673 reproduction can be affected. Mortality of pre-adult forms, leading to reduced recruitment, or
4674 developmental effects leading to reduced embryonic and larval survival, or to a decrease in successful
4675 metamorphosis, can also reduce reproductive outcome if compensation mechanisms are not enough
4676 to overcome such losses.

4677 Alterations of behaviour and activity are frequently studied effects of pollutants across all life stages of
4678 amphibians (except embryos), including terrestrial stages. Alteration of behaviour is usually regarded
4679 as an indicator of neurotoxicity, although the mechanisms of how the neurotoxic action of a chemical
4680 may end up in detectable behavioural effects are very variable. Alteration of reproductive behaviours
4681 has been discussed above, but there are other behavioural displays that have been studied in
4682 amphibians. Simple locomotor activity, especially swimming performance in larvae, has been tested by
4683 recording the percentage of animals moving, the time that animals move spontaneously or as a
4684 response to prodding, or even calculating the swimming speed (e.g. Brunelli et al 2009; Denoël et al
4685 2013). For instance, exposure of *Bufo bufo* tadpoles to endosulfan at 10 and 50 µg/L (nominal) *via*
4686 the ambient water from shortly after hatching to completed metamorphosis resulted in altered
4687 swimming activity as soon as four days after hatching (Brunelli et al 2009). Larval activity can be used
4688 as a way of testing anti-predator escape responses in tadpoles (e.g. Ortiz-Santaliestra et al 2010).
4689 Prey capture and feeding behaviours can also be studied in both aquatic and terrestrial amphibians. In
4690 general, recordings of the experimental enclosures allow for determining all kind of abnormalities in
4691 the behaviour of exposed animals. The entire feeding process in juveniles or adults can be split into
4692 prey detection (i.e. the time that the animal takes to detect the prey), approach (time since detection
4693 until actual capture), and manipulation (time since capture until complete swallowing) (Burke et al
4694 2010), and different patterns of alteration could suggest different types of alteration at the sensory
4695 and/or neuromuscular levels. Behaviours related to movements and orientation have been
4696 experimentally studied in amphibians; in particular, the different factors governing orientation have
4697 been addressed by leaving animals in circular arenas with different availability of potential orientation
4698 sources (Phillips et al 2010). Although the effects of pollution on orientation or homing behaviours

4699 have not been analysed, the fact that experimental studies have been designed to investigate how
4700 these specific behaviours are displayed (e.g. Diego-Rasilla et al 2015) provides the possibility of
4701 implementing them as endpoints in toxicological studies.

4702 Endpoints at the sub-organismal level in the amphibian ecotoxicological literature are not as common
4703 as in bird or mammal studies, although some studies have applied the best known metabolic
4704 responses from studies with other wildlife species to amphibians. These endpoints include
4705 quantification of metabolic and detoxifying enzymes (e.g. cytochrome P450, glutathione-S-
4706 transferase), oxidative stress biomarkers, or biomarkers of exposure or effects related to specific
4707 substances, such as inhibition of cholinesterase activity (e.g. Zhang et al 2013, Sparling et al 2015).
4708 Indicators of genotoxicity, and especially the micronucleus test, have also been widely observed in
4709 amphibians (e.g. Pollo et al 2016). Toxicodynamics studies that are commonly conducted with other
4710 wildlife specimens, analysing pollutant residues in different organs and tissues, also exist for
4711 amphibians, although limited to substances with certain bioaccumulation potential like metals or POPs
4712 (Huang and Karasov 2000). Most of the currently used pesticides have never been studied in this
4713 regard in amphibians.

4714 8.5.2 Reptiles

4715 Apart from the data on reproductive and endocrine disruption presented above (Section 8.4), data on
4716 sublethal responses of reptiles to pollutants are very sparse. In the field of growth and developmental
4717 effects, hatching parameters after an *in ovo* exposure have been reported in both lizards (Marco et al
4718 2004b) and turtles (Sparling et al 2006b). Neuman-Lee *et al* (2014) also studied biometry of offspring
4719 after maternal exposure of snakes to atrazine and several studies have quantified body-mass gain
4720 after long-term exposures (e.g. Salice et al 2009). Fluctuating asymmetry is regarded as a common
4721 indicator of developmental stress and has been used as a measure of stress in some toxicological
4722 studies. In contrast with amphibians, reptiles have some clearly recognisable, paired structures other
4723 than biometrical measures that can be used in quantification of fluctuating asymmetry; these include,
4724 for example, numbers of several types of scales or femoral pores (Amaral et al 2012b, Neuman-Lee et
4725 al 2014).

4726 The most widely used endpoints of sublethal effects in reptiles are those related to behaviour. With
4727 the use of video-recordings, prey-capture behaviour (Amaral et al 2012c), feeding rate (Peveling and
4728 Demba 2003, Salice et al 2009) or sprint speed after prodding of individuals introduced in a straight
4729 track (Amaral et al 2012c) have been recorded as behavioural indicators of pollutant exposure in
4730 lizards. As a very specific behaviour, it is worth mentioning the time to righting of turtles after being
4731 turned on their backs, which was proposed and used by Sparling et al (2006b) as an integrating
4732 endpoint on the basis that it is a process requiring coordination, stamina, and strength.

4733 Some sub-organismal responses have also been studied using techniques including histopathological
4734 evaluations of exposed individuals (Özelmas and Akay 1995, Neuman-Lee et al 2014), plasma
4735 biochemistry (Suski et al 2008, Salice et al 2009), oxidative stress biomarkers (Amaral et al 2012d),
4736 detoxification and metabolic enzymes (Yawetz et al 1983) or specific biomarkers like cholinesterase
4737 inhibition (Yawetz et al 1983). Some studies have managed to quantify some of these biomarkers
4738 without killing the animals, which constitutes a very important step in the application of these
4739 endpoints for monitoring of wild populations (Sanchez-Hernandez et al 2004).

4740 The use of more complex designs to study pollutant effects in reptiles is scarce in laboratory
4741 ecotoxicological studies. Amaral et al (2012a) conducted a long-term, mesocosm experiment and
4742 recorded some of the endpoints listed above, in lizards collected from the mesocosms at the end of
4743 the assay (i.e. growth, behaviour, biomarkers, histopathology). The high mortality recorded during the
4744 experiment unfortunately means that it is difficult to draw conclusions from extrapolation of
4745 laboratory-observed effects to responses in the field. Anyway, this study showed that terrestrial
4746 mesocosms could be adapted to reptilian ecotoxicological studies.

4747 8.6 Amphibian and reptilian model organisms for toxicity studies

4748 *Xenopus laevis* (African clawed frog) is the model species originally suggested for both OECD standard
4749 tests (AMA, LAGDA) as well as for FETAX. Both the AMA and the FETAX protocols have also been
4750 applied to other amphibian species. There are many advantages to the use of *Xenopus* as an

4751 experimental system, including the availability of abundant, externally developing embryos. The
4752 embryo-larval development in *Xenopus* has been divided into 66 discrete stages: Nieuwkoop & Faber
4753 (NF) stages 1 – 66 (Nieuwkoop & Faber, 1956) (see Table 25: for staging table). NF stage 66 is
4754 reached when the tail has completely regressed i.e. metamorphosis is completed. This staging table is
4755 very handy in toxicity studies as it enables exposure and analysis of effects at specific developmental
4756 stages. *Xenopus tropicalis* (Western clawed frog) has emerged as a useful model organism because of
4757 its short generation time and diploid, sequenced genome (Hirsch et al. 2002; Berg et al. 2009;
4758 Hellsten et al. 2010). The generation time of *Xenopus tropicalis* is about 4-6 months compared to 12-
4759 24 months in *Xenopus laevis* (Hirsch et al. 2002; Olmstead et al. 2009) and has therefore proven useful
4760 in life cycle studies (Pettersson et al 2006; Gyllenhammar et al 2009; Kvarnryd et al, 2011; Porter et al
4761 2011). The use of *Xenopus tropicalis* as an alternative to *Xenopus laevis* as a test species in the AMA
4762 and the FEATX tests has been evaluated (Carlsson & Norrgren, 2007; Fort et al 2004). Both studies
4763 concluded that there were no substantial differences between the species in terms of type of effects
4764 or sensitivity to the test substances evaluated, suggesting that *X. tropicalis* could be used effectively
4765 as an alternative test organism for the AMA and FETAX tests (Carlsson & Norrgren, 2007; Fort et al
4766 2004).

4767 Prior to the year 2000, most of the reptilian ecotoxicological literature focused on the endocrine
4768 disruption of alligators living in contaminated areas of North America. Based on the knowledge
4769 accumulated, Crain & Guillette (1998), and Crews et al (2003) proposed using reptiles as models for
4770 studying endocrine disruption. Later, Talent et al (2005) proposed the Western fence lizard
4771 (*Sceloporus occidentalis*) as a model species for ecotoxicological assessment, which could lead to
4772 further implementation of standard tests using this species. There has been no further developmental
4773 of tests, however, perhaps because reptilian tests are at present not required for regulatory purposes.

4774 8.6.1 Species differences in susceptibility to reproductive toxicity in 4775 amphibians

4776 Inter-species comparisons of susceptibility to developmental and reproductive toxicity in amphibians
4777 are difficult because of a lack of data. Most comparative studies investigate effects of ethynylestradiol
4778 (EE2) a potent estrogenic pharmaceutical and environmental pollutant, or atrazine. The susceptibility
4779 of *Xenopus tropicalis* and *Rana temporaria* to estrogen-induced disruption of gonadal differentiation
4780 was investigated in (Pettersson & Berg, 2007). Larvae of the two species were exposed to to
4781 ethynylestradiol (EE2), a potent estrogenic pharmaceutical and environmental pollutant, from shortly
4782 after hatching until completed metamorphosis. Larval EE2 exposure caused female-biased sex ratios
4783 at similar concentrations: 18 ng/L (0.06 nM) in *Xenopus tropicalis* and 27 ng/L (0.09 nM) in *Rana*
4784 *temporaria*. This study indicates that the effect of larval estrogen exposure was similar i.e. male-to-
4785 female sex reversal, and that the sensitivity of the two species to EE2 was comparable.

4786 Tamschick et al. (2016) investigated the susceptibility of sex differentiation to estrogen-induced
4787 disruption in three divergent anuran families, *Xenopus laevis* (Pipidae), *Hyla arborea* (Hylidae) and
4788 *Bufo viridis* (Bufonidae). The tadpoles were exposed to EE2 at the concentrations of 0, 50, 500 and
4789 5000 ng/L. The lowest exposure concentration that caused gonadal effects was 500 ng/L in all three
4790 species, but the effects differed between the species i.e. gonadal sex-reversal was shown in *Xenopus*
4791 *laevis* and *H. arborea* whereas mixed-sex (intersex) gonads was a more pronounced effect in *B.*
4792 *viridis*. Hence the sensitivity of the three species to estrogen-induced gonadal effects seems
4793 comparable although the nature of the effect differed. This in turn may be due to inter-species
4794 differences in exposure period relative to the gonadal differentiation period. It has been shown in
4795 *Xenopus laevis* tadpoles that the timing of estrogen exposure determines the extent of sex-reversal of
4796 the testis as described above (Chang & Witschi, 1956; Villapando & Merchant-Larios 1990).

4797 Mackenzie et al (2003) investigated effects of larval exposure to estrogenic and antiestrogenic
4798 compounds ($\mu\text{g/L}$ -concentrations) on gonadal differentiation in northern leopard frogs (*Lithobates*
4799 *pipiens*) and wood frogs (*Lithobates sylvaticus*). Exposure to the test substances induced alterations
4800 of gonadal differentiation in both species. Comparisons between the two species indicated that *R.*
4801 *pipiens* was more susceptible to sex reversal and development of intersex gonads.

4802 Hayes et al (2002; 2003) examined effects of atrazine on sexual development in *Xenopus laevis* and
4803 *Lithobates pipiens*. Larvae were exposed to atrazine (0.01–200 $\mu\text{g/L}$) by immersion throughout larval
4804 development. Atrazine exposure ($\geq 0.1 \mu\text{g/L}$) induced hermaphroditism in *Xenopus laevis* males

4805 (Hayes, 2002). In *Lithobates pipiens* atrazine exposure ($>$ or $=$ to 0.1 $\mu\text{g/L}$) resulted in retarded
4806 gonadal development (gonadal dysgenesis) and testicular oogenesis (hermaphroditism). Hence, these
4807 studies suggest that *Xenopus laevis* and *Lithobates pipiens* exhibited comparable sensitivity to atrazine
4808 (Hayes et al 2003).

4809 8.7 Conclusions

4810 Laboratory experiments have shown that there is a range of toxicological responses in amphibians and
4811 reptiles that are potentially useful as test endpoints for impaired embryo/larval survival,
4812 developmental rate, gonadal differentiation, spermatogenesis, oogenesis, fertility rate, and behaviour.

4813 Three standardized tests are available for amphibians: the Larval Amphibian Growth and
4814 Developmental Assay (LAGDA), the Amphibian Metamorphosis Assay (AMA), and the Frog Embryo
4815 Teratogenesis Assay – *Xenopus* (FETAX). Of these, LAGDA is the most extensive test with an
4816 experimental design that allows detection of disrupted metamorphosis as well as sexual development
4817 in the model species *Xenopus laevis*. AMA is designed to detect effects of chemical exposure on
4818 metamorphosis but the exposure period does not encompass the sensitive windows of sex
4819 determination and gonadal differentiation in *Xenopus laevis*. None of the above tests, however, covers
4820 the reproductive ability of amphibians. A full life-cycle test with amphibians (e.g. with *Xenopus*
4821 *tropicalis*, which has a shorter generation time than *Xenopus laevis*) could be very useful in a risk
4822 assessment context because it enables the identification of impaired reproductive function following
4823 exposure during a sensitive window of development.

4824 No standard test guidelines exist for reptiles and there is a lack of toxicity data for this group of
4825 vertebrates. This makes it very difficult to compare the toxicological sensitivity among different reptile
4826 species. Standard test protocols should be developed for reptiles in order to close these knowledge
4827 gaps in future.

4828 The potential of relying on other vertebrates as surrogates for amphibians and reptiles to cover
4829 toxicity of PPPs is compromised by some particular biological processes typical of these animals,
4830 including metamorphosis in amphibians or hormone-dependent sex determination and sex organ
4831 development in both amphibians and reptiles. Thus, impacts of pesticides need to be assessed for
4832 specific, sensitive time windows within the amphibian aquatic development. It is suggested that
4833 research is conducted to develop in-vitro tests for acute and chronic effects.

4834

4835 9. Exposure assessment in the environment

4836

4837 9.1. Introduction

4838 The ecological attributes of the specific protection goals (SPGs) have been defined for amphibians and
4839 reptiles both at the level of individuals and the population (Chapter 5). At an individual level, both the
4840 individual juvenile and adult amphibians and reptiles are to be protected, while at the population level,
4841 all life stages (including eggs) are potentially important. This implies that possible exposure routes
4842 during all life stages of amphibians and reptiles need to be considered.

4843 Amphibians have an aquatic as well as terrestrial habitat, while reptiles mainly live terrestrially. Below
4844 we first consider the exposure for amphibians, both aquatic and terrestrial, and next, the exposure for
4845 reptiles. Because the SPGs concern in-field as well as off-field habitats, the exposure needs to be
4846 assessed in-crop and off-crop. In the following the different environments (aquatic, terrestrial, in-crop,
4847 off-crop) have been addressed separately. An individual may, however, be exposed in multiple
4848 environments throughout its life span. Note that the tables summarising the exposure routes in the
4849 sections 9.2 and 9.3 have been based on expert judgement and are not rigorously based on data, as
4850 these are insufficiently available. In the following, the different routes of exposure will be addressed
4851 separately. An individual may, however, be exposed through multiple routes at the same time or also
4852 throughout its life span.

4853 Tables have been drawn below for the Exposure Assessment Goals, including their specific elements,
4854 as well as the exposure routes that are coherent with the SPGs defined at the level of individuals (no
4855 mortality) for both the aquatic and terrestrial environment of amphibians and for reptiles. For
4856 population persistence, SPG tables on the Exposure Assessment Goal and exposure routes were drawn
4857 for amphibians in the aquatic environment only, based upon the two standardized amphibian test
4858 guidelines available within the OECD framework: the Amphibian Metamorphosis Assay (AMA) and the
4859 Larval and Growth Development Assay (LAGDA). We thus implicitly assume that the SPG of population
4860 persistence with its attributes of abundance/biomass, distribution and population growth can be
4861 evaluated on the basis of these two tests.

4862 The Working Group did not draw tables for Exposure Assessment Goals and exposure routes that are
4863 coherent with the SPGs of population persistence with its attributes of abundance/biomass,
4864 distribution and population growth for amphibians in the terrestrial environment and for reptiles,
4865 except for eggs in nests. At present no detailed and quantitative definitions of the SPG population
4866 persistence for its attributes exist for EU registration, especially for amphibians in the terrestrial
4867 environment and for reptiles other than their nests; it is therefore not yet possible to define
4868 satisfactorily e.g. the spatial unit and its statistical population. This may be possible in a later stage.
4869 Population modelling presented in Chapter 4 may be an alternative way to evaluate the SPGs of
4870 population persistence in the future.

4871

4872 9.2. Exposure of amphibians

4873 9.2.1. Aquatic environment

4874

4875 The entirely aquatic life stages of amphibians are aquatic eggs, hatchlings and larvae (tadpoles). At
4876 the end of the larval stage, the larvae undergo metamorphosis and transform into terrestrial juveniles
4877 and, next, adults. Juveniles and adults also stay part of their time in the aquatic environment and
4878 some even hibernate in the sediment of water bodies. So, all life stages may be exposed to pesticides
4879 *via* the aquatic environment.

4880 *Type of aquatic habitats*

4881 A variety of water body types may be the aquatic habitat of amphibians. Ponds or pools, but also
4882 ditches, canals, small and bigger streams and even (artificial) lakes may host amphibians. Ponds may
4883 be isolated, but also linked to an inflow or outflow (Figure 27:). Amphibians have a preference for
4884 environments without predators such as fish, so temporary ponds are preferred habitat .

4885 As temporary ponds are generally shallow, it is important to account for evaporation of water in the
4886 exposure assessment. As a result of the shrinking water volume, concentrations may not decrease as
4887 is usually expected in water bodies; in extreme cases (e.g. no degradation or other dissipation
4888 processes), concentrations might even increase over time in such shallow, temporary ponds.

4889 The depth of the water bodies may be as low as a few centimetres. Permanent water bodies mostly
4890 occur off-field, whereas temporary water bodies may occur in the middle of a treated field as well as
4891 off-field. The formation of temporary ponds depends on the amount of rainfall and soil characteristics.
4892 They may be recharged by rainfall (southern Europe) or groundwater (northern Europe. Drainage may
4893 also recharge temporary ponds in the field (personal communication DE and CH).

4894 There is little knowledge on the distribution of ponds where amphibians dwell (see also Appendix C on
4895 dimensions etc of ponds in Spain, UK and Switzerland). Surrounding agricultural land use of such
4896 ponds is important to be able to assess exposure concentrations, e.g. in ponds located in
4897 grassland/meadows, exposure to pesticides will be much lower than in ponds located in arable land,
4898 such as potatoes or cereals. For instance, in a country like the Netherlands more amphibian ponds can
4899 be found in habitats surrounded by grassland than in habitats surrounded by agricultural crops.

4900



4901

4902 **Figure 27:** Pond having inflow and outflow in the UK (source: Williams et al, 2010)

4903

4904 *Dimensions and surrounding land use of amphibian ponds in Spain and Switzerland and ponds in the*
 4905 *UK and comparison to water bodies of the EU FOCUS surface water scenarios*

4906 In Appendix C, the working group gathered data on surface area, water depth and water volume of
 4907 ponds serving as aquatic habitat for amphibians, as well as on land use in their immediate
 4908 surroundings. The dimensions of amphibian ponds are important to be able to determine pesticide
 4909 concentrations in the ponds. By assessing whether agricultural land use occurs in the immediate
 4910 surroundings of the ponds, we are able to evaluate whether the amphibians are likely to be exposed
 4911 in their aquatic habitat. In addition, the dimensions of the amphibian ponds were compared to the
 4912 dimensions of the so-called FOCUS surface-water bodies (pond, ditch and stream) that are currently
 4913 used in the risk assessment for the aquatic ecosystem at EU level. This was done in order to assess
 4914 whether the PEC values are really 'worst case' in the FOCUS water bodies used in the regulatory
 4915 process.

4916 In Spain, 794 water bodies serving as amphibian breeding sites were monitored from 2010 onwards.
 4917 The water bodies have been classified as ponds (421), artificial pool (152), dam/reservoir (66),
 4918 lagoon/lake (21), river (30), stream (85) and wetland/marsh (19).

- 4919 • In 18% of the water bodies, water depth was 30cm or less (30cm is the minimum water
 4920 depth of the FOCUS ditches and streams).
- 4921 • In 70% of the water bodies, water depth was 1m or less (the depth of the standard FOCUS
 4922 pond).
- 4923 • In 59% of the water bodies, water surface area was less than or equal to 100 m² (the surface
 4924 area of the 100m*1m FOCUS ditches and streams).
- 4925 • In 87% of the water bodies, surface area was less than 900 m² (the surface area of the
 4926 30m*30m FOCUS ponds).
- 4927 • Land use data were available for 151 Spanish ponds used by amphibians. For 70 ponds
 4928 agricultural fields were present within 100 m distance, out of which 13 ponds were entirely
 4929 surrounded by agricultural fields. These data demonstrate that a non-negligible proportion of
 4930 the ponds in Spain in which amphibians live, are likely to receive pesticides residues.

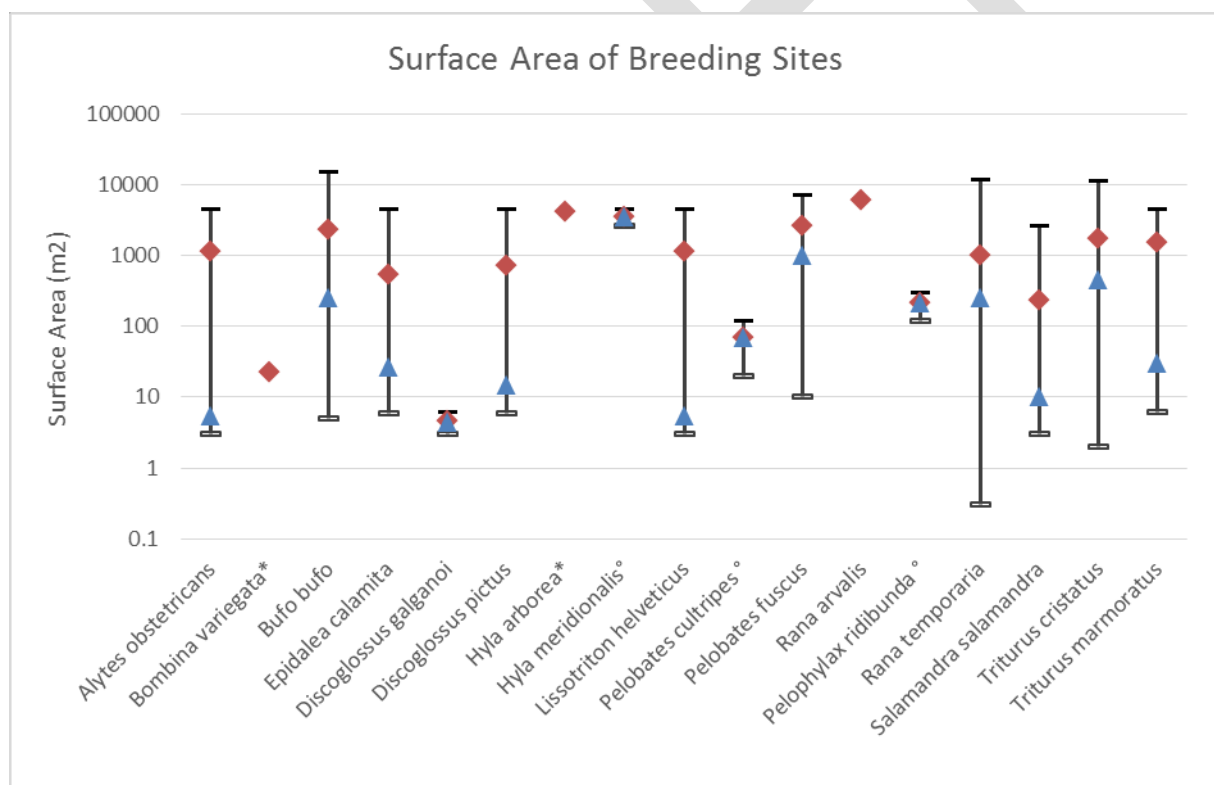
4931 In the Aargau canton of Switzerland, there has been an amphibian-monitoring program since 2006.
 4932 Eight amphibian species were surveyed. Ponds were selected based on the occurrence of one of these
 4933 eight species and in total 754 water bodies are surveyed. Volunteers estimated pond surface areas
 4934 during the period mid-June to end of July (with some exceptions between March and September) but
 4935 water depths were not recorded.

- 4936 • In 52% of the water bodies, surface area was 100 m² or less (the area of the FOCUS ditches
4937 and streams)
- 4938 • In 89% of the water bodies, surface area was 900 m² or less (the area of the FOCUS ponds).
- 4939 • There are no data for the Swiss canton ponds that specify the land use in their immediate
4940 surroundings. Aargau is a canton with intensive agriculture and a good distribution of
4941 amphibian populations, therefore a number of the ponds in the survey are likely to represent
4942 amphibian habitats that may receive pesticide residues.
- 4943 In the United Kingdom, the current state of ponds was surveyed and described in the Countryside
4944 Survey of 2007 (Williams et al, 2010). A pond was defined as a body of standing water of 25 m² to 2
4945 ha, in area, which usually holds water for at least four months of the year. Ponds smaller than 25 m²
4946 were not recorded, although they might have been present. The survey made an inventory of all
4947 ponds (as defined above), including ponds where amphibians may not be present. It covered a total
4948 of 591 1x1 km square samples spread across England, Scotland and Wales. The ponds may be
4949 isolated or not. The survey demonstrated that almost two-thirds (63%) of ponds were directly linked
4950 to the stream network and that a third of these ponds had an inflow but no outflow, suggesting that
4951 many ponds intercept and retain drainage water. Water surface area was recorded for 257 ponds,
4952 mostly in the period May-October 2007.
- 4953 • 23% of the ponds surveyed had a surface area of 100 m² or less (the surface area of FOCUS
4954 ditches and streams)
- 4955 • 79% of ponds surveyed had a surface area of 900 m² or less (the surface area of the FOCUS
4956 ponds).
- 4957 • The depth was measured for 109 ponds. The maximum depth was not measured in the
4958 remaining 150 ponds, because it was too deep to wade; thus the data on water depth are
4959 biased with maximum water depth over 1m not represented. For 69 of the 109 ponds where
4960 depth was measured, the water depth was below 0.3m or less, and these 69 ponds represent
4961 27% of the total of 257 ponds.
- 4962 • Depending on the classification of arable land use (presence in the 0-100 m perimeter around
4963 the ponds, or the regionally-based land use classification of the ITE, Institute of Terrestrial
4964 Ecology) approximately 20% (59 of the 259 ponds) or nearly 50% of the ponds (115 of the
4965 259 ponds) had significant arable land use in the vicinity of the ponds.
- 4966 • The presence of amphibians (e.g. tadpoles, frogs, newts) was recorded. Amphibians were
4967 observed in 49 of the 259 sampled ponds. This number is expected to be an underestimation
4968 of reality, as the observation of amphibians will depend on the expertise on amphibians of the
4969 surveyor as well as on the time of the year of the survey (often between April/May to
4970 October/November).
- 4971 The analysis of the dimensions of the Spanish and Swiss amphibian ponds and the CountrySide
4972 Survey ponds in the UK (Appendix C) demonstrates that most of them (70% - 90%) are considerably
4973 smaller than the FOCUS ponds. This means that we expect peak concentrations in FOCUS ponds *not*
4974 to be conservative estimates for the ponds in the surveys. It is more complicated to compare the peak
4975 concentrations in FOCUS ditches and streams with the ponds in the surveys, therefore the working
4976 group was unable to make a general statement on whether or not peak concentrations in FOCUS
4977 ditches and streams are conservative for the ponds in the surveys. In view of the higher flow-through
4978 rates in the FOCUS ditches and streams, however, the pesticide concentrations are expected to
4979 decline more rapidly in the FOCUS ditches and streams and thus they probably underestimate chronic
4980 exposure in the surveyed ponds. The Panel therefore expects that the FOCUS ditches and streams are
4981 *not conservative* for the chronic risk assessment of exposure in ponds used by amphibians in the EU.
- 4982 The Working Group compiled Appendix C, which gives an overview of pond characteristics across the
4983 EU in order to be able to make a spatio-temporal statistical distribution of environmental
4984 concentrations. From such a distribution, ponds with the desired percentile 'worst-caseness' in
4985 concentration could be selected and used to perform the amphibian risk assessment.
- 4986

4987 *Surface area and water depth of ponds for 17 and 14 amphibian species in Europe*
 4988 A literature search on amphibian breeding sites resulted in the type and size of water body within
 4989 Europe in which different amphibian species prefer to breed (Annex B, Appendix E). In 104
 4990 publications the size measurements of the water bodies were reported, of which 61 contained data
 4991 suitable for further analysis. Data were analysed from studies where it was explicitly specified that
 4992 ponds were used as breeding sites or wherever the presence of juveniles, tadpoles or eggs was
 4993 reported. Surface area and water depth were determined: minimum and maximum values, as well as
 4994 means and medians for various amphibian species. The surface area of breeding sites and water
 4995 depth were evaluated for 17 and 14 amphibian species, respectively (Figure 28:). Median surface
 4996 areas ranged from 4.50 m² to 3500 m² for *Discoglossus galganoi* (n=3) and *Hyla meridionalis* (n=2),
 4997 respectively (Garcia-Gonzalez & Garcia-Vazquez 2012; Ruhi et al., 2012). The smallest median depths
 4998 were reported for *Discoglossus pictus* (n=10) and *Epidalea calamita* (n=14) with 0.18 m (Ruhi et al.,
 4999 2012; Sebastian et al., 2015). The maximum median depth was 1.20 m, reported both for *Bufo bufo*
 5000 (n=6) and *Pelobates fuscus* (n=23) (Eggert & Guyetant 1999; Nystrom et al., 2007; Ruhi et al., 2012;
 5001 Sebasti & Carpaneto 2004; Sztatecsny & Holdl 2009). The compiled data can aid to add certainty to
 5002 the characterization of breeding habitats. The data give a rough estimate of the ranges of breeding
 5003 pond sizes within which species occurred, but this analysis does not rule out that habitats of different
 5004 sizes might also be suitable for the respective species.

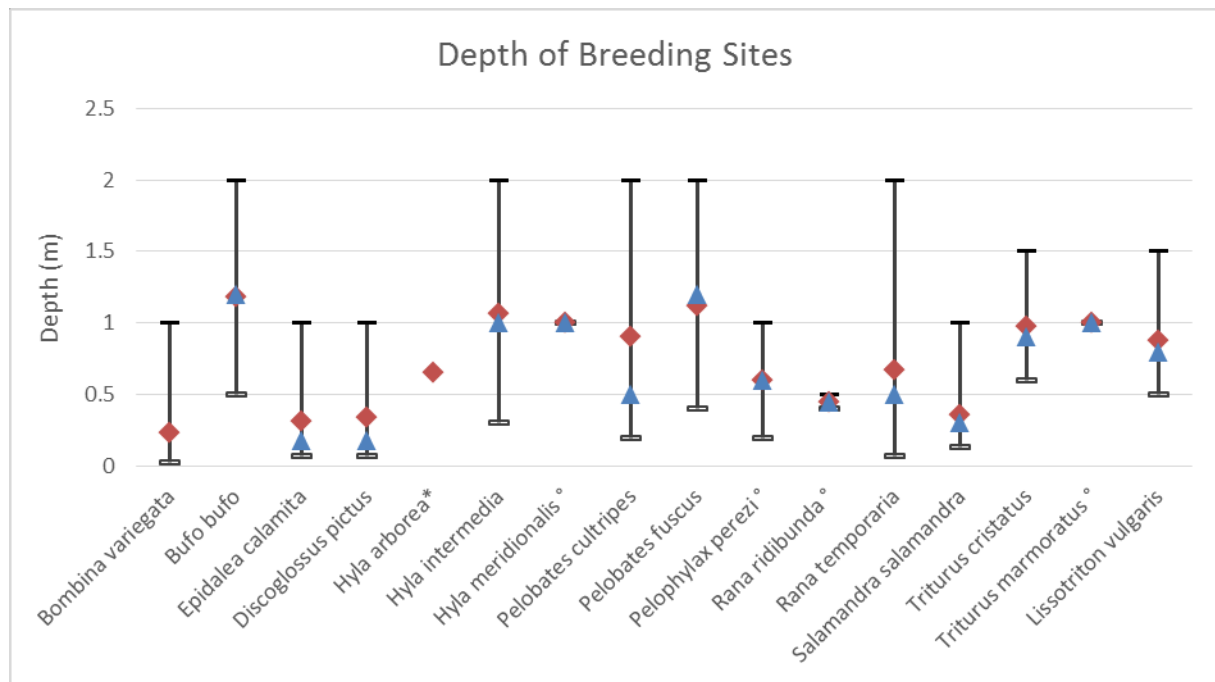
5005 Surface-area and water-depth measurements for sites in which the use as breeding sites was not
 5006 explicitly stated, or only the presence of adults was reported, were not considered in the two Figure
 5007 28: presented below.

5008



5009

5010



5011

5012

5013 **Figure 28:** Ranges of surface area (A) and depth measurements (B) of breeding sites reported in
 5014 literature for different amphibian species. Medians (blue triangle) and means (red diamond) were
 5015 calculated from literature values for $n \geq 2$. Species for which only 2 data points were available are
 5016 marked with a °; species for which only a single mean value was reported are marked with a *.

5017

5018 For the ponds used for breeding by 17 amphibian species, the mean surface area ranged from 4.6 to
 5019 4160 m², while the mean water depth ranged from 0.23 to 1.18 m (Appendix E).

5020

5021 *Paddy fields*

5022 Paddy fields, i.e. flooded fields with a rice crop, were only briefly discussed in the Working Group,
 5023 because they were judged not to be a major habitat of amphibians. It is clear that paddy fields
 5024 receive agricultural inputs such as fertilisers and pesticides, exposure to pesticides is expected to be
 5025 considerable and that paddy fields require a separate exposure-assessment procedure, that will differ
 5026 from other types of water body. For example, overspray with pesticides will be the rule, while drained
 5027 water, containing pesticides, from other paddy fields may also be a likely entry route.

5028 *Ponds versus puddles*

5029 In the context of this Opinion on amphibians and pesticide regulation, the WG considers ponds in
 5030 locations that may be in the vicinity of agricultural land (edge-of-field or somewhat farther, but still
 5031 part of the habitat that is relevant in landscape modelling) or within agricultural fields. In the latter
 5032 case the (temporary) ponds are surrounded by crops and may receive spray-drift deposition, but they
 5033 should never receive any overspray, as pesticide regulation assumes Good Agricultural Practices.

5034 Puddles were defined as being temporary water bodies only, that may be located in-field and may
 5035 receive overspray. This implies that exposure in puddles will be considerably higher in puddles than in
 5036 ponds, thus in greater risks for amphibians. It is a risk manager's decision whether or not to include
 5037 puddles in the risk assessment and to decide on (a) potential mitigation measures, and (b) to judge
 5038 what consequences this may have with respect to the safe use of the pesticide as well as the farmer's
 5039 behaviour with respect to puddles. For example, an obligation to establish buffer zones around
 5040 puddles might lead farmers to fill in the puddles.

5041

5042 *Sediment*

5043 Bed sediment might be an important environmental compartment for amphibians as they may feed on
5044 it as well as hibernate in it. Amphibians may become exposed to pesticides *via* pore water, the organic
5045 matter content or a combination of both. The distribution of PPP between pore water and sediment
5046 organic matter depends to a great extent on the sorption capacity of the pesticide and is generally a
5047 function of depth in the sediment. Over a year, or years, pesticides may accumulate in sediment. This
5048 implies that the maximum PEC in the sediment is generally not immediately after application and
5049 accompanying spray-drift deposition on the water column, but later. The exact timing depends on e.g.
5050 the sorption capacity of the compound, the degradation rate in the sediment, the degradation rate in
5051 the overlying water column (promoting back diffusion from the sediment into the water), the number
5052 of applications, or the organic matter content of the upper sediment.

5053 The sediment is characterised by its properties of dry bulk density, porosity and organic
5054 matter/organic carbon content. These properties are a function of depth. There are, however, few
5055 data on this, especially for water bodies that host amphibians.

5056 Sediment properties were measured in four watercourses in the Netherlands, located immediately
5057 adjacent to an arable or horticultural field and distributed across the country (Adriaanse et al, 2015).
5058 They were sampled twice, in June/July and in September 2013, carried water all year round and had a
5059 minimum water depth of 20 cm. Sediment cores were sampled by pushing transparent PVC tubes into
5060 the sediment; these were frozen and then cut with a belt saw into segments of 0-1 cm, 1-2 cm, 3-5
5061 cm and 5-10 cm. For each location, five cores were sampled in June/July and again in September.
5062 Averages and standard deviations were calculated for dry bulk density, porosity and organic matter
5063 content for each segment. The properties vary relatively little with depth. The sediment bulk densities
5064 are very low compared to soil bulk densities (0.9 to 1.8 g/ml, excluding peat soils): they range from
5065 0.09 to 0.40 g/ml for the top 0-1 cm and 0.37 to 0.66 g/ml for the lower 5-10 cm sediment. For
5066 porosity, the numbers range from 0.71 to 0.93 for the upper cm and 0.75 to 0.84 for the 5-10 cm
5067 segment. For the organic matter content, the numbers are 8.9 to 30.2% for the 0-1 cm segment and
5068 6.5 to 22.6% for the 5-10 cm segment.

5069 In the USA, bed sediment was sampled from perennial or seasonal ponds containing actively breeding
5070 native amphibian populations and located in proximity to agricultural or urban areas where pesticides
5071 were being applied (Smalling et al, 2012). Samples were collected in 2009 and 2010, in early spring
5072 and summer during the amphibian breeding season in the states of California, Colorado and Oregon
5073 (undeveloped, remote areas without direct pesticide applications) and Georgia, Idaho, Louisiana and
5074 Maine (where the sampling sites were in close proximity to either agricultural or suburban areas). The
5075 bed-sediment samples were collected from areas of active sediment deposition. A stainless steel scoop
5076 was used to collect the top 2cm of bed material from multiple points within approximately 1m² area,
5077 which was passed through a 4mm mesh sieve before analysis. Percent organic carbon ranged from
5078 0.2 to 36.0% for the 42 sites and was below 10 for the large majority of sites. In exposure
5079 assessment for pesticide-risk assessment, 90th percentile worst-case exposures are often considered.
5080 Depending on whether the exposure is mainly *via* the pore water or via the organic matter content the
5081 90th percentile worst-case sediment has 1.2% or 12% organic matter, respectively.

5082



5083

5084 **Figure 29:** Tadpoles of the common toad (*Bufo bufo*) feeding by grazing on reed and its biofilms
5085 (source: Christian Fisher, CreativeCommons)

5086 *Macrophytes, algae, biofilms, zooplankton*

5087 Amphibians have a variable diet composition (section 2.2.6). Newt and salamander larvae mostly feed

5088 on zooplankton, while anuran larvae are vegetarian and feed mainly on periphyton, grazing on
 5089 sediment, plants or biofilms present on the plants (Figure 29:). They may thus become exposed to
 5090 pesticides sorbed or taken up by the plants/biofilms. Filtering phytoplankton or skimming the scum at
 5091 the water surface are also very common among anuran tadpoles. Aquatic habitats generally contain
 5092 one or more types of plants, such as macrophytes (rooted or floating), macroalgae (submerged and
 5093 floating), floating microalgae or biofilms present on macrophytes. Pesticides sorb to plants (e.g. Crum
 5094 et al, 1999), or may be taken up by shoots or roots of plants (e.g. Burešová et al, 2013). The mass
 5095 sorbed (and thus concentration) depends on the sorption capacity of the compound and need to be
 5096 assessed on a case by case basis as no standard, accepted process description and compound-specific
 5097 parameters are available.

5098

5099 9.2.2. Exposure assessment goals and exposure routes for aquatic 5100 environment

5101 The elements of the exposure-assessment goals linked to the Specific Protection Goal for individual
 5102 amphibians (no mortality, see section 5) in their aquatic environment are described in Table 23: . The
 5103 corresponding exposure routes are based upon Table 3: of section 2.4 and given in Table 24: . The
 5104 EREQs mentioned in Table 24: are proposals. As standard ecotoxicological experiments for
 5105 amphibians are not yet available for the pesticide-registration procedure, the final EREQ choice can
 5106 only be made after a deliberate selection of the most relevant exposure concentration in the possibly
 5107 future ecotoxicological experiment to express the endpoint. (For reasons of simplification the word
 5108 'ponds' is used in the tables for all temporary or (semi-) permanent water bodies hosting amphibians).

5109 The size of the spatial unit in the tables below, i.e. the ponds with surface area of 10 m² to 2 ha, was
 5110 based upon the following considerations. The minimum pond surface area was set to 10 m² as the WG
 5111 judged that (i) smaller ponds usually do not persist sufficiently long to allow larvae to develop into
 5112 juveniles, (ii) farmers tend to fill in very small ponds, so they do not exist long in the agricultural
 5113 landscape and (iii) no species could be identified that are specialised in small ponds only. The
 5114 maximum surface area of 2 ha was mentioned as the upper limit in the pond definition of the UK
 5115 Countryside Survey (Williams et al, 2007) and was judged to correspond sufficiently with the tendency
 5116 of amphibians to lay their eggs not too far from the coast or edge of the pond.

5117

5118 **Table 23:** Elements of the exposure assessment goal for individual juvenile or adults amphibians
 5119 (SPG: no mortality) in the aquatic environment (in-field, plus edge-of-field)

Element	Description	Remarks
EREQ	See Table 24:	
Temporal dimension of EREQ	See Table 24:	
Spatial unit (SU), type and size (if relevant)	Ponds with surface area of 10 m ² to 2 ha	Implicitly averaging over whole surface area of pond is considered acceptable
Statistical population of SUs	All ponds in-field or edge-of-field of treated agricultural fields in area of use of substance	

Multi-year temporal statistical population of EREQ values for one spatial unit	Series of tens of years of annual maximum concentration in pond water in years of treatment	Consider only concentrations in periods that are relevant, i.e. when juveniles and adults are present in the ponds
Desired spatio-temporal percentile of the statistical population of EREQ values	Overall 90 th percentile of the statistical population of each EREQ	Percentile can easily be changed if needed

5120

5121 **Table 24:** Exposure routes and the definition of the EREQs for individual juvenile or adult
5122 amphibians (SPG: no mortality) in the aquatic environment (in-field, plus edge-of-field).

Exposure route		Source/ location	EREQ	Temporal dimension of EREQ	Remarks
Contact exposure	Pond water	Spray drift	Concentration dissolved in pond water	Maximum in relevant period of the year	Important route for in-field ponds, less important for edge-of-field ponds
		Runoff			Important route, also for edge-of-field ponds receiving runoff water from treated fields
		Drainage			May be important route in case of macropore drainage
		Atmospheric deposition			Minor route for in-field ponds. Negligible route for edge-of-field ponds
Oral exposure		Food, plants, water	Daily mass taken in by individual	Maximum in relevant period of the year.	Minor for individuals in ponds, except newts
Inhalation		Air	-	-	Expected to be a minor route compared to oral and contact exposure.

5123 * In chronic risk assessment realistic worst-case time-weighted average concentrations may also be used. Annual exposure
5124 profiles may be needed e.g. to allow the use of TK-TD models to predict the effects of the realistic exposure profile.

5125

5126 Similar tables linked to the Specific Protection Goal for the population persistence
 5127 (abundance/biomass, distribution and population growth rate, see section 7) and their corresponding
 5128 exposure routes have been made (Table 25: and Table 26:). This covers all life stages of amphibians
 5129 including eggs, larvae, juveniles and adults. These tables are made consistent with the two
 5130 standardized amphibian test guidelines available within the OECD framework: the Amphibian
 5131 Metamorphosis Assay (AMA), lasting 21 days and the Larval Growth and Development Assay (LAGDA,
 5132 see section 8.2.1), spanning the period from the embryo stage before hatching (NF 8-10) up to 10
 5133 weeks after the median time to reach NF stage 62 in the control group.

5134 **Table 25:** Elements of the exposure-assessment goal for all life stages of amphibian populations
 5135 (SPG: population persistence) when they are in the aquatic environment (in-field, plus
 5136 edge-of-field)

Element	Description	Remarks
EREQ	See Table 26:	
Temporal dimension of EREQ	See Table 26:	
Spatial unit (SU), type and size (if relevant)	Ponds with surface area of 10 m ² to 2 ha	Implicitly averaging over whole surface area of pond is considered acceptable
Statistical population of SUs	All ponds in-field or edge-of-field of treated agricultural fields in area of use of substance	
Multi-year temporal statistical population of EREQ values for one spatial unit	Series of tens of years of annual maxima of time-weighted average concentration in pond water in years of treatment	Consider only concentration durations and periods that are relevant, i.e. for AMA (21 d) and LAGDA (NF 8-10 up to 10 weeks after NF 62, i.e. approximately 4 months) in their relevant periods (spring/early summer)
Desired spatio-temporal percentile of the statistical population of EREQ values	Overall 90 th percentile of the statistical population of each EREQ	Percentile can easily be changed if needed

5137

5138 **Table 26:** Exposure routes and the definition of the EREQs for all life stages of amphibian
 5139 populations (SPG: population persistence) in the aquatic environment (in-field, and edge-
 5140 of-field)

Exposure route	Source/location	EREQ	Temporal dimension of EREQ	Remarks
•				

Contact exposure	Pond water	Spray drift	Concentration dissolved in pond water	Maximum or maximum moving TWA* over specified time window for AMA or LAGDA test in the relevant period of the year (length of the time window depends on the endpoint considered e.g. growth or sex ratio)	Important route for in-field ponds, less important for edge-of-field ponds located not immediately adjacent to crops
		Runoff			Important route, also for edge-of-field ponds receiving runoff water from treated fields
		Drainage			Possibly an important route
		Atmospheric deposition			Minor route for in-field ponds. Negligible route for edge-of-field ponds
Oral exposure		Food, plants, sediment, water	The daily mass taken in by individuals of the population.	Maximum in relevant period of the year	May be important for a compound with high adsorption capacity such as pyrethroids. No spiked food in AMA and LAGDA tests, therefore the main exposure route in AMA and LAGDA tests is via contact (water)
Breathing		Air	-	-	Expected to be a minor route of exposure compared to contact and oral exposure for juveniles and adults, for aquatic eggs, hatchlings and larvae expected to be unimportant as they do not or hardly breath

5141 * See the Aquatic Guidance Document (section 4.5.1 in EFSA, 2013) for criteria when TWA concentrations may be used

5142

5143 The four tables above focus on the exposure of amphibians in their aquatic environment *via* water
 5144 mainly. As mentioned in Table 3: of section 2.4, however, the underlying sediment may also be an
 5145 important exposure route, especially for tadpoles feeding on sediment, and for adults that may
 5146 hibernate in sediment. This is important for compounds that accumulate in sediment, especially when
 5147 concentrations in the overlying water column are low so that the primary route of exposure is *via*
 5148 sediment. There are no standard tests to assess toxicity of pesticides to adults hibernating in
 5149 sediment, so relating exposure in the field to ecotoxicological effects observed in standard toxicity
 5150 tests is not possible. From an ecotoxicological point of view, exposure during hibernation is probably

5151 mainly *via* the dermal route as metabolism of the amphibians is low, and, in view of their permeable
 5152 skin, the ecotoxicological relevant concentration is probably the pore-water concentration (and not the
 5153 concentration sorbed to the sediment or the total concentration). The working group found two test
 5154 guidelines for tadpoles from the USA to evaluate risks of pesticides to amphibians, both considering
 5155 tadpoles exposed by ingestion of and contact with the sediment: (i) the ASTM Whole sediment toxicity
 5156 tests with amphibians E2591-07 (2013) and (ii) the EPA Tadpole/sediment subchronic toxicity test
 5157 OPPTS 850.1800 (1996). The ASTM Guideline uses spiked sediment tests of 10 d with recently
 5158 hatched tadpoles, sediment concentrations are expressed in mg of active ingredient per kg dry
 5159 sediment and the overlying water may be renewed by continuous replacement (flow through) or static
 5160 renewal. The EPA guideline uses spiked sediment of three different natural sediments (organic matter
 5161 content of 0.1-0.2%, 0.5-1.0% and 2.0-3.0%) and tadpoles before metamorphosis, characterized by
 5162 the emergence of hind paddles and respiration by gills. The tadpoles are exposed by ingestion, either
 5163 by direct dosage of spiked slurry into their buccal cavity at the beginning of the test in test chambers
 5164 with only clean dilution water or by allowing tadpoles to ingest contaminated sediment *ad libitum* in
 5165 chambers containing 3-5 cm contaminated sediment and 10-20 cm overlying water. Sediment (and
 5166 water) concentrations are measured at $t=0$ and every 10 d thereafter and sediment concentrations
 5167 are expressed in mg a.i per kg sediment (dry weight). Based upon the EPA Guideline, which was most
 5168 explicit in its description with respect to the exposure in the test system, we defined below the
 5169 elements of the exposure-assessment goals linked to the Specific Protection Goal for population
 5170 persistence (abundance/biomass, distribution and population growth rate) and the corresponding
 5171 exposure routes in the two Table 27: and Table 28: .

5172

5173 **Table 27:** Elements of the exposure-assessment goal for individual tadpoles (SPG: population
 5174 persistency) in the sediment of the aquatic environment (in-field, plus edge-of-field)

Element	Description	Remarks
EREQ	See Table 28:	
Temporal dimension of EREQ	See Table 28:	
Spatial unit (SU), type and size (if relevant)	Ponds (with their sediment) with surface area of 10 m ² to 2 ha	Implicitly averaging over whole surface area of sediment in the pond is considered acceptable
Statistical population of SUs	All ponds (with their sediment) in-field or edge-of-field of treated agricultural fields in area of use of substance	
Multi-year temporal statistical population of EREQ values for one spatial unit	Series of tens of years of annual maximum TWA 30d concentration in sediment in years of treatment	Consider only concentrations in periods that are relevant, i.e. when tadpoles are present in the ponds
Desired spatio-temporal percentile	Overall 90 th percentile of the statistical population	Percentile can easily be changed if

of the statistical population of EREQ values	of each EREQ	needed
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5175

5176

5177 **Table 28:** Exposure routes and the definition of the EREQs for individual tadpoles (SPG: population
5178 persistence) in the sediment of the aquatic environment (in-field, plus edge-of-field).

Exposure route		Source/ location	EREQ	Temporal dimension of EREQ	Remarks
Oral exposure					Main exposure route
		Sediment	Total concentration in upper x mm layer sediment	Maximum or maximum moving TWA* over specified time window in the relevant period of the year (length of the time window depends on the endpoint considered e.g. growth or sex ratio)	Important route, always present but may depend on feeding mode of tadpole
		Periphyton on waterplants	Total concentrations in biofilms on waterplants		Importance of route may depend on presence and type of plants and feeding mode of tadpoles
		Other food items (e.g. zooplankton or phytoplankton)	Total concentration in other food items		Importance of route may depend on presence and type of other food items and feeding mode of tadpoles
		Water (i.e. respiration/gill filtration)	Total concentration in water (incl sorbed on suspended solids)		Important route compared to other oral routes (ingestion)
Contact exposure	Pond water		Concentration dissolved in pond water	Maximum or maximum moving TWA* over specified time window in the relevant period of the year (length of the time window depends on the endpoint considered e.g. growth or sex ratio)	Less important route than oral route (ingestion)
	Sediment		Concentration in sediment pore water		Less important route than oral route (ingestion)

5179

* See the Aquatic Guidance Document (section 4.5.1 in EFSA, 2013) for criteria when TWA concentrations may be used

5180

5181 9.2.3. Terrestrial environment

5182 This section focusses on exposure of adult and juvenile amphibians in their terrestrial habitat only, as
5183 these are the only terrestrial life stages of amphibians.

5184 *Type of terrestrial habitats*

5185 As mentioned in section 2.2.5. in Europe 38 amphibian species (43% of the amphibian diversity)
5186 inhabit arable lands. Off-field sites are a preferred habitat, but occasionally the occupation of arable
5187 areas can be dominant. In other cases, the use of arable fields is restricted to particular activities,
5188 such as feeding or moving. Home ranges are generally small and, for many amphibian species, they
5189 change with season.

5190 *Movement patterns*

5191 Section 2.2.5 describes the two most typical movement patterns of amphibians: (i) the breeding
5192 migration and (ii) the dispersal of juveniles after metamorphosis. Both movements are characterised
5193 by being massive, implying that if exposure to pesticides is possible, probably high proportions of the
5194 population may become exposed. During the breeding season, animals tend to concentrate around
5195 water bodies whereas they occupy terrestrial environments during the rest of activity, where they
5196 search for food. With respect to breeding habitats (i.e. aquatic), amphibians have a site fidelity,
5197 migrating year after year to the same breeding site. These migrations may follow the shortest way or
5198 can run along more suitable corridors, which affects the chance that the animals may cross arable,
5199 cropped fields and risk becoming exposed to pesticides. Distances may be short (few hundreds of
5200 meters) or rather long, e.g. 4 km. The second most typical movement is the dispersal of juveniles
5201 after metamorphosis. Dispersal movements tend to be orientated non-randomly towards the most
5202 suitable habitat patches. The movements may go over distances of 1.5 km.

5203 *Exposure in agricultural areas*

5204 Section 2.2.5 mentions that crossing arable fields during breeding migration may happen and may
5205 coincide with pesticide application periods. As migration generally occurs during the night, the risk of
5206 direct overspray of amphibians is relatively low. When the animals cross shortly after pesticide
5207 application, however, dermal exposure by contact with the oversprayed soil surface may be a
5208 significant pathway of pesticide exposure of adult or juvenile amphibians. When the animals migrate
5209 in early spring while the vegetation cover in crop fields is still very low, this favours easy and quick
5210 displacement of the animals and the risks of contact to pesticide residues intercepted by the very low
5211 vegetation is probably relatively small compared to the exposure *via* the soil surface. During daytime
5212 resting periods, animals tend to look for refuge in densely vegetated areas, which will often be off-
5213 field. Some species, however, dig themselves to shelter; as ploughed, loose fields facilitate this
5214 strategy, individuals may stay for entire days inside crop fields during their breeding migrations. In
5215 previously treated fields they may thus become exposed to pesticide residues *via* e.g. dermal contact
5216 or inhalation.

5217 Off-crop (in-field and outside fields) the animals may also become exposed *via* dermal contact with
5218 the soil, or residues intercepted by plants. As the deposition rates off-crop are lower than in-crop we
5219 expect the exposure to be generally lower off-crop than in-crop. The deposition rates off-crop
5220 generally depend on many factors, such as crop type and height, spraying equipment, but also wind
5221 speed and wind direction related to field orientation (e.g. in The Netherlands the most common wind
5222 direction is southeast, and with the main row orientation of fruit trees being north-south, the spray
5223 drift deposition is not randomly distributed around the fruit orchards).

5224 Exposure of amphibians in agricultural areas therefore depends on a range of spatial factors (e.g. in-
5225 crop, off-crop, distance to crops, numbers of crops cultivated and associated application techniques,
5226 soil types), and temporal factors (e.g. amphibian life stage, crop development stage), as well as
5227 amphibian species with their characteristic habitat and movement traits.

5228 *The two exposure routes direct overspray to indirect exposure by contaminated soil*

5229 For terrestrial amphibians exposure to pesticides through dermal contact is a primary route of
5230 exposure in agricultural landscapes. As well as direct overspray or contact with pesticide intercepted
5231 on plants followed by uptake through their permeable skin, as uptake from contaminated soils, mainly
5232 by their ventral seat patch, are two possible exposure scenarios. Unlike amniotes, amphibian skin is
5233 used both for gas and for water exchange. A ventral seat patch, a highly vascularized region of ventral
5234 skin, and aquaporins assist with water movement across the skin. Many amphibians actively place

5235 their seat patch in direct contact with a moist substrate and this may contribute to increased
 5236 susceptibility to pesticides and other contaminants. In particular, applications of pre-emergent
 5237 pesticides, which are applied to fields early in the growing season with newly germinated plants, can
 5238 put amphibians in direct contact with relatively high amounts.

5239

5240 9.2.4. Exposure assessment goals and exposure routes for terrestrial 5241 environment

5242 The elements of the exposure-assessment goals linked to the Specific Protection Goal for individual
 5243 amphibians (no mortality) in their terrestrial environment (in-crop) are described in Table 29: . The
 5244 corresponding exposure routes are based upon Table 3: of section 2.4 and given in Table 30: .Table
 5245 31: and Table 32: present this for the off-crop, terrestrial life stage. Note that the in-crop and off-
 5246 crop exposure-assessment goals and exposure routes have been described in separate tables,
 5247 because the statistical populations of Spatial Units and exposure routes are different. The EREQs
 5248 mentioned in Table 30: and Table 32: are proposals. As standard ecotoxicological experiments for
 5249 amphibians are not yet available for the pesticide-registration procedure, the final EREQ choice can
 5250 only be made after a deliberate selection of the most relevant exposure concentration in the possibly
 5251 future ecotoxicological experiment to express the endpoint.

5252

5253 **Table 29:** Elements of the exposure-assessment goal for individual juvenile or adult amphibians
 5254 (SPG: no mortality) in their terrestrial environment (in-crop)

Element	Description	Remarks
EREQ	See Table 30:	
Temporal dimension of EREQ	See Table 30:	
Spatial unit (SU), type and size (if relevant)	Individual amphibians	This implies that each individual amphibian has its own in-crop exposure depending e.g. on migration day with respect to application day
Statistical population of SUs	All individual amphibians in all treated agricultural fields (generally around 1 to 5-10 ha) in area of use of substance	Risk managers may also opt for the alternative of all individuals in all agricultural fields grown with the crop on the pesticide label
Multi-year temporal statistical population of EREQ values for one spatial unit	Series of tens of years of annual maxima of EREQ of individual amphibians in years of treatment	
Desired spatio-temporal percentile of the statistical	Overall 90 th percentile of the statistical population	Percentile can easily be changed if needed

population of EREQ values	of each EREQ	
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5255

5256

5257 **Table 30:** Table 9.8. Exposure routes and the definition of the EREQs for individual juvenile or adult
5258 amphibians (SPG: no mortality) in the terrestrial environment (in-crop)

Exposure route		Source/ location	EREQ	Temporal dimension of EREQ*	Remarks
Dermal exposure	Direct	Overspray	Mass of substance deposited per individual amphibian divided by its body mass	Maximum in relevant period of the year (i.e. when individual amphibians may be present in agricultural fields)	Only important route if migration or other movement occurs during daytime
	Soil	Residues on soil surface	Concentration dissolved in pore water of upper x cm soil or mass taken up by the individual amphibian	Maximum in relevant period of the year.	Important route
	Water in puddle on field	Runoff from treated field	Concentration in runoff water** or mass taken up by the individual amphibian divided by its body mass)	Maximum in relevant period of the year.	If puddles are formed by runoff in the treated field, this route may be relevant
	Plants	Residues on plant leaves	Dislodgable foliar residue or mass taken up by the individual amphibian (expressed as mass per body mass)	Maximum in relevant period of the year.	May be important especially immediately after spray on low crops (e.g. early growth stages of cereals, all growth stages of salads)
Oral exposure		Food (generally small arthropods)	Daily mass of compound taken in by individual amphibians (mass per body mass)	Maximum in relevant period of the year.	See chapter 10 for importance of this route
Inhalation		Air	-	-	Inhalation exposure is expected to be a minor route compared to dermal and oral exposure.

5259 *Annual exposure profile may be needed to predict effects by the use of TK-TD modelling
 5260 ** See Appendix I of Opinion on bees (2012)

5261

5262

5263 **Table 31:** Elements of the exposure-assessment goal for individual juvenile or adult amphibians
 5264 (SPG: no mortality) in the terrestrial environment (off-crop)

Element	Description	Remarks
EREQ	See Table 32:	
Temporal dimension of EREQ	See Table 32:	
Spatial unit (SU), type and size (if relevant)	Individual amphibians	
Statistical population of SUs	All individual amphibians in all off-crops strips of land of x m wide in area of use of substance	Risk managers may also opt for the alternative of all individuals in all off-crop strips of land adjacent to fields grown with the crop on the pesticide label This implies that each individual amphibian has its own off-crop exposure, depending on its distance to the treated crop
Multi-year temporal statistical population of EREQ values for one spatial unit	Series of tens of years of annual maxima of EREQ of individual amphibians in years of treatment	Consider only EREQs in periods that are relevant, i.e. individual amphibians may be present in off-crop strips of land
Desired spatio-temporal percentile of the statistical population of EREQ values	Overall 90 th percentile of the statistical population of each EREQ	Percentile can easily be changed if needed

5265

5266

5267 **Table 32:** Exposure routes and the definition of the EREQs for individual juvenile or adult
 5268 amphibians (SPG: no mortality) in the terrestrial environment (off-crop)

Exposure route	Source/ location	EREQ	Temporal dimension of EREQ	Remarks
•			•	

Dermal exposure	Direct	Spray drift	Mass of substance deposited per individual amphibian divided by body mass	Maximum in relevant period of the year (i.e. when individual amphibians may be present in agricultural fields)	Important route only if migration or other movement occurs in off-crop strips of land during spray events.
		Atmospheric deposition	Mass of substance deposited per individual amphibian divided by body mass	Maximum in relevant period of the year	Expected to be a minor route and only if migration or other movement occurs in off-crop strips of land During the periods immediately after spraying.
	Soil	Residues on soil surface	Concentration dissolved in pore water of upper x cm soil or mass taken up by the individual amphibian	Maximum in relevant period of the year	Important route
	Water in puddle on field	Runoff from treated field	Concentration in runoff water* or mass taken up by the individual amphibian	Maximum in relevant period of the year.	If puddles are formed by runoff in the close vicinity of sprayed crops this route may be important.
	Plants	Residues on plant leaves	Dislodgable foliar residue or mass taken up by the individual amphibian divided by body mass	Maximum in relevant period of the year.	Expected to be less important as in crop.
Oral exposure		Food (generally small arthropods)	Daily mass of compound taken in by individual amphibian	Maximum in relevant period of the year	See chapter 10 for importance of this route

5269

5270

5271 9.3. Exposure of reptiles

5272 9.3.1. Life stages and habitats

5273 This section focuses on reptile life stages and habitats, based upon sections 2.3.4 and 2.3.5, and the
 5274 possible ways of pesticide exposure of reptiles. For most reptiles all life stages are terrestrial.
 5275 Reproduction can be broadly divided into two groups: viviparity (approximately 20% of squamates)
 5276 and oviparity (most common). Viviparity is an adaptation to cold climates and viviparous species
 5277 generally give birth in early summer. Oviparous species generally lay eggs in late spring or early

5278 summer and hatching occurs in late summer. The relevant exposure window may differ between
 5279 these two groups. Egg nests are located at carefully selected sites, because egg development depends
 5280 on favourable environmental conditions, such as temperature, water and oxygen availability. Reptilian
 5281 eggshells are permeable to water diffusion and water is used in the yolk metabolism. This implies that
 5282 pesticides may enter the egg with the absorbed water and potentially affect the embryonic
 5283 development. This may occur especially for agroecosystem-inhabiting populations that sometimes
 5284 prefer loose soils for nesting, such as freshly ploughed or otherwise cultivated soils.

5285 The majority of continental reptiles are generally sedentary. Their home ranges are better defined
 5286 than for amphibians and territorialism is not uncommon. Their exposure pattern may therefore be
 5287 relatively stable: animals inhabiting exposed areas will be almost chronically exposed, while individuals
 5288 inhabiting non-exposed areas will have little chances of entering in contact with pesticides. Dermal
 5289 exposure of juveniles or adults may occur *via* direct overspray or spray-drift deposition during
 5290 pesticide application or contact with contaminated soils, including granules or treated seeds. Dermal
 5291 exposure by contact with water can happen in puddles inside fields. In addition, some species like
 5292 terrapins or water snakes are semi-aquatic and spend long periods of time in water bodies. Thus the
 5293 exposure routes of runoff, drainage, drift, or atmospheric deposition to water bodies may be relevant
 5294 for reptiles in a number of cases.

5295 As for amphibians in agricultural areas, exposure for reptiles needs to be assessed in-crop as well as
 5296 off-crop, (in-field and outside fields). The exposure is dependent on a range of spatial factors (e.g. in-
 5297 crop, off-crop, distance to crops, numbers of crops cultivated and associated application techniques,
 5298 soil types) and temporal factors (e.g. reptile life stage, crop development stage), as well as reptile
 5299 species with their characteristic habitat and movement traits.

5300

5301 9.3.2. Exposure assessment goals and exposure routes

5302 The WG limited the exposure-assessment goals for the reptiles to the terrestrial habitat, as this is
 5303 most frequent for reptiles; the WG did not consider semiaquatic species. The elements of the
 5304 exposure-assessment goals linked to the Specific Protection Goal for individual reptiles in-crop (no
 5305 mortality) are described in Table 33: . The corresponding exposure routes are based upon section 2.4
 5306 for reptiles and given in Table 34: , Table 35: and Table 36: present this for the off-crop habitat.
 5307 Similar to what was done for the terrestrial environment of amphibians, we described the in-crop and
 5308 off-crop exposure-assessment goals and exposure routes for reptiles in separate tables, because the
 5309 statistical populations of Spatial Units and exposure routes are different. The EREQs mentioned in
 5310 Table 34: and Table 36: are proposals. As standard ecotoxicological experiments for reptiles are not
 5311 yet available for the pesticide-registration procedure, the final EREQ choice can only be made after a
 5312 deliberate selection of the most relevant exposure concentration in the possibly future ecotoxicological
 5313 experiment to express the endpoint.

5314

5315 **Table 33:** Elements of the exposure-assessment goal for individual juvenile or adult reptiles in-crop
 5316 (SPG: no mortality)

Element	Description	Remarks
EREQ	See Table 34:	
Temporal dimension of EREQ	See Table 34:	
Spatial unit (SU), type and size (if	Individual reptiles	This implies each individual reptile has its own in-crop exposure depending e.g. on migration day

relevant)		with respect to application day
Statistical population of SUs	All individual reptiles in all treated agricultural fields in area of use of substance	Risk managers may also opt for the alternative of all individuals in all agricultural fields grown with the crop on the pesticide label
Multi-year temporal statistical population of EREQ values for one spatial unit	Series of tens of years of EREQ of individual reptiles in years of treatment	As reptiles are quite sedentary, they may be in-crop during the entire year, so no relevant periods are to be distinguished as is needed for amphibians
Desired spatio-temporal percentile of the statistical population of EREQ values	Overall 90 th percentile of the statistical population of each EREQ	Percentile can easily be changed if needed

5317

5318

5319 **Table 34:** Exposure routes and the definition of the EREQs for individual juvenile or adult reptiles in-crop (SPG: no mortality)

5320

Exposure route		Source/location	EREQ	Temporal dimension of EREQ	Remarks
Dermal exposure	Direct	Overspray	Mass of substance deposited per individual reptile divided by body mass	Annual maximum	Annual maximum may occur after several consecutive applications. Animals may stay in the field the whole year.
	Soil	Residues on soil surface.	Total concentration in specified soil layer	Annual maximum	Possibly important – refer to experiments of Scott Weir –the ventral skin was more permeable than the dorsal skin.
	Plants	Residues on plants	Mass deposited on plants	Annual maximum	Possibly important, e.g. when residing in grass
	Water in puddle on field	Runoff from treated field	Concentration in runoff water*	Annual maximum	Likely to be minor route of exposure.
Oral exposure		Food (including	Daily mass of compound taken	Annual maximum	Important route, see chapter 10 (coverage

	secondary poisoning)	in by individual reptile		of reptiles by birds and mammals)
	Water	Concentration in runoff water*	Annual maximum	Highest concentrations are expected for drinking water from puddles in crop.
	Soil	Daily mass of compound taken in by individual reptile	Annual maximum	Probably accidental ingestion of soil (occasionally surpassing the 5% of the diet)
Inhalation	Air	-	-	Inhalation exposure is expected to be a minor route compared to dermal and oral exposure.

5321 * See Appendix I of Opinion on bees (2012)

5322

5323 **Table 35:** Elements of the exposure-assessment goal for individual juvenile or adult reptiles off-crop
 5324 (SPG: no mortality)

Element	Description	Remarks
EREQ	See Table 36:	
Temporal dimension of EREQ	See Table 36:	
Spatial unit (SU), type and size (if relevant)	Individual reptiles	This implies that each individual reptile has its own off-crop exposure, depending on its distance to the treated crop
Statistical population of SUs	All individual reptiles in all off-crops strips of x m wide and adjacent to treated fields	Risk managers may also opt for the alternative of all individuals in all off-crop strips of land adjacent to fields grown with the crop on the pesticide label
Multi-year temporal statistical population of EREQ values for one spatial unit	Series of tens of years of EREQ of individual reptiles in years of treatment	As reptiles are quite sedentary, they may be in-crop during the entire year, so no relevant periods are to be distinguished as is needed for amphibians
Desired spatio-temporal percentile	Overall 90 th percentile of the statistical population	Percentile can easily be changed if needed

of the statistical population of EREQ values	of each EREQ	
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5325

5326

5327 **Table 36:** Exposure routes and the definition of the EREQs for individual juvenile and adult reptiles
 5328 off-crop (SPG: no mortality)

Exposure route		Source/ location	EREQ	Temporal dimension of EREQ	Remarks
Dermal exposure	Direct	Spray drift	Mass of substance deposited per individual reptile divided by body mass	Annual maximum	Different exposure on the ground or on vertical structures (trees or stone walls)
		Atmospheric deposition	Mass of substance deposited per individual reptile divided by body mass	Annual maximum	Expected to be a minor route during periods immediately after spraying.
	Soil	Residues on soil surface	Total concentration in specified soil layer	Annual maximum	Expected to be minor route, close to crops where spray drift deposition onto soil occurred
	Plants	Residues on plants	Mass deposited on plants	Annual maximum	Expected to be minor route, close to crops where spray drift deposition onto plants occurred
Oral exposure		Food (including secondary poisoning)	Daily mass of compound taken in by individual reptile	Annual maximum	Important route, see chapter 10 (coverage of reptiles by birds and mammals)
		Water	Concentration in runoff water*	Annual maximum	Highest concentrations are expected for drinking water from puddles formed by runoff water from treated fields.
		Soil	Daily mass of compound taken in by individual	Annual maximum	Probably accidental ingestion of soil.

		reptile		
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5330

5331 Additional tables linked to the Specific Protection Goal for population persistence (abundance/biomass,
 5332 distribution and population growth rate) and their corresponding exposure routes are, for reptiles,
 5333 limited to the eggs for the in-crop situation. The off-crop situation for reptile eggs is expected to be of
 5334 minor importance compared to the in-crop situation. As no standard accepted methods exist for
 5335 pesticide registration to evaluate the effects of egg development on abundance/biomass, distribution
 5336 and population growth of reptiles, the EREQs presented in Table 37: and Table 38: are proposals that
 5337 may need to be adapted later.

5338 **Table 37:** Elements of the exposure assessment goal for reptile eggs in nests in-crop (SPG:
 5339 population persistence)

Element	Description	Remarks
EREQ	See Table 38:	
Temporal dimension of EREQ	See Table 38:	
Spatial unit (SU), type and size (if relevant)	A nest with eggs	This implies that each nest has its own in-crop exposure
Statistical population of SUs	All nests in all treated agricultural fields in area of use of substance	Risk managers may also opt for the alternative of all nests in all agricultural fields grown with the crop on the pesticide label
Multi-year temporal statistical population of EREQ values for one spatial unit	Series of tens of years of EREQ of nests in years of treatment	Consider only EREQs in periods that are relevant, i.e. when nests with eggs are present in the agricultural fields (late spring to late summer)
Desired spatio-temporal percentile of the statistical population of EREQ values	Overall 90 th percentile of the statistical population of each EREQ	Percentile can easily be changed if needed

5340

5341

5342 **Table 38:** Exposure routes and the definition of the EREQs for reptile eggs in nests in-crop (SPG:
 5343 population persistence)

Exposure route	Source/	EREQ	Temporal dimension of	Remarks
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		location		EREQ	
Contact exposure	Soil	Residues in soil.	Total mass of substance in specified soil layer absorbed by all eggs in nest	Maximum in relevant period in the year.	Only (and thus most important) route of contact exposure of eggs

5344

5345

5346 9.4 Conclusions

5347

5348 The analysis of the dimensions of the Spanish and Swiss amphibian ponds and the CountrySide
 5349 Survey ponds in the UK (Appendix C) demonstrates that most of them (70-90%) are considerably
 5350 smaller than the FOCUS ponds, used at present in the aquatic risk assessment at EU level. We
 5351 therefore expect peak concentrations in FOCUS ponds not to be conservative estimates for those in
 5352 the analysed ponds. For peak concentrations in FOCUS ditches and streams, the WG was unable to
 5353 make a general statement on their conservativeness compared to those in the analysed ponds. In
 5354 view of the higher flow-through rates in the FOCUS ditches and streams, the pesticide concentrations
 5355 are expected to decline rapidly and thus they probably represent underestimates of chronic exposure
 5356 in ponds in the areas surveyed.

5357 The use of Exposure Assessment Goals defines the spatial unit with its Ecotoxicologically Relevant
 5358 Exposure Quantities (EREQs). Whereas exposure routes allow for an explicit and systematic
 5359 methodology to calculate Predicted Exposure Concentrations (PECs) in the field, for amphibians in
 5360 their aquatic and terrestrial environment and for reptiles.

5361

5362

5363

5364 10. Coverage of risk to amphibians and reptiles by existing RA for other 5365 groups of organisms (including human RA)

5366 10.1. Introduction

5367 It has been assumed up to now that the risk to amphibians and reptiles is covered by the current risk
5368 assessment on surrogate species such as birds, mammals or fish. When analyzing this assumption,
5369 the question arises as to what exactly is implied by this. The regulation (EC) No 1107/2009 for plant
5370 protection products requires that "substances should only be included in plant protection products
5371 where it has been demonstrated that they present a clear benefit for plant production and they are
5372 not expected to have any harmful effect on human or animal health or any unacceptable effects on
5373 the environment". In order to assess the acceptability of the effects, the toxicity of the substance to
5374 non-target organisms needs to be compared with exposure levels that may result from the application
5375 of a specific compound in the environment, while considering the uncertainties in the approach, which
5376 evolve from the assumptions and extrapolations done for the assessment. Ultimately, the acceptability
5377 of the effects is defined by the protection goals.

5378 The uncertainties are addressed by applying assessment factors (AF) in the risk assessment.
5379 According to the Technical Guidance Document for Chemicals (EC, 2003) the following has to be taken
5380 into account when choosing the appropriate assessment factor:

- 5381 • Intra- and inter-laboratory variation of toxicity data
- 5382 • Intra- and inter-species variation of toxicity data
- 5383 • Short-term to long-term/chronic toxicity extrapolation
- 5384 • Extrapolation from single species laboratory data to field impact on ecosystems

5385 Whilst there are substantial data that demonstrate the uncertainty for the first three bullet points for
5386 fish (EC, 2002), there are limited data available for amphibians and reptiles. It is therefore unknown
5387 at present whether the same assessment factor as for fish can be applied to amphibians and reptiles.

5388 A comparison of toxicity endpoints from fish with amphibians was done in the aquatic guidance
5389 document (EFSA PPR Panel, 2013) where it was concluded that "rainbow trout is a good surrogate
5390 test species for predicting the acute toxicity of PPPs for larval stages of amphibian species living in the
5391 aquatic compartment of the environment. By using the same AFs as have been applied for fish, the
5392 achieved level of protection will be the same for both groups of organisms." It is important, however,
5393 to differentiate between the coverage of risk and the coverage or even predictability of toxicity.

5394 Even though the comparison is considered appropriate and valuable, the comparison of toxicity
5395 endpoints does not allow a judgement on the achieved level of protection. This is due to the rather
5396 limited data set available for amphibians, focusing on one species (*X. laevis*, 87 of 253 data points
5397 with 48 species, 34%). A range of unresolved issues stem from this, such as the representativeness of
5398 the tested species as well as the variability in sensitivity between species, populations and life stages.

5399 Furthermore, the statement implies that the exposure of amphibians in the aquatic environment is
5400 comparable to fish without providing further details. Therefore, in the following, first an evaluation of
5401 the available toxicity studies with surrogate species and then an assessment of the suitability of the
5402 available exposure models is provided before addressing the coverage of amphibians and reptiles in
5403 the current risk assessment scheme.

5404 In the current risk assessment, a limited number of species and life stages are tested mainly for direct
5405 effects *via* limited and separate routes of exposure, but predictions are made for entire populations
5406 living in a landscape. The coverage and conservatism of the current scheme for all substances and
5407 non-target species in the long term is unknown at present.

5408 **10.2. Coverage of aquatic life stages of amphibians and reptiles in**
5409 **the current risk assessment for aquatic organisms**

5410 This section analyses endpoints and exposure models used in the aquatic risk assessment and makes
5411 a comparison with likely exposure and effects in amphibians and reptiles.

5412 **10.2.1. Extrapolation of endpoints observed in fish to amphibians and**
5413 **reptiles**

5414 Standardised test protocols are available for fish and the endpoints could be available in the dossiers
5415 as standard requirements (see Appendix E). According to the data requirement (Commission
5416 Regulation (EU) No 283/2013), an acute toxicity testing is always required for rainbow trout
5417 (*Oncorhynchus mykiss*). Chronic studies (early life stage or fish full life cycle) are required depending
5418 on the stability of the active substance. A bioconcentration study is required depending on the log P_{ow}
5419 and the stability of the active substance. Further studies may be required if the substance is a
5420 potential endocrine disruptor. When accumulation of an active substance in aquatic sediment is
5421 indicated or predicted by environmental fate studies, the impact on a sediment-dwelling organism
5422 needs to be assessed by determining the chronic risk to *Chironomus riparius* or *Lumbriculus* spp..

5423 The observed effects in studies with fish can be summarised into six categories, namely effects on
5424 survival, appearance, size, behaviour and reproduction as well as effects on the endocrine system.
5425 Endocrine and reproductive toxicity are discussed in chapter 8 and will not be further addressed here.

5426 Acute endpoints, which are based on mortality, are considered comparable and it is desirable in order
5427 to limit animal testing to use the LC50 from fish to cover the acute sensitivity of amphibians and
5428 reptiles. However, it needs to be defined what percentage of data points needs to be covered. Is the
5429 sensitivity of amphibians and reptiles covered by fish, based on a statistical evaluation of the acute
5430 endpoints, if all, the majority or the mean of all endpoints is higher than for fish? The question is
5431 whether the toxicity of new substances can be predicted based on the available data.

5432 Sublethal endpoints are more difficult to compare as the exposure time may not be identical. Also the
5433 significance of a sublethal effect observed in the laboratory on a population in nature is difficult to
5434 predict. Sublethal concentrations of pesticides may affect survival of amphibians by increasing the
5435 susceptibility of eggs and larvae to pathogens or diseases (Carey and Bryant, 1995) by altering the
5436 immune system (Mann et al., 2009). By retarding growth and metamorphosis, the time the young
5437 depart the breeding pond may be affected or the vulnerability to size-specific predation. Furthermore,
5438 the ability of young to avoid predators may be inhibited by sublethal concentrations, i.e. by causing
5439 deformities in the body or tail or by reducing swimming performance (Carey and Bryant, 1995).

5440 Missing endpoints, which cannot be covered by fish and might be needed due to the special biology of
5441 amphibians, are for instance effects on metamorphosis and certain effects on the reproductive system
5442 (see chapter 8). The reproductive physiology of amphibians shows a closer relationship with higher
5443 vertebrates than with that of fish. For instance, in amphibians and higher vertebrates, testosterone
5444 and dihydrotestosterone are the main androgenic sex hormones, whereas in fish it is 11-
5445 ketotestosterone (reviewed by Kloas et al, 2006). Another major difference between fish and
5446 amphibians is that the Müllerian ducts, which are the embryonic precursors of the female reproductive
5447 tract (uterus, oviducts) in vertebrates, are absent in teleost fish (in which the sex duct has a different
5448 ontogenetic origin) (Adkins-Regan, 1987). It is therefore not possible to extrapolate the effects of
5449 chemical exposure from fish to amphibians with regard to impact on the development of the female
5450 reproductive system due to fundamental anatomical differences. But that does not exclude the
5451 possibility that the sensitivity of the endpoints in sexual development toxicity tests in fish is
5452 comparable to those in amphibian tests, to certain chemical exposures. Ethinyl estradiol exposure
5453 affects sexual development in fish and amphibians at comparable exposure levels (about 1 ng/L). the
5454 effects however are different; in fish testicular development is affected whereas in amphibians (and
5455 reptiles, birds, mammals) both testicular and female oviduct development are affected. Thus, the
5456 hazard of chemicals that specifically target female reproductive development may be overlooked using
5457 fish tests.

5458 An exposure of aquatic life-stages can lead to effects that are carried over into adult amphibians.
5459 Whereas decrease in mass at metamorphosis in the aquatic environment after a single exposure to a

5460 short-lived contaminant (carbaryl) was overcome in the terrestrial environment by spring emergence
5461 (Boone, 2005; Distel and Boone 2009, 2010), other studies observed that sublethal effects on larval
5462 growth and development impaired amphibian populations permanently (Jones et al., 2010; Relyea
5463 2005a, Relyea 2005b). Embryonic and larval exposure to atrazine altered the behaviour and increased
5464 the dehydration rate of postmetamorphic *Ambystoma barbouri* eight months after exposure (Rohr and
5465 Palmer, 2005). Endocrine effects may lead to skewed sex ratio or sterility in adults (Pettersson et al.,
5466 2006; Gyllenhammar et al., 2009; Hayes et al., 2010; Kvarnryd et al., 2011) and thus affect
5467 reproduction. Due to the biphasic life history amphibians face a double jeopardy of exposure
5468 stemming from terrestrial and aquatic environments (Todd et al., 2011). Thus long-term and carry-
5469 over (postexposure) effects spanning several life stages, which may have greater consequences on
5470 populations than transient effects, need to be addressed.

5471 As with fish, ontogenic variation in vulnerability to pesticides has been reported, but may be difficult
5472 to generalise. Whereas the jelly coat of the eggs may protect embryos from some substances,
5473 insufficient protection by the jelly coat has been observed after the exposure of *Rana arvalis* eggs to
5474 cypermethrin (Greulich and Pflugmacher, 2003) leading to abnormalities in the hatched embryos. The
5475 sensitivity of larvae may be determined by the development of organs and thus depends on the mode
5476 of action of the active substance. The time of metamorphosis may be particularly sensitive due to the
5477 physiological demands through that developmental time. Also the ability to detoxify pollutants in
5478 different life stages affects the sensitivity as well as the thickness of the skin.

5479 A further, significant difference may be the duration to effects after exposure due to different rates of
5480 metabolism. The metabolic rate is influenced by temperature and is thus very variable. Toxicants may
5481 be metabolised faster and thus not reach a threshold value, but a faster metabolism could also
5482 increase the energetic demand and thus increase uptake of a chemical orally or by inhalation

5483 **10.2.2. Potential coverage in toxicity – comparison of fish toxicity with** 5484 **toxicity values for amphibians and reptiles**

5485 A limited number of published comparisons is available and will be summarized in the immediately
5486 following text. Further comparative work will be conducted within the framework of the procurement
5487 OC/EFSA/PRAS/2015/01 and may be considered at a later point.

5488 **Acute data:**

5489 Acute toxicity data of freshwater species developed by the U.S. Fish and Wildlife Services from 1965
5490 to 1986 were analyzed with regards to taxonomic differences (Mayer and Ellersieck 1986). In this data
5491 set of 14 insecticides, amphibians were the least sensitive group compared to insects, crustaceans and
5492 fish. Testing three species (*Daphnia*, *Gammarus* and rainbow trout) provided the lowest toxicity value
5493 88% at the time. The suggestion was that, by testing a limited set of species, the range of sensitivity
5494 of all species could be determined. The working assumption for U.S. EPA is therefore, that toxicity
5495 data for fish are broadly protective of aquatic-phase amphibians and that oral/dietary toxicity data for
5496 birds are broadly protective of terrestrial-phase amphibians.

5497 Hoke and Ankley (2005) concluded, based on a limited data set (atrazine, malathion, parathion), that
5498 traditional aquatic test species (primarily cladoceran, fish) are generally more sensitive than FETAX (*X.*
5499 *laevis*) when comparisons are based on lethality data. They also pointed out, however, that growth is
5500 the more sensitive endpoint from FETAX.

5501 Birge *et al.* (2000) compared the toxicity of fish and amphibians for organic compounds (atrazine,
5502 phenol, chloroform, carbon tetrachloride, NTA and methylene chloride) and concluded that
5503 amphibians were more sensitive than rainbow trout in 35% of the comparisons.

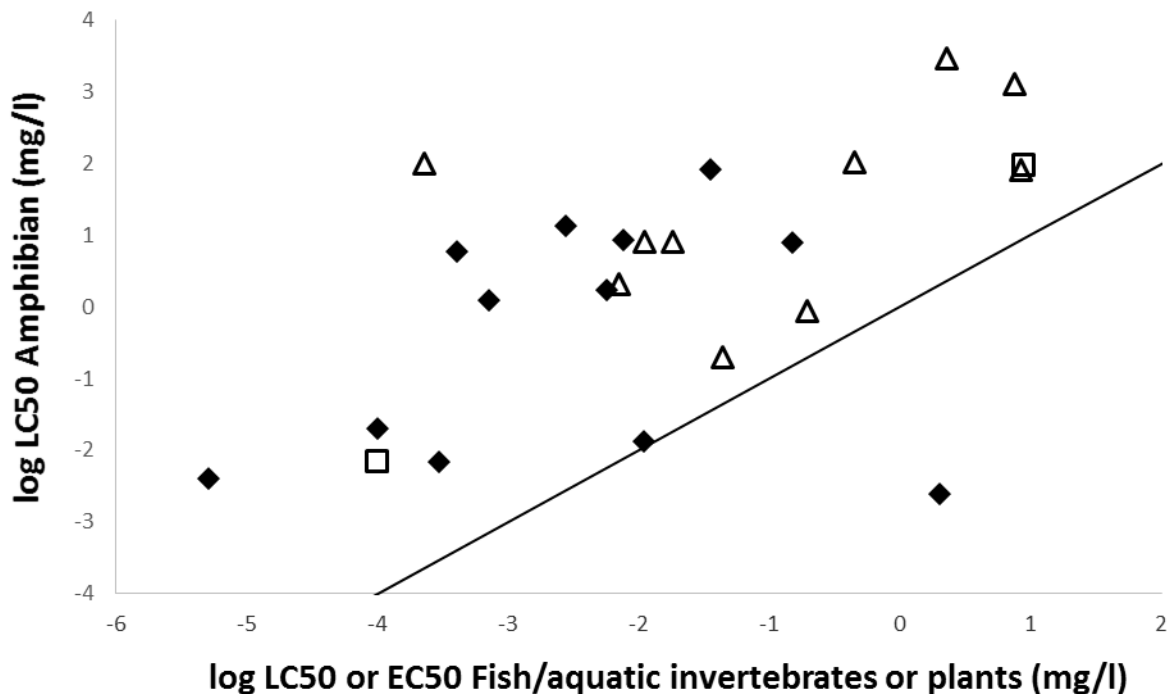
5504 Kerby et al. (2010) noted that a number of authors have investigated the sensitivity of amphibians to
5505 pollutants, but no clear indication of the overall sensitivity could be derived. They conducted an
5506 extensive review using a species-sensitivity-distribution (SSD) approach and analyzed the median
5507 lethal concentration (LC50, 24-96 h) data from almost 24,000 studies with aquatic species retrieved
5508 from the AQUIRE database of the U.S. Environmental Protection Agency (EPA) to compare the
5509 amphibian sensitivity of 44 species with that of other groups of organisms. They concluded that
5510 amphibians (eggs and larvae) are of low to moderate sensitivity to metals, inorganic chemicals, and
5511 pesticidal active substances (pyrethroids, carbamates, organophosphates, or organochlorines) when

5512 compared with 13 other classes of organisms, including fishes. The estimated HC50 values were
 5513 above the average estimates for all taxa analysed signifying an overall low relative acute sensitivity.
 5514 They found, however, that amphibians were highly acutely sensitive to three phenolic chemicals and
 5515 add that the average low sensitivity of amphibians does not mean that highly vulnerable amphibian
 5516 species are not impacted.

5517 Toxicity values for fish and amphibians have been compared (Aldrich, 2009; Weltje et al., 2013; EFSA
 5518 PPR Panel, 2013) to determine whether standard tests with fish required for the dossier would be
 5519 likely to cover the potential risk to amphibians present in the surface water.

5520 In the study by Aldrich (2009), acute endpoints for aquatic invertebrates were also included in the
 5521 comparison as the first-tier, acute aquatic risk assessment is triggered by the lowest endpoint of all
 5522 aquatic organisms. Here the data were extended with endpoints for aquatic plants (Figure 30:). The
 5523 majority of data was found for herbicides and insecticides and 24 substances could be compared. In
 5524 11 cases aquatic invertebrates reacted most sensitively, in two cases fish, in 10 cases aquatic plants
 5525 and in one amphibians were most sensitive (dimethoate) (see Figure 30: below). For dimethoate the
 5526 variability in the published endpoints for amphibians was rather large.

5527



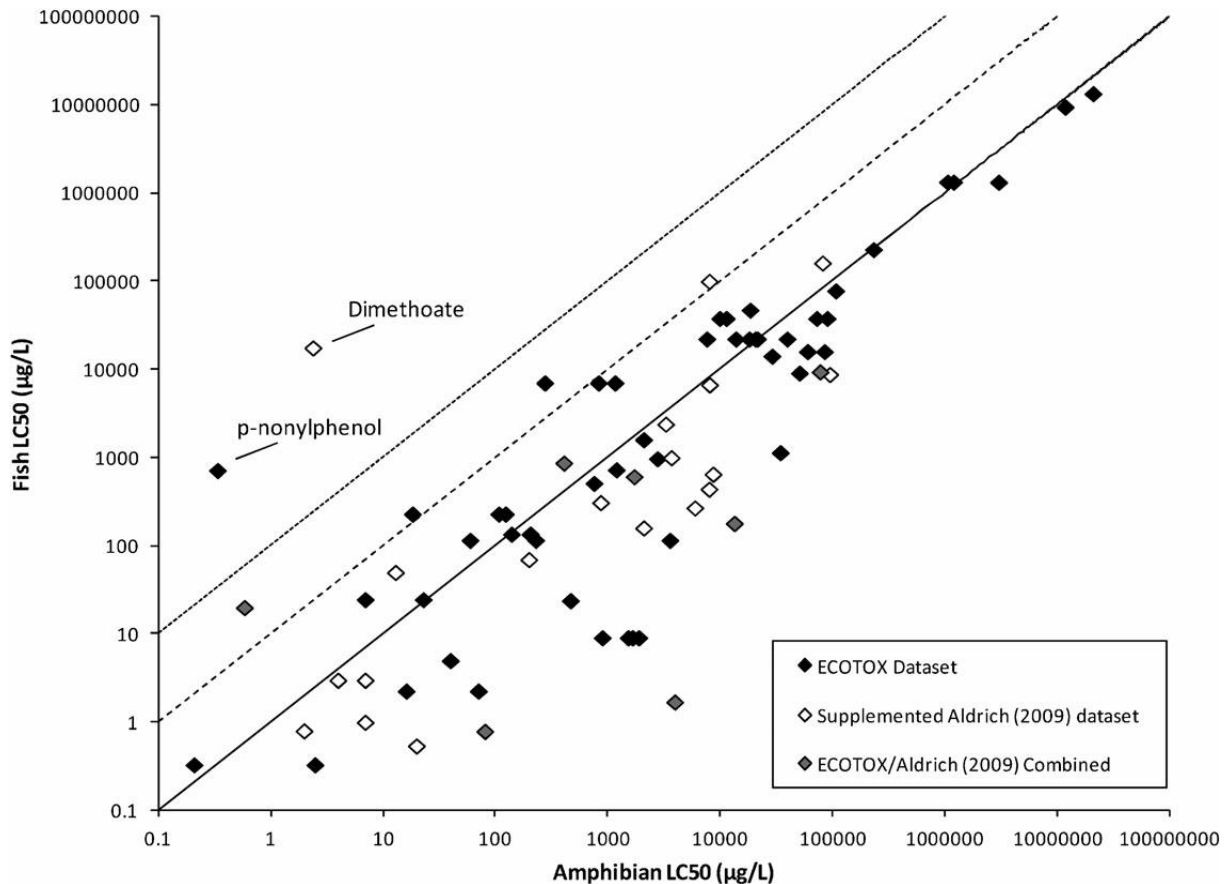
5528

5529 **Figure 30:** Comparison of the sensitivity (log LC50) of amphibians with the lowest endpoint of
 5530 fish (open square), aquatic invertebrates (filled diamond) or aquatic plants (open triangle) for 24
 5531 active substances.

5532 A review published by industry (Weltje et al., 2013) compared acute and chronic toxicity data
 5533 obtained from the U.S. Environmental Protection Agency (U.S. EPA) ECOTOX database supplemented
 5534 with data from the scientific and regulatory literature. Data were collected for amphibian species and
 5535 either rainbow trout (*Oncorhynchus mykiss*) or fathead minnow (*Pimephales promelas*) (Figure 31:).
 5536 Only tests from ECOTOX that reported measured concentrations of test chemical were included. A
 5537 geometric mean value was calculated if more than one 96-h LC50 value was available for the same
 5538 chemical and species. When data on several amphibian species were available, only data for the most
 5539 sensitive amphibian species were selected for further analysis. The comparison was based on 55
 5540 chemicals (eight inorganic chemicals and 47 organic chemicals, of which 32 were pesticidally active).
 5541 The overall outcome was that fish and amphibian toxicity data were highly correlated using
 5542 Spearman's correlation ($r_s = 0.81$). Amphibians were more sensitive than fish in 16 of 55 cases
 5543 (29%). For four of the 55 chemicals, amphibians were between 10- and 100-fold more sensitive than

5544 fish and for two chemicals more than 100-fold more sensitive. After a detailed inspection of these two
 5545 cases, however, the authors concluded that fish and amphibians showed a similar acute sensitivity.

5546



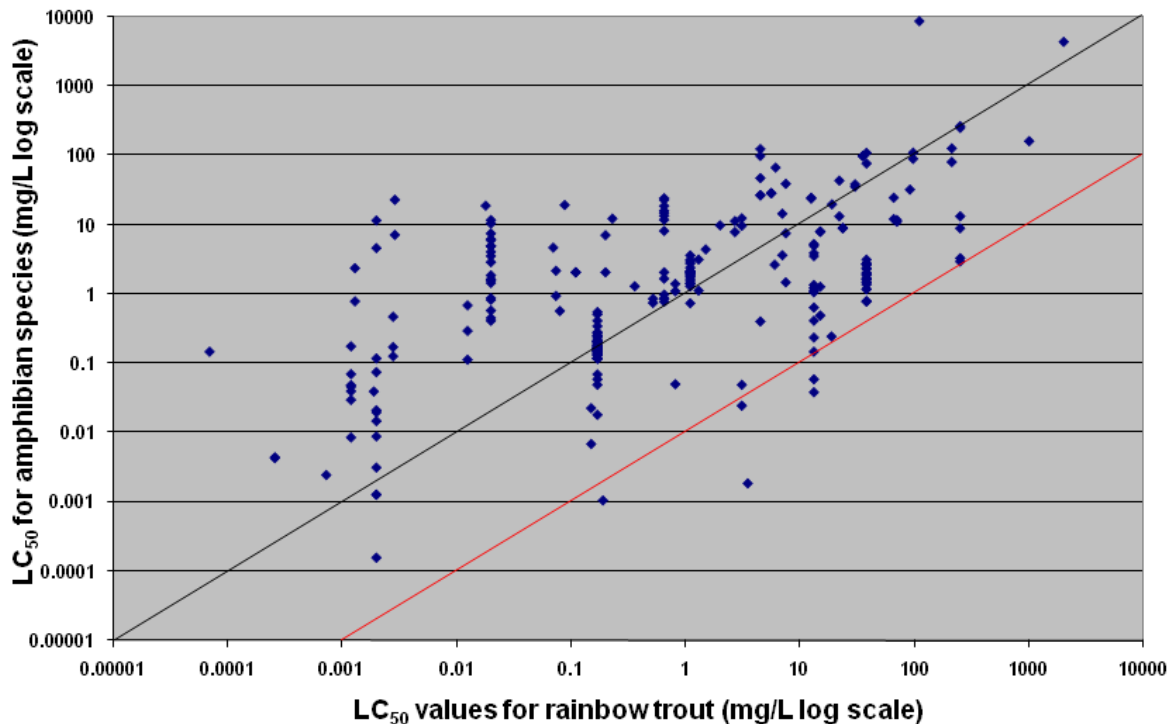
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5548 **Figure 31:** Relationship between amphibian and fish median lethal concentration (LC50) values.
 5549 The solid line delineates the 1:1 relationship. The dashed line delineates a factor of 10. The
 5550 dotted line delineates a factor of 100, which is the standard EU assessment factor applied to fish
 5551 acute toxicity data for plant protection products. (Weltje et al., 2013)

5552

5553 The comparison in the aquatic guidance document (EFSA PPR Panel, 2013) used the data collected by
 5554 Fryday and Thompson (2012) on amphibian species exposed in water. In total, 253 data points for 48
 5555 amphibian species including several life stages (e.g. tadpoles/larvae and embryos) with corresponding
 5556 rainbow trout values were available, from tests with an exposure duration of 96 hours on 60 different
 5557 pesticidal active substances performed under either a flow-through or a static-renewal system. Most
 5558 of the tested species belonged to the subclass of Anura (frogs and toads) and seven of the tested
 5559 species to the subclass Caudata (salamanders or newts). No data for adults were included in the
 5560 analysis.

5561 The comparison of the data revealed that in 62% of the cases the rainbow trout is more sensitive
 5562 than the amphibian species (points above the 1:1 line on Figure 32:) and thus in 38% of the cases
 5563 amphibian species are more sensitive than rainbow trout. If the assessment factor of 100 used for the
 5564 acute risk assessment of fish is applied, then in 2% of the cases the amphibian test species is more
 5565 than a factor of 100 more sensitive than the rainbow trout (values below the red line in Figure 32:).
 5566 In those cases the LC50 for amphibians would be lower than the RAC based on the rainbow trout.
 5567 Repeating this analysis but splitting it by life stage (i.e. keeping embryos and larvae separate) gave a
 5568 comparable result to the assessment on the whole dataset. Therefore, the results were considered to
 5569 be valid for both embryos and larvae.

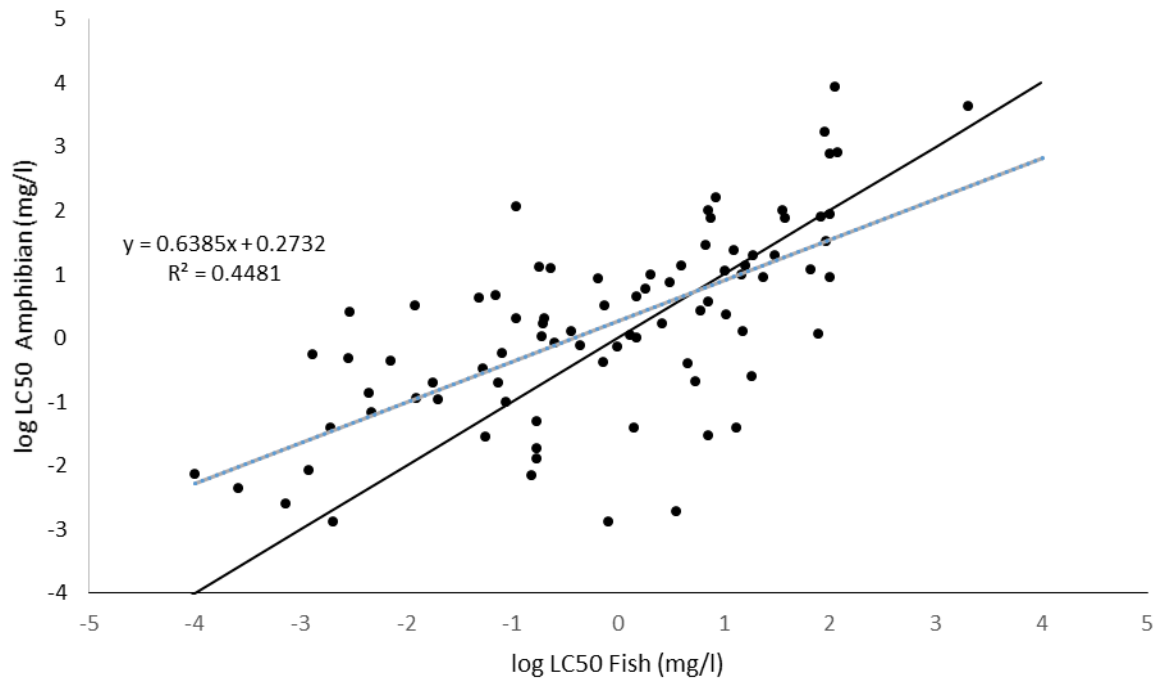


5570

5571 **Figure 32:** Comparison of each amphibian toxicity value with the respective toxicity value for
 5572 rainbow trout (*Oncorhynchus mykiss*). The black line is the 1:1 line, i.e. the line indicating that the
 5573 outcome for rainbow trout and amphibians would be exactly the same. The red line considers the
 5574 assessment factor of 100 applied in the acute risk assessment for fish, i.e. where toxicity to an
 5575 amphibian would be exactly 100 times higher than toxicity to the rainbow trout. (EFSA PPR Panel,
 5576 2013)

5577 Based on the same LC50 data for amphibians collected by Fryday and Thompson (2012) and using the
 5578 acute LC50 for fish listed in the Pesticide Properties DataBase (PPDB database) supplemented by LC50
 5579 for thirteen older active substances listed in the aquatic guidance document (EFSA 2013), the
 5580 correlation is further investigated here. Only the lowest acute endpoint for amphibians is compared for
 5581 each of the 85 pesticidally active substances. Unbounded endpoints (>) were included in the
 5582 comparison. The statistical correlation was investigated with Spearman and Pearson correlation.
 5583 Whereas the Spearman correlation assesses the relationship between two variables using a monotonic
 5584 function, the Pearson correlation assesses linear relationships. For the Spearman's correlation $r_s =$
 5585 0.66 ($p < 0.0001$), whereas for the Pearson correlation $r^2 = 0.48$ (95% confidence interval 0.30-0.63, p
 5586 < 0.0001) and $y = 0.6385x + 0.2732$, indicating a weak correlation. The linear regression line has a
 5587 slope < 1 indicating that for substances where fish react very sensitive amphibians react less sensitive
 5588 and at concentrations > 1 mg/l amphibians may react more sensitive than fish (see Figure 33:).

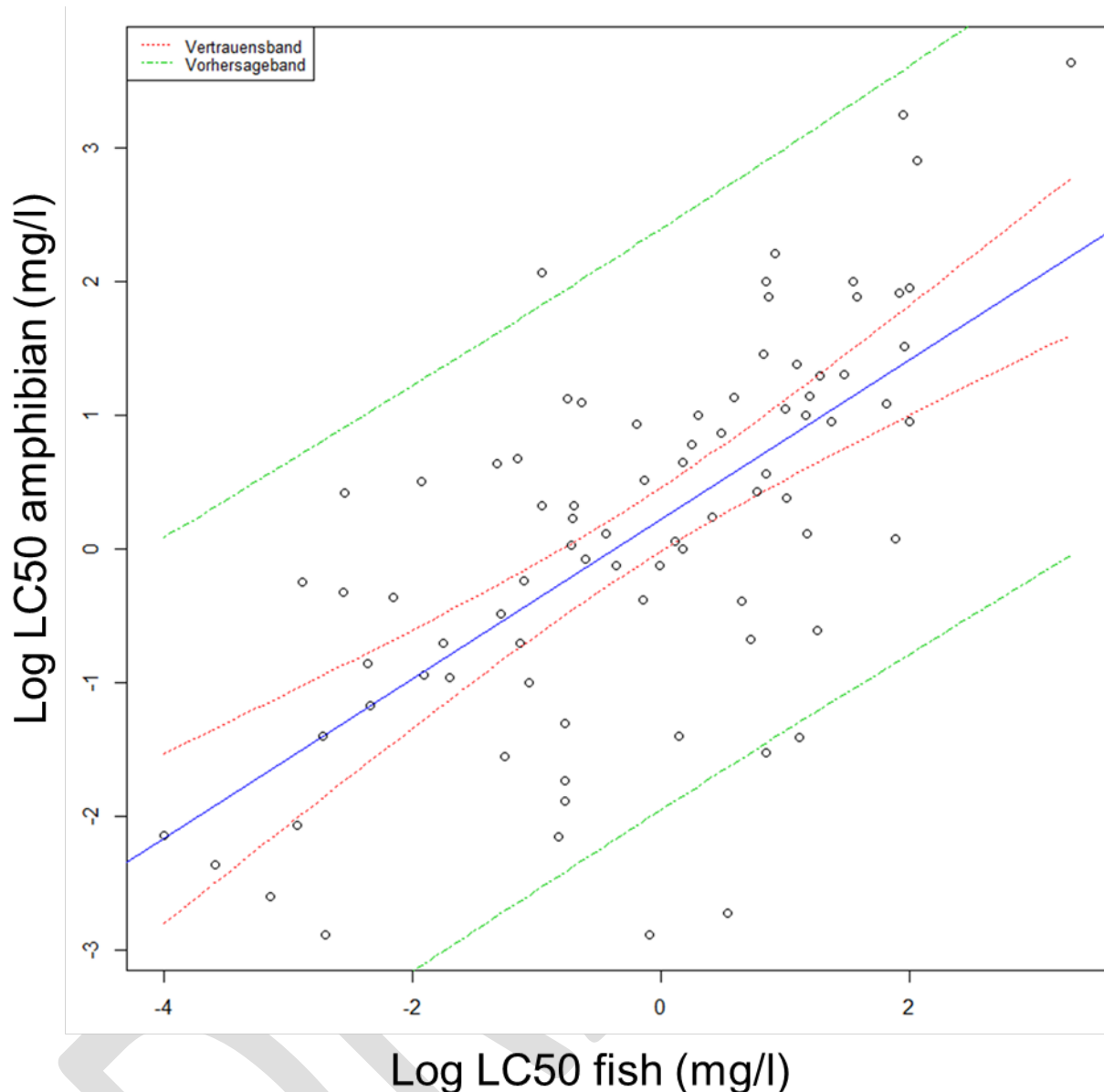
5589



5590

5591 **Figure 33:** Comparison of the lowest amphibian toxicity value with the respective lowest toxicity
5592 value for fish for 85 active substances. The blue line is the linear regression ($r^2 = 0.448$; $y =$
5593 $0.6385x + 0.2732$) and the black line is the 1:1 line, i.e. the line indicating that the outcome for
5594 rainbow trout and amphibians would be exactly the same.

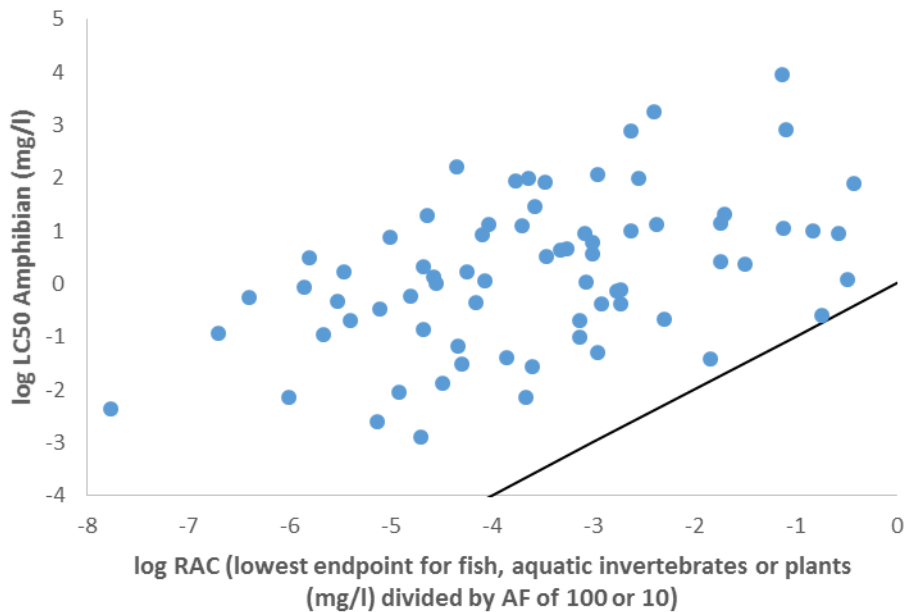
5595 The uncertainty in the linear regression is presented in Figure 34: with the confidence interval and
5596 prediction interval. The width of the prediction interval indicates that a factor of at least 100 is a
5597 suitable extrapolation factor to predict the LC50 for amphibians based on the LC50 for fish.



5598

5599 **Figure 34:** Comparison of the lowest amphibian toxicity value with the respective lowest toxicity
 5600 value for fish for 85 active substances. The blue line is the linear regression ($r^2 = 0.448$; $y =$
 5601 $0.6385x + 0.2732$), the red lines are the confidence interval of the mean and the green lines
 5602 indicate the width of prediction. For a new substance with a measured LC50 for fish the LC50 for
 5603 amphibians can be predicted to lie within the green lines. As the green lines are straight and not
 5604 curved the prognosis is good. The distance between the blue and the green line indicates that a
 5605 factor of at least 100 is a suitable extrapolation factor.

5606 In the current procedure, the aquatic risk assessment is triggered by the aquatic species showing the
 5607 greatest risk, which may be based on fish, aquatic invertebrates or aquatic plants (macrophytes and
 5608 algae). Hence, the above data were extended with endpoints obtained from the Pesticide Properties
 5609 DataBase (PPDB database) for aquatic invertebrates and aquatic plants; thus 72 substances could be
 5610 compared. In nine cases (14%), the lowest endpoint for amphibians was lower than the lowest
 5611 endpoint of the current surrogate species. If the RAC is used (by including the current assessment
 5612 factors of 100 for fish and aquatic invertebrates and 10 for aquatic plants) the sensitivity of
 5613 amphibians is covered (Figure 35:). However, the assessment factors are used to assess the toxicity
 5614 to exposure ratio and need to cover more than the species variability in toxicity.



5615

5616 **Figure 35:** Comparison of the lowest RAC (regulatory acceptable concentration, i.e. lowest
 5617 endpoint for fish, aquatic invertebrates or plants divided by the current assessment factors of 100
 5618 resp. 10) with the respective amphibian acute endpoint. The black line is the 1:1 line, i.e. the line
 5619 indicating that the outcome for the surrogate species (with assessment factor) and amphibians
 5620 (without assessment factor) would be exactly the same.

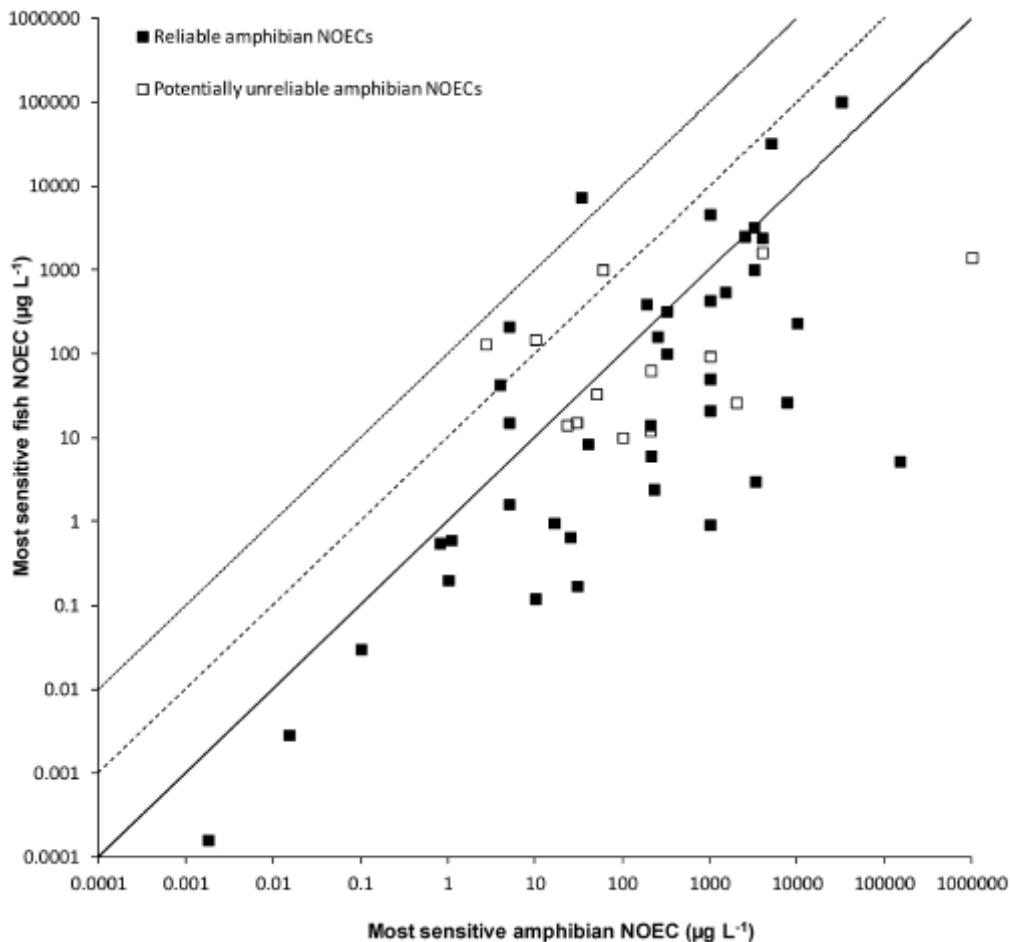
5621

5622 The question remaining is what extrapolation factor (if any) should be applied to LC50 for fish to
 5623 cover the acute toxicity for amphibians considering the limited data available with regards to species
 5624 (largest number of studies conducted with *X. laevis*) and larval stages tested? The most frequently
 5625 tested species in the literature is the African Clawed Frog (*Xenopus laevis*), whose sensitivity in
 5626 comparison to European species is not clear. Whereas Birge et al (2000), Hoke and Ankley (2005) and
 5627 Kerby et al (2010) do not consider *X. laevis* to be particularly sensitive to any type of chemical,
 5628 Wagner et al (2013) describe *X. laevis* as a sensitive species. Therefore, the sensitivity of European
 5629 or local species might give a more reliable – and different - estimate of the impacts of PPP on
 5630 amphibian species. Furthermore, comparing dose-response functions rather than just endpoints would
 5631 give more information about the respective sensitivities.

5632 **Chronic data:**

5633 Chronic toxicity data for fish were available for 52 chemicals (10 inorganic chemicals and 42 organic
 5634 chemicals, of which 20 were pesticidally active substances) (Weltje et al., 2013). Chronic toxicity data
 5635 were taken from US EPA databases and scientific literature, for fish also from EU or US regulatory
 5636 dossiers. Data were retained for analysis if they were from studies of at least a 10d duration for
 5637 amphibians and 21d duration for fish, employed either static renewal or flow-through aqueous
 5638 exposure-study designs, and reported apical endpoints of potential population relevance (i.e. they
 5639 were related to survival, growth, development [including metamorphosis], or reproduction). Most of
 5640 the fish NOEC values are based on measured exposure concentrations, whereas most of the
 5641 amphibian data are based on nominal concentrations. The amphibian data comprised studies involving
 5642 14 amphibian species (predominantly studies using species from the genera *Rana*, *Bufo*, and
 5643 *Xenopus*). If the NOEC was unbound (i.e., where the highest tested concentration is reported as the
 5644 NOEC) or the spacing factor was ≥ 100 the studies were considered to be potentially unreliable by the
 5645 authors, because in these cases, the true NOEC may be higher than the reported value. Amphibians
 5646 were more sensitive than fish in 11 of 52 cases (21%) (Figure 36:). Amphibians were between 10-
 5647 and 100-fold more sensitive than fish for five substances and greater than 100-fold more sensitive for
 5648 one chemical (sodium perchlorate). The authors concluded that additional amphibian testing is not
 5649 necessary during chemical risk assessment (acute and chronic). It has to be pointed out though, that

5650 endpoints derived from different effects (survival against sublethal effects), different exposure times
 5651 or specifications of concentration (for fish mainly measured and for amphibians mainly nominal
 5652 concentrations) were compared. The exposure time for the fish studies was potentially longer (21-396
 5653 d) compared to the amphibian studies (10-210 d).



5654

5655 **Figure 36:** Relationship between chronic amphibian and fish no observed effect concentrations
 5656 (NOECs). The solid line delineates the 1:1 relationship. The dashed line delineates a factor of 10,
 5657 which is the standard EU assessment factor applied to fish chronic toxicity data for plant
 5658 protection products. The dotted line delineates a factor of 100. (Weltje et al., 2013)

5659 The U.S. Environmental Protection Agency (EPA) used ecotoxicity data for a standard amphibian test
 5660 species (*Xenopus laevis*), which they received as part of the Endocrine Disruptor Screening Program,
 5661 for a comparative analysis of chronic effects (Aubee and Glaberman, unpublished data). Although
 5662 these studies are primarily designed to inform a determination of potential thyroid interaction within
 5663 the context of other endocrine screening studies, they also contain valuable data on survival and
 5664 growth, which can be compared with existing fish data for a given chemical. Endpoints were extracted
 5665 for survival, body weight, and length from EPA reviews (preliminary or final) of industry data
 5666 submissions for 43 different pesticidal active substances. The analysis considered only pesticidal active
 5667 substances toxicity and not PPP toxicity. The dataset included studies with *O. mykiss*, *P. promelas*,
 5668 and *X. laevis*. Each laboratory study included at least 21 days of chemical exposure and was
 5669 conducted according to a standard EPA test design. Data generated according to amphibian
 5670 metamorphosis assay (AMA), fish short-term reproduction assay (FSTRA), and fish early life stage
 5671 (ELS) toxicity test designs were considered. All comparisons were done based on the No Observed
 5672 Adverse Effect Concentration (NOAEC) for a given effect and all endpoints were based on measured
 5673 test concentrations (mean measured or time-weighted average). The lowest test concentration was
 5674 divided by two in cases where the endpoint was nondefinitive (<) because effects were seen at all
 5675 treatment levels. Unbounded NOECs were not adjusted, but included in the analysis.

5676 In a preliminary analysis of the 43 pesticidally active substances tested in both an amphibian
 5677 metamorphosis assay and a short-term, fish reproduction assay, statistically significant effects on
 5678 mortality were seen in amphibians (*Xenopus laevis*) at lower test concentrations than in fish
 5679 (*Pimephales promelas*) 42% of the time (Table 39:). Growth parameters in amphibians were affected
 5680 at lower test concentrations than in fish in 53% (body weight, n=43) and 59% (length, n=39) of
 5681 studies, respectively. (Length was not reported in all fish studies.) The fish NOAEC underestimated the
 5682 amphibian NOAEC by a factor of at least ten in 8% of cases for mortality, 13% of cases for body
 5683 weight, and 29% of cases for length. In cases where the amphibian endpoint was lower, particularly
 5684 for length, the average difference between the amphibian and fish endpoint appeared to be
 5685 considerably greater than the difference when the amphibian was not more sensitive. Therefore it
 5686 seems worthwhile to investigate cases where amphibians are more sensitive than fish. On the whole,
 5687 however, there were no statistically significant differences in NOAEC values for mortality, body weight,
 5688 and length in amphibians as compared to fish.

5689 **Table 39:** Summary statistics for the one-tailed hypothesis: Are amphibians more sensitive than
 5690 fish?

Apical Effect (n)	Amphibian NOAEC < Fish			Amphibian NOAEC > Fish			p-value
	% of Cases	Mean Diff.	Max Diff.	% of Cases	Mean Diff.	Max. Diff.	
Mortality (n=43)	42%	-44%	-98%	58%	45%	10E3%	0.94
Body Weight (n=43)	53%	-62%	-97%	47%	17%	12E3%	0.90
Length (n=39)	59%	-70%	-99%	41%	13%	11E3%	0.09

5691

5692 The results of this exploratory analysis indicate that, overall, chronic fish ecotoxicity data for exposure
 5693 to pesticidal active substances provide a reasonable approximation of central tendency for ecotoxicity
 5694 in amphibians, using model test species. There were no statistically significant differences between
 5695 NOAEC values for fish and amphibians with respect to mortality, body weight, or length. In cases
 5696 where amphibians were less sensitive than fish, they were at times far less sensitive – with orders of
 5697 magnitude in the difference between species. Aubee and Glaberman conclude that, if the purpose of a
 5698 surrogate is to provide an average representation of effects levels across taxa, then these results
 5699 indicate that the use of fish to represent aquatic amphibians under chronic exposure conditions
 5700 remains appropriate.

5701 It is noteworthy, however, that a lower endpoint was derived for amphibians in 42-59% of
 5702 comparisons. While not statistically significant, this suggests that amphibians may be more sensitive
 5703 to certain exposures on an individual chemical basis, and, according to the authors, it challenges the
 5704 traditional notion in ecological risk assessment that fish are consistently more sensitive. Such
 5705 differences may have an impact in individual chemical risk conclusions, depending on the magnitude
 5706 of the difference and the environmentally relevant exposure levels as a chronic risk assessment based
 5707 on fish may not cover the toxicity for amphibians.

5708

5709 **Conclusion of the comparative studies with fish**

5710 Based on the limited data and studies available, the comparison of the acute and chronic sensitivity of
 5711 amphibians with the standard fish test species, suggests that the sensitivity of amphibians may be
 5712 covered by fish in some, though not all, cases. Around 30% of the acute endpoints for amphibians are
 5713 lower than for fish and for the chronic endpoints around 50%. In order to cover the acute toxicity to
 5714 amphibians, an extrapolation factor of at least 100 is suggested based on the current database. The

5715 limitation of the current analysis is that a comparable endpoint does not indicate a comparable
5716 sensitivity when the slope of the dose-response-curve is considered. A steep dose-response-curve
5717 introduces a greater uncertainty in the risk assessment than a flat dose-response-curve. The
5718 preliminary conclusion is based on a limited data set for less than one hundred pesticidal active
5719 substances, especially for the chronic exposure and with regards to the life stages tested. Possibly not
5720 the most sensitive chronic endpoint of the most sensitive life stage was used for the comparison due
5721 to lack of data questioning the general validity of the comparison. The majority of endpoints are
5722 available for *X. laevis* and thus limited conclusions can be drawn about variability in sensitivity
5723 between species. Whereas *O. mykiss* is generally regarded as one of the most sensitive fish species,
5724 the most sensitive amphibian species is currently not known. One question is whether individual cases
5725 where amphibians are more sensitive than fish are a reason of concern. Another question is whether
5726 the sensitivity of amphibians can satisfactorily be predicted by using fish data and possibly an
5727 extrapolation factor. By comparing one endpoint for single species, the distribution in sensitivity for all
5728 species cannot be adequately predicted and the whole range may not be covered.

5729 Major gaps in knowledge with regards to toxicity are:

- 5730 • Variability in sensitivity of amphibian species
- 5731 • Representativeness of the tested species for indigenous species
- 5732 • Lack of comparable chronic toxicity data
- 5733 • Comparability of chronic endpoints with regards to their significance for the population level
- 5734 • Significance of low endpoints for amphibians for the selective sensitivity towards specific
5735 mode of actions
- 5736 • Sensitivity of different life stages not easily maintained in the laboratory

5737

5738 **10.2.3. Potential coverage of the exposure assessment – analysis of** 5739 **available exposure models for aquatic organisms and suitability for** 5740 **amphibians and reptiles**

5741 The exposure of aquatic vertebrates to pesticides may be *via* the water column, organic substrates
5742 (i.e. algae, leaf litter, etc.) and inorganic substrate (i.e. sediments).

5743 **Simulation of the exposure in laboratory studies with surrogate species:**

5744 In acute laboratory toxicity studies, fish are exposed *via* water, while no feed is provided (OECD 203).
5745 Therefore fish are taking up the test substance dissolved in water *via* their skin or gills and not orally
5746 *via* feed. In long term toxicity studies, fish are fed daily (OECD 204, 215) or *ad libitum* (OECD 210).
5747 However, as the feed is freshly added and not treated, it does not represent a worst-case oral
5748 exposure. This is of minor relevance for the exposure of fish *via* the oral or dietary route, as the
5749 primary route of uptake of pesticides is *via* gills (Rankin et al., 1982). In fish, gills are critical organs
5750 for respiratory, osmoregulatory and excretory functions.

5751 Oral and dermal exposure *via* sediment is routinely tested with aquatic invertebrates (i.e. *Chironomus*
5752 sp., OECD 218, 219) or *Lumbriculus sp.*, OECD 225) and not fish, even though higher tier studies with
5753 fish, especially for highly adsorbing substances, may be conducted in the presence of sediment. Test
5754 guidelines for amphibians have been developed, see chapter 8. Tadpole oral and dermal exposure *via*
5755 sediment is studied in the ASTM Whole sediment toxicity tests with amphibians E2591-07 (2013) and
5756 the EPA Tadpole/sediment subchronic toxicity test OPPTS 850.1800 (1996).

5757

5758 **Routes of exposure of amphibians and reptiles:**

5759 The exposure of amphibians to PPP differs from fish and varies throughout their life cycle. Whereas
5760 eggs are laid in the central part of the water column (attached to submerged plants) and hence are
5761 mainly exposed *via* water, later terrestrial stages are also exposed *via* oral uptake of food, contact
5762 with soil or plants, air or direct overspray. Even though directly immersed in water, eggs are expected

5763 to have a limited uptake of contaminants from the water column due to their gelatinous coating.
5764 There is some evidence, however, showing that the gelatinous coating may not protect against all
5765 pesticides as it was demonstrated that isoproturon and cypermethrin could enter the eggs despite this
5766 coating and have some detrimental effects on tadpole development (Greulich and Pflugmacher, 2002;
5767 Greulich and Pflugmacher, 2004). The uptake of chemicals can begin shortly after egg deposition, as
5768 water moves into the egg capsule (Birge et al., 2000); in the jelly mass, 0.7% of total radioactivity
5769 was detected, while 2% was measured in the egg. The thickness of the gelatinous coating may
5770 change over time. In very shallow ponds, eggs may be in contact with the sediment. Eggs may also
5771 be contaminated *via* maternal transfer. Little is known about this route of exposure at present,
5772 however. Some earlier work indicates that lipophilic compounds may concentrate in eggs *via* maternal
5773 transfer, reducing the concentrations in female frogs, and reach higher concentrations in eggs than in
5774 mothers. This is especially true for POPs including organochlorine pesticides (Kadokami et al., 2004;
5775 Wu et al., 2012). Eggs of reptiles may also be exposed *via* soil contact. There is evidence showing
5776 that eggs laid in agricultural areas with local OC pesticide use will have a higher burden of these
5777 contaminants, compared with eggs laid in non-exposed areas (Stocker et al., 2011). Experimental
5778 evidence of soil transfer has been published in snapping turtle eggs (*Chelydra serpentina*) exposed *via*
5779 the soil to atrazine, simazine, metolachlor, azinphos, dimethoate, chlorpyrifos, carbaryl, endosulfan,
5780 captan and chlorotalonil. Except for chlorotalonil, all other pesticides were detected above
5781 quantification limit in the eggs. The main drivers for egg transfer appeared to be duration of
5782 exposure, the soil concentration, a low sorption to organic carbon and lipid, and a high water
5783 solubility. The exact mechanisms of transfer are still unknown, although it is speculated that the
5784 primary route of exposure could be the vapour and not the dissolved phase in soil (De Solla and
5785 Martin, 2011). After hatching of the eggs, the hatchlings start feeding and can thus additionally be
5786 exposed *via* sediment and feed. As the hatchlings grow and become larvae, internal gills are formed,
5787 they start breathing and exposure *via* air becomes a possible (even though expectedly low) route of
5788 exposure. During metamorphosis, feeding stops, lungs are developing, but the main routes of
5789 exposure are *via* sediment and water (see also section 2.4).

5790 The dermal exposure of reptiles *via* water is much lower than compared with fish or amphibians, apart
5791 from some water-dwelling snakes and terrapins. Some aquatic turtles rely on water held in their
5792 buccal cavity for oxygen uptake and this may also provide a pathway for entry of dissolved chemicals
5793 (Linder et al., 2010). The oral uptake of water from drinking or feeding is considered relevant.

5794

5795 Therefore the routes of uptake are

- 5796 • dermal *via* water, sediment, soil, plants or air,
- 5797 • oral *via* feed or water and
- 5798 • inhalation.

5799

5800 In the aquatic system, the relevant routes of exposures are considered to be:

- 5801 • dermal exposure to water for amphibians (eggs, hatchlings, larvae, metamorphics, juveniles,
5802 adults) and reptiles (water dwelling snakes and terrapins),
- 5803 • dermal exposure to sediment for amphibians (hatchlings, larvae, metamorphosis, and
5804 juveniles and adults hiding or over wintering on the sediment)
- 5805 • oral exposure *via* sediment for amphibians (larvae)
- 5806 • oral exposure *via* feed in the water column for amphibians (hatchlings, larvae) and
- 5807 • oral exposure *via* water while feeding of amphibians (hatchlings) and reptiles (juveniles,
5808 adults).

5809

5810 The current risk assessment is intended to cover the use of a single PPP in a limited time frame. This
5811 is especially problematic for amphibians, which may be exposed in the water and transfer the body

5812 burden to the terrestrial environment. Also, the terrestrial exposure of adults may *vice versa* lead to
5813 maternal transfer to eggs (Kadokami et al., 2004; Pagano et al., 1999).

5814 A hypothesis is that *X. laevis* may show greater effects than in fish if exposed *via* diet for lipophilic
5815 compounds, as the dietary uptake is more important for amphibians than for fish. The relevant
5816 exposure therefore needs to be determined in laboratory studies in order to achieve an optimal and
5817 realistic uptake.

5818 For lipophilic compounds, a possible exposure during metamorphosis is the release of substances
5819 accumulated and stored in body tissue during tail resorption (Bernabo et al., 2016).

5820 **Suitability of the laboratory studies with aquatic surrogate species to simulate the**
5821 **exposure of amphibians and reptiles in the water system:**

5822 The dermal exposure in water is likely to be adequately reflected by laboratory studies with fish
5823 (OECD 203). Dermal and oral exposure to sediment are simulated in the study design with
5824 invertebrates (i.e. *Chironomus* sp., OECD 218, 219 and *Lumbriculus variegatus*, OECD 225) only. The
5825 oral exposure to pesticides *via* the dietary route is only partially covered in the long-term studies with
5826 fish. The oral exposure *via* water is considered to be covered in the acute and long-term studies with
5827 fish as freshwater and marine fish pass water through their stomachs and excrete urine.

5828 Hence the current aquatic studies with surrogate vertebrates do not adequately cover the dietary
5829 route of exposure and dermal exposure *via* contact to sediment for amphibians and reptiles, whereas
5830 the dermal exposure in the water column is adequately reflected. The oral uptake of sediment is
5831 expected to be covered by longterm studies with *Lumbriculus variegatus*. The relative importance of
5832 the dietary route should be determined.

5833 **Table 40:** Possible exposure concentrations for amphibians in temporary and permanent ponds
5834 (edge of field and in field) for the entry pathways drift, run-off and drainage:

Life stage	medium	Available model*	unit	Description of ecotoxicological exposure quantity
Eggs, hatchlings, larvae, metamorphs, juveniles, adults	Water	FOCUS-sw models	mg/L	mass of ai dissolved per volume water at average depth (mixed water column)
Eggs, hatchlings, larvae, metamorphs, juveniles, adults	Sediment	FOCUS-sw models	mg/kg	Total concentration in top layer of sediment
Eggs	Submerged plant	TOXSWA	mg/kg dry weight macrophyte	Eggs adhered to plants may adsorb the concentration per mass of water plants modelled by TOXSWA
Eggs	Maternal transfer	Metabolism studies	mg/kg in egg	
hatchlings, larvae	Food	- (TK/TD)	mg/kg food	Concentration in periphyton, planktonic algae and invertebrates

5835 *which needs to be adjusted to adequately predict the exposure for amphibians with regards to size of pond, distance to
5836 crop, movement of water, field to pond ratio, organic carbon content in the sediment, bulk density and texture of
5837 sediment, adsorption coefficient for water plants.

5838

5839 In order to determine the uptake of sediment or food by larvae, the ingestion rate needs to be known.

5840 Shallow ponds (<1 m) are considered to be quite homogenous as the temperature difference will lead
5841 to a daily mixing of the entire water column. So consecutive daily inputs *via* drift and run-off will be
5842 rapidly and homogeneously distributed in the ponds. Although the amount of suspended matter may be
5843 relatively high in these ponds, adsorption and resuspension of pesticides are insignificant for the size
5844 of the PEC in the water layer except for compounds with high sorption capacities, such as pyrethroids.

5845 In order to predict the concentration in the aquatic system, not only hydrological parameters, but also
5846 the physical-chemical properties of the substance are taken into account such as degradation
5847 (DegT50) and adsorption (Koc). Depending on the route of entry, the adsorption to the sediment may
5848 take days or be instantaneous. Temporary water bodies differ from permanent water bodies in many
5849 ways (Lahr, 1997) with fluctuating physical (temperature, light, water level) and chemical (oxygen,
5850 pH, ionic strength) characteristics. These characteristics may affect the concentration and
5851 bioavailability of the PPP in the water system.

5852

5853 **Adequacy to predict the concentration in surface waters used by amphibians by the**
5854 **FOCUS models (Comparison of scenarios and parameters):**

5855 Assessing the exposure for amphibians in all fifteen FOCUS water bodies would be more in line with
5856 the current aquatic risk assessment at EU level. In the current EU registration procedure ponds are
5857 considered as being half a scenario, because in the same scenario a pond or stream is situated as
5858 well. A safe scenario needs to have low risk for all water bodies defined for that scenario. So, if, e.g.
5859 in both the pond and the ditch of the R6 scenario the PEC values are lower than the calculated RAC,
5860 there would be low risk for the aquatic ecosystem in both waterbodies and the R6 scenario would be
5861 classified as having low risk. However, if the PEC in the pond is lower than the RAC, while the PEC in
5862 the ditch is higher than the RAC, then the R6 scenario would be classified as presenting a risk for the
5863 aquatic ecosystem. Note that, at present, it is common practice in the assessment procedure by EFSA
5864 to include the use of mitigation measures according to FOCUS Landscape and Mitigation (FOCUS,
5865 2007) in the PEC calculation in order to reduce risks. And in EFSA's conclusions for the evaluated
5866 compound a 'critical area of concern' is not identified for the aquatic risk assessment when, for at
5867 least one of the representative uses assessed, more than half the FOCUS scenarios specified for that
5868 use indicate low risk. If less than half the scenarios for all the representative uses assessed indicate
5869 low risk, then EFSA indicates 'critical area of concern'.

5870 Moreover, the exposure concentrations in FOCUS ditches and streams are considerably higher than
5871 those in FOCUS ponds. Concentration peaks triggered by spray drift depositions are considerably
5872 higher in FOCUS ditches and streams than they are in FOCUS ponds, because the 1-m width water
5873 surfaces of the ditches and streams receive higher depositions than the 30-m wide pond surface area,
5874 while their water depths (often 0.3 m) are lower than the water depth of the ponds (1 m). Also
5875 concentration peaks by drainage or runoff entries are considerably higher in FOCUS ditches and
5876 streams than the peaks in FOCUS ponds, because the treated land:water ratio of ditches and streams
5877 (100:1) is much higher than the treated land:water ratio for ponds (5:1), while the available water
5878 volume for dilution is smaller for FOCUS ditches and streams than for FOCUS ponds (FOCUS, 2001).

5879 As explained above, amphibians prefer to breed in temporary ponds without predators such as fish.
5880 Often their water depth is shallow and so, the FOCUS pond with its 1 m water depth is expected to
5881 result in non-conservative PEC_{sw} , i.e. not to be a realistic worst-case exposure scenario. In order to
5882 form realistic worst case exposure scenarios for amphibians, the FOCUS ponds especially need to be
5883 calibrated to a higher tier, having more realistic PEC_{sw} , obtained with the aid of spatio-temporal
5884 statistical populations for relevant PEC_{sw} values, as explained in section 7.7 on the exposure
5885 assessment goals. The adjusted pond scenarios will probably consist of smaller ponds than the current
5886 30*30 m ponds with a water depth smaller than the current 1m. The analysis on the amphibian ponds
5887 in Spain, the canton Aargau in Switzerland and the ponds of the Countryside Survey in the UK also
5888 indicate that it seems unlikely that the FOCUS pond of 30*30 m with its 1 m water depth is protective
5889 for the majority (e.g. 90%) of all ponds hosting amphibians in agricultural areas (Appendix C). The
5890 majority of analysed ponds (70-90%) are smaller in size than the FOCUS ponds with regards to water
5891 depth, surface area and volume. Therefore the FOCUS ponds are not considered to be conservative

5892 enough to estimate the concentration in amphibian ponds due to entries *via* drift, run-off or drainage.
5893 The entry *via* drift in the FOCUS ditches and streams is estimated to be greater than in amphibian
5894 ponds due to the short crop-edge to water distance and narrow 1m widths of the FOCUS ditches and
5895 streams, but on the other hand, the amphibian ponds may still be shallower than 0.3 m. With regards
5896 to volume, the amphibian ponds may be similar to the FOCUS ditches and streams, but the land:water
5897 ratio is unknown and the contribution of the surrounding fields is difficult to compare. Therefore, we
5898 are unable to evaluate *a priori* whether the FOCUS ditches or streams result in conservative acute
5899 exposure concentrations for amphibians. With regards to the chronic exposure assessment, the
5900 FOCUS ditches and streams are expected to underestimate the exposure as they are slowly flowing in
5901 comparison to standing amphibian ponds. In conclusion, we recommend evaluation of exposure for
5902 amphibians, not only in FOCUS ponds but in all FOCUS surface-water bodies; we are unable to predict
5903 whether the acute exposure will be conservative for amphibians in their aquatic environment, but we
5904 expect the chronic exposure to be non-conservative. Final conclusions on the use of FOCUS scenarios
5905 can be drawn, when exposure calculations are possible for spatio-temporal statistical populations of
5906 amphibian ponds and other relevant water bodies defined with the aid of the Exposure Assessment
5907 Goal methodology, described in Chapter 9.

5908 As stated before, the FOCUS step 3 scenarios intend to represent 'realistic worst case' scenarios for
5909 the PECs in the water layer and not for the PECs in sediment. Generally speaking, due to the partition
5910 between water and sediment, high concentrations in the water layer are associated with low
5911 concentrations in the sediment and *vice versa*. The prediction of the sediment concentrations in the
5912 FOCUS surface water scenarios is therefore expected to result in non-conservative estimates, as in
5913 principle worst-case exposure in water is associated with best-case exposure in sediment. Note that
5914 this may not be true if the pore water in sediment is the Ecotoxicologically Relevant type of
5915 Concentration. Moreover, PPPs cannot accumulate in the sediment of the FOCUS scenarios, as the
5916 simulation time is only one year. This is an additional reason why sediment concentrations are not
5917 expected to be conservative. For the time being, therefore, the EFSA Scientific Opinion on effect
5918 assessment on sediment organisms proposes a simple and conservative approach to calculate
5919 sediment concentrations in FOCUS step 3 scenarios that takes multi-year applications into account
5920 (EFSA, 2015).

5921 Major gaps in knowledge with regards to exposure are:

- 5922 • Distribution of actual size and depth of (temporary) amphibian ponds in Europe
- 5923 • Habitat preference of different species with regards to type of pond and agricultural area in
5924 order to determine focal species
- 5925 • Monitoring data of PPP concentrations in ponds being habitats for amphibians (water column
5926 and sediment)

5927

5928 **References:**

5929

5930 **10.3. Coverage of terrestrial life stages of amphibians and reptiles in** 5931 **the current risk assessment for birds and mammals and** 5932 **humans**

5933

5934 **10.3.1. Extrapolation of endpoints observed in birds and mammals to** 5935 **amphibians and reptiles and potential coverage of toxicity:**

5936 Endpoints on acute and short term toxicity (mortality) and effects on reproduction are available from
5937 standard tests included in the dossiers for pesticidally active substances (an overview on available
5938 endpoints can be found in Annex E). In order to avoid additional vertebrate testing with amphibians
5939 and reptiles, it would be highly desirable to use endpoints from birds and mammals as surrogates.

5940 Due to the lack of toxicity data with reptiles and adult (terrestrial) stages of amphibians, it is not,
 5941 however, possible to make statistically robust comparisons of endpoints among the different taxa.
 5942 Therefore the following includes interpretation of available information and general considerations on
 5943 the use of surrogate endpoints from birds and mammals.

5944 *Comparison of reptile and bird and mammals endpoints*

5945 The assumption that birds are more toxicologically sensitive than reptiles cannot be fully supported.
 5946 Indeed, as demonstrated by Weir et al (2010 (Table 41:), completing the work by Pauli et al. (2000),
 5947 toxicity may vary greatly depending on the compound, the class of compound and the tested species.
 5948 Out of 17 chemicals for which comparable acute toxicity data could be found, Weir et al (2010)
 5949 observed that birds and reptiles were of equivalent susceptibility for 6 out of 17 chemicals, birds were
 5950 more susceptible for 3 out of 17 and reptiles were more susceptible for 8 out of 17 chemicals (many
 5951 of which were pyrethroids but not all). As a consequence, the limited information available shows
 5952 that acute toxicity data from birds may not cover the range of susceptibility of reptiles, especially
 5953 considering the very limited number of species for which data were available.

5954

5955 **Table 41:** Range of LD50 in mg/kg bw (acute toxicity studies) (adapted from Weir *et al*, 2010)

	Bird-low	Bird High	Reptile -Low	Reptile-High
Malathion	167	1485	170	2324
Propoxur	3,8	60,4	15	15
Parathion	1,3	24	8,9	10
Methyl-Parathion	3	60,5	83	83
Azinphos	8,25	136	98	98
Pyrethrins	5620	5620	15	78
I-cyhalothrine	3950	3950	10	10
Allethrin	2000	2000	30	30
Resmethrine	2000	2000	30	30
Fipronil	31	1065	30	30
1080	3	17,7	200	200
Rotenone	133	1000	2	2
Diphacinone	58,8	2265	30	30
Methyl thiophanate	4640	4640	900	900
DNT	55	55	380	577

5956 Green : reptiles less susceptible ; yellow: equally susceptible ; red: reptiles more susceptible

5957

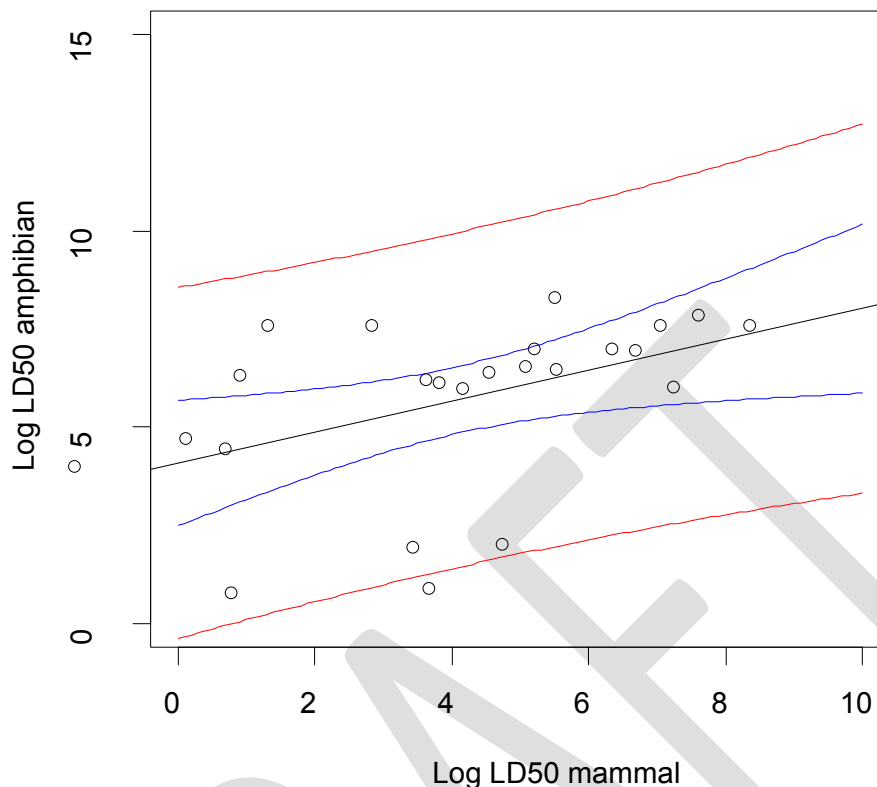
5958 A significant correlation was found between reptile and bird acute LD50 values for the lizard
 5959 *Sceloporus occidentalis*. (Weir et al. 2015). This analysis is however based on only one species and
 5960 eight different pesticidally active substances. Further data (more species and substances tested) are
 5961 therefore needed before a general conclusion can be drawn on the potential of extrapolating acute
 5962 toxicity endpoints from birds to reptiles.

5963 No comparison of endpoints from long-term studies was available for reptiles.

5964 In a recent publication (Crane et al 2016), acute toxicity data from oral exposure of amphibians were
5965 compared with bird and mammal data. There was a general tendency of amphibians being less
5966 sensitive than mammals or birds. Amphibians were more susceptible than mammals and birds in 5/26
5967 comparisons. If an assessment factor of 10 is applied to the bird and mammal endpoints, then only
5968 two amphibian endpoints would be lower than the birds and mammals endpoint. It should be noted
5969 that the dataset was limited and some uncertainties remain. It is unclear whether substances with
5970 other mode of actions than the ones tested are covered. Organochlorine substances were
5971 overrepresented and some of the substances are not used as pesticidal actives. Another uncertainty is
5972 the extrapolation to European species since most tests were conducted with bullfrog (*Lithobates*
5973 *catesbeiana*). The LD50 for DDT of common frog (*Rana temporaria*) was three orders of magnitude
5974 lower than the one observed for bullfrog. It is unclear however if this is due to differences in
5975 sensitivity or due to the different carrier substances used in the studies with common frog and
5976 bullfrog. The above mentioned uncertainties would need to be addressed before the findings with
5977 regard to coverage of the toxicological sensitivity of amphibians by mammal and bird endpoints can be
5978 generalised.

5979 Another aspect is the potential extrapolation from birds and mammal acute LD50 endpoints to
5980 amphibians. This is only possible if a correlation exists between the endpoints. A weak and statistically
5981 significant correlation was found between mammal and amphibian LD50s but no correlation was found
5982 between amphibian and bird acute toxicity in the study of Crane et al (2016). The WG investigated
5983 further whether the mammal data could be used to predict the amphibian endpoints. The correlation
5984 was weak and hence large boundaries for 95th percentile predictions were obtained (+/- 4.5 of the
5985 regression mean at log scale, equivalent to a factor of 30000 at non-logarithmic scale) (see Figure 37:
5986 below). This means that a factor of about 30000 would need to be applied to the mammal acute LD50
5987 endpoint to cover 95% of the amphibian acute LD50 endpoints. In addition there are the uncertainties
5988 with regard to species sensitivity and representativeness of tested substances as discussed above.
5989 Therefore it is not considered meaningful to extrapolate from mammal acute LD50 endpoints to
5990 amphibian acute LD50 endpoints based on the available dataset.

5991



5992

5993 **Figure 37:** Comparison of amphibian and mammal acute LD50 values based on the dataset
 5994 presented in Crane et al. (2016). The blue lines are the 95% confidence intervals for the
 5995 regression mean and the red lines are the 95%tiles for predictions. $\text{Log amphibianLD50} = \text{Log}$
 5996 $\text{mammalLD50} \times 0.393 + 4.0899$, $R^2 = 0.2144$, $p = 0.0227$

5997

5998 *General considerations on coverage of endocrine and reproductive effects in amphibians and reptiles*
 5999 *by bird and mammals*

6000 Amphibians have a unique phase in their life cycle with a complete transformation of the animal
 6001 (metamorphosis) and therefore potential detrimental effects on metamorphosis in amphibians cannot
 6002 be covered in bird or mammal reproductive toxicity studies.

6003 In amphibians and reptiles, gonadal differentiation is affected by factors such as hormone levels or
 6004 temperature in addition to the genetic mechanisms for determination of sex (see chapter 6). Gonadal
 6005 differentiation in reptiles and amphibians is therefore a plastic process that may be more susceptible
 6006 to impact of endocrine-disrupting chemicals than in birds and mammals. Reproductive toxicity studies
 6007 in birds and mammals (such as OECD 416) include endpoints with potential information relating to
 6008 identification of endocrine-disrupting effect (Estrogen, Androgen, Thyroid). There is evidence showing
 6009 that gonadal alterations observed after 17β estradiol exposure in the painted turtle (*Chrysemis picta*)
 6010 were comparable to alterations observed in birds after *in ovo* exposure (Berg et al., 1999, 2001a),
 6011 suggesting that endocrinedisrupting chemicals may induce similar effects on gonadal differentiation
 6012 in birds and reptiles. In principle, it might be possible to extrapolate certain effects from bird long-term
 6013 studies to effects on reptiles. Reproductive toxicity studies in birds (OECD 206) usually do not include
 6014 thorough histopathological examination. This information would be highly valuable and serve as a
 6015 good indicator of potential detrimental effects on gonadal/Müllerian duct differentiation and

6016 development in reptiles (Crain et al., 1999, Berg et al., 2001b). Critical routes of exposure at critical
6017 windows of development may not, however, be comparable in birds, mammals, reptiles and
6018 amphibians, which makes potential extrapolation of effects uncertain.

6019 *In vitro* tests to detect endocrine effects are available for mammals (e.g. ER-transactivation assay
6020 OECD 455, aromatase assay OECD (US EPA) and other tests are still being developed (e.g. androgen
6021 receptor transactivation assay). As already pointed out by EFSA (2013), mechanistic information on
6022 the endocrine pathways and availability of internationally standardised test methods is limited for
6023 amphibians and very limited for reptiles. Hence it is unclear whether observations related to
6024 endocrine effects in the studies with mammals can be extrapolated to amphibians and reptiles. There
6025 is a need for specific tests and/or information on the endocrine pathway and susceptibility of
6026 amphibians and reptiles at various life stages, including *in vitro* data. Standard tests exist for
6027 amphibians to detect effects on the thyroid axis and development of sex organs (see section 8) but
6028 they are lacking for reptiles.

6029

6030 *General consideration on coverage of behavioural effects*

6031 The ongoing behaviour of non-target species should be protected according to the EU regulation
6032 1107/2009. The tests with birds and mammals do not systematically investigate behavioural effects.
6033 Some non-standardised test with amphibians and reptiles exist (see section 8). Further investigation is
6034 needed into how relevant behavioural effects observed under laboratory conditions are to wild
6035 populations.

6036

6037 *General considerations on coverage of dermal toxicity*

6038 At present, the Bird and Mammal Guidance Document (EFSA 2009b) does not require any specific
6039 information with respect to inhalation and dermal toxicity and hence no surrogate endpoints are
6040 available for extrapolation to amphibians and reptiles. Dermal toxicity is currently investigated only for
6041 human risk assessment. It covers several aspects, including systemic toxicity *via* the dermal route of
6042 exposure, local effects as dermal irritation and skin sensitisation. For the estimation of the systemically
6043 available exposure levels in cases of non-dietary exposure, the dermal absorption of the active
6044 ingredients as well as of the dilution(s) of the plant protection products is also measured for the
6045 establishment of the appropriate absorption factors for human risk assessment.

6046 Coverage of amphibian and reptilian dermal toxicity (OECD 402, 410) and absorption (OECD 427, 428)
6047 by routine rodent tests may not be completely adequate, especially with respect to amphibians.

6048 There is a general agreement on the high permeability of amphibian skin, much higher than
6049 mammalian dermal absorption (Quaranta et al 2009), (Kaufmann & Dohmen, 2016). *Ex vivo* skin-
6050 absorption tests could be used to estimate adequate dermal absorption factors instead of default
6051 values (100%). Furthermore, amphibian skin plays an important role as a respiratory organ and some
6052 studies with pesticides clearly show that dermal exposure may be lethal, even without any systemic
6053 exposure, as a result of respiration defects (Johnson et al., 2016). Overall, the current dermal toxicity
6054 tests in rodents do not cover the specific risk for amphibians, while reptiles appear more similar to
6055 mammals (Weir et al., 2015).

6056 Testing for skin irritation in mammals, both *in vitro* (e.g. OECD 431) and *in vivo* (OECD 404), is
6057 probably appropriate for the estimation of the irritation potential of PPPs and active ingredients for
6058 amphibians and/or reptiles, considering that irritation is a mechanism of toxic response, which may
6059 occur in all vertebrate species. Skin irritation is commonly described as a result of toxicant exposure
6060 in amphibians (Pessier, 2002).

6061 Skin-irritation tests in mammalian species cannot, however, provide any information in relation to
6062 mucus-layer degradation of amphibians, which may occur, and there are no published data evaluating
6063 chemical characteristics of toxicants that might lead to the degradation of the mucus layer. It is
6064 suspected that some chemicals may have a detrimental impact on this layer without being irritant
6065 (pyraclostrobin case study). Furthermore, surfactants, for instance, may have an important impact on
6066 the mucus layer in amphibians without any significant or strong irritation potential in mammals. In the

6067 case of reptiles, the skin structure appears to be similar to mammalian skin and dermal irritation tests
6068 (e.g. OECD 404 or 431) could cover this particular risk (Weir et al., 2015).

6069 With respect to skin sensitization, there is no evidence or data to support the applicability of tests to
6070 amphibians or reptiles.

6071 Many studies show that dermal exposure may result in severe immunological disorders in amphibians
6072 and that this is not adequately covered by existing tests (Johnson et al., 2016). It is also
6073 demonstrated that exposure in the field may result in secondary bacterial or fungal contamination
6074 leading to delayed death (Pessier, 2002). Several authors have demonstrated that peptides present in
6075 skin secretions may have an important immune-protective effect. (Conlon et al., 2011). None of the
6076 existing tests covers this issue. A dermal overspray test would appear appropriate to estimate this
6077 kind of damage.

6078 In conclusion, there is a need for adapted protocols to evaluate dermal toxicity, dermal absorption and
6079 indirect consequences of dermal exposure in amphibians and reptiles. Further research is warranted to
6080 establish appropriate protocols and endpoints for evaluation. This is especially needed for amphibian
6081 skin as it is too different in its anatomy and function from mammalian skin to to exclude skin effects in
6082 amphibians even if there are no effects observed in tests with mammalian skin. Endpoints from skin
6083 tests to be used in a risk assessment context are skin lesion (irritation/corrosion) and dermal
6084 adsorption. Such a test could be used in a first tier screening. In order to minimize animal testing it
6085 would be highly desirable to develop in-vitro methods. For measuring skin permeability a test design
6086 similar to the one in Kaufman and Dohmen (2016) could be used. For skin irritation it might be an
6087 option to develop a test for measuring skin irritation in amphibian keratinocyte cell cultures similar to
6088 OECD 439 and measuring viability of cells with an MMT assay. However, there would be uncertainty
6089 with regard to effects on the whole animal as there is no active uptake in-vitro and not accounting for
6090 effects on respiratory function. Therefore in-vivo tests with amphibians would still be needed. Only if a
6091 scientific research project would generate enough test results with in-vivo/in-vitro outcomes for
6092 different types of pesticidal active substances and PPPs, then it could be looked into whether it is
6093 possible to extrapolate from the in-vitro to in-vivo effects. The results of such a research programme
6094 could also be used to set up and test TK/TD models for amphibians.

6095

6096

6097 *Influence of temperature on toxicity*

6098 Biological activity of amphibians and reptiles usually increases with higher temperatures (under
6099 temperate climates) because of poikilothermy. As a consequence, the FIR will increase, exposure will
6100 increase and toxicity may reach its peak. There is no direct relationship, however, between metabolic
6101 rates, FIR and external temperature (Harvey-Pough, 1983). Enzyme activity may vary as a result of
6102 external temperature modifications. It has been shown for instance, that cholinesterase activity is
6103 highly dependent of temperature, with increased basal activity in frogs raised at 19°C vs frogs raised
6104 at 8°C (Johnson et al 2005). As a consequence, lower temperatures could be associated with
6105 increased susceptibility of amphibians or reptiles, as also commonly observed for pyrethrins (Talent,
6106 2005) in lizards, glyphosate (formulated PPP) in common toads (Baier et al., 2016), or copper sulfate
6107 toxicity in amphibians (Chiari et al 2015). This is not true for all active substances or commercial
6108 formulations, and other reports are available indicating a higher susceptibility of amphibians and
6109 reptiles to PPP active substances such as endosulfan (Broomhall, 2002), methomyl (Lau et al 2015),
6110 carbaryl and malathion (Rumschlag et al 2014) with increasing temperatures. There is also one
6111 published evidence of non-monotonic response to an active substance (glyphosate) (Gandhi & Cecala,
6112 2016). In a recent study in tropical frogs ((Alza et al 2016)), temperature alone has a positive
6113 influence on tadpole growth, but chlorotalonil toxicity is not modified by changes in diurnal
6114 temperature (limited range of +1 to +9°C difference).

6115 It is also important to mention the major role of external temperature on the egg development and
6116 gender determination in reptiles, which cannot be evaluated with current toxicological endpoints from
6117 birds, mammals or human toxicity studies. It has been shown, for instance, that very low
6118 environmental exposure of eggs to xeno-estrogens such as DDT may lower the temperature threshold
6119 on sex determination in alligators ((Milnes et al 2005).

6120 Overall, there are only limited data available to describe the relationship between temperature and
6121 toxicity in amphibians and reptiles, and there is no evidence of a general dose-response curve. It is
6122 impossible to predict the impact of temperature on the toxicity of a given PPP (either as an active
6123 substance or as a commercial product) in amphibians or reptiles. Furthermore, the current risk
6124 assessment in birds and mammals or human beings is not adapted to integrate ectothermy and its
6125 potential effects on toxicity or exposure and any extrapolation from birds, mammals or human risk
6126 assessment should include some evaluation of the potential influence of ectothermy.

6127

6128 **Overall conclusion**

6129 The acute oral toxicity of pesticidally active substances to amphibians and reptiles might be covered
6130 by studies with birds and mammals. Available data are very limited, however, in terms of substances
6131 and amphibian and reptile species tested. Any general conclusion on potential coverage and
6132 assessment factors would be highly uncertain. The main issues that need to be addressed are
6133 potential differences in species sensitivity and relevance of the different modes of action of pesticidally
6134 active substances in respect of differences in susceptibility between species. Correlation between
6135 endpoints from birds and mammals with endpoints from amphibians and reptiles is a prerequisite for
6136 extrapolating and using birds and mammal endpoints as surrogates in a risk assessment. The datasets
6137 are very limited. A weak correlation was found between acute oral endpoints between mammal and
6138 amphibian endpoints. A strong correlation was found between bird and reptile oral toxicity. The
6139 underlying datasets were, however, too limited to recommend extrapolating acute oral toxicity from
6140 mammal and birds to amphibians and reptiles, respectively.

6141 Even greater uncertainty exists regarding potential coverage of long-term toxicity. For example,
6142 detrimental effects on amphibian metamorphosis are not covered. It might be possible to use effects
6143 observed in bird-reproduction studies (with some modifications of the test protocol, e.g. adding
6144 histopathological observations) in the risk assessment for reptiles.

6145 Amphibians and reptiles are poikilothermic and therefore have lower metabolic rates compared to
6146 birds and mammals. Ambient temperature is an important factor modifying toxicity in poikilothermic
6147 animals. In case that metabolic activation of a substance leads to increased toxicity (e.g.
6148 anticoagulants), then amphibians and reptiles may react less sensitively or with delayed effects
6149 compared to birds and mammals because of their slower metabolism. If initial metabolism reduces
6150 toxicity of a pesticidally active substance, then fast metabolisers such as birds and mammals will react
6151 less sensitively. In this case, temperature will have strong effects on the toxicity of the compound to
6152 amphibians and reptiles with enhanced toxicity at lower temperatures. Therefore the specific mode of
6153 action of a pesticidal active substance and the interplay between metabolic rates and temperature
6154 need to be taken into account when using birds and mammal toxicity data as surrogates.

6155 Coverage of endocrine effects in amphibians and reptiles by available studies with birds and mammals
6156 is uncertain because critical routes of exposure and critical windows of development may be different.
6157 Specific test protocols exist for amphibians but are lacking for reptiles. It needs to be investigated
6158 further whether endocrine effects on reptiles are covered by mammals and birds.

6159 Dermal toxicity and adsorption data from mammals may be used as surrogates for reptiles but not for
6160 amphibians. Amphibian skin has important functions such as respiration, water regulation and immune
6161 function. The structure of amphibian skin with its mucus layer is very different from mammalian skin
6162 and therefore it is not possible to extrapolate to effects on amphibians. A test to investigate local
6163 effects on amphibian skin is needed. Systemic effects from dermal exposure also need to be
6164 addressed. Ideally such tests should be in-vitro studies to avoid animal testing. The WG recommends
6165 developing in-vitro tests with amphibian skin for inclusion in future risk assessment schemes.

6166

6167 *Please note that EFSA has outsourced an activity on collecting data from literature to allow a*
6168 *comparison of endpoints observed in studies with amphibians and reptiles with endpoints from bird*
6169 *and mammal studies. The final report of this outsourced activity was not available at the time when*
6170 *the draft opinion was produced. The conclusion regarding potential coverage and the use of endpoints*
6171 *from surrogate species will be updated with the information from the procurement.*

6172

6173
6174
6175

6176 **10.3.2. Potential coverage of the exposure assessment-analysis of**
6177 **available existing exposure models for birds, non-human mammals**
6178 **and humans, and suitability for amphibians and reptiles exposure**
6179 **assessment for oral uptake**

6180

6181 **Oral exposure**

6182 **Oral exposure via food uptake**

6183 The risk assessment considers oral uptake of contaminated food items and drinking water. In addition
6184 there is a risk assessment for bioaccumulation and food chain behaviour focused on earthworm and
6185 fish eating birds (see Annexes D and E for details on the current risk assessment for birds and
6186 mammals)

6187 Residues in different food items, percentage of contaminated food in the diet and food uptake rates
6188 determine the oral exposure. All three parameters need to be considered when comparing oral
6189 exposure across taxa. Provided that the same food items are consumed and 100% contaminated food
6190 is consumed, then the comparison of food-intake rates provide an indication of differences in oral
6191 exposure.

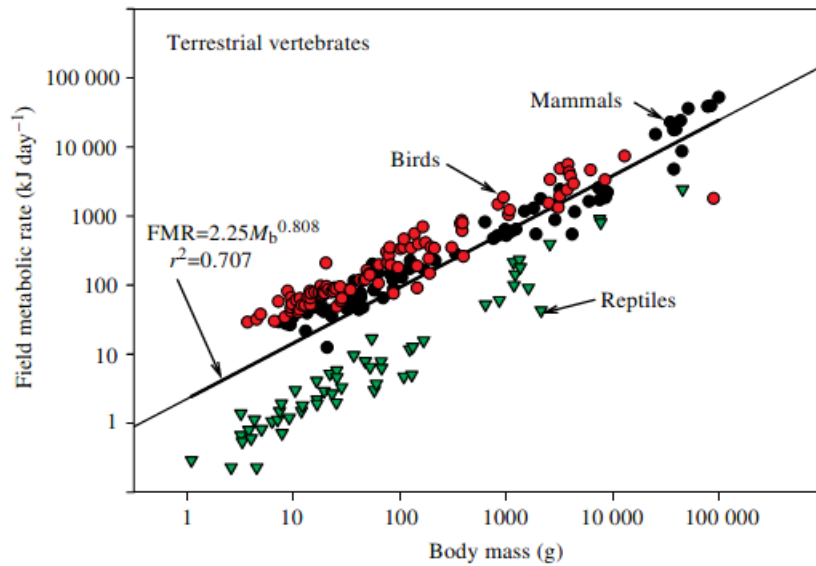
6192 The Table 42: below compares food intake rates from birds, herbivorous mammals and herbivorous
6193 and insectivorous reptiles.

6194

6195 **Table 42:** Estimated Food Intake Rates (adapted from Nagy, 1987 in USEPA, 1993)

Group	FIR (100g animal)
Birds-passerines	20.05
Birds-Non passerines	15.11
Seabirds	12.54
Rodents	8.33
Herbivores	48.24
Iguanid-Herbivore	1.77
Iguanid-Insectivore	0.42

6196
6197



6198

6199 **Figure 38:** Field metabolic rates for birds, mammals and reptiles. (from Nagy 2005)

6200

6201 Food intake rates (FIR) are greater for birds and mammals than for amphibians and reptiles, by at
 6202 least one order of magnitude (USEPA 1993). Specific FIR equations are used by the USEPA in order to
 6203 adapt their exposure model from birds to reptiles or amphibians

6204 Field metabolic rates of birds and mammals are about 12-20 times higher than for equally sized
 6205 reptiles (Nagy 2005) (Figure 38:). The food intake rates for reptiles and amphibians are considerably
 6206 smaller than for birds or mammals. Therefore it is concluded that the oral exposure estimates for birds
 6207 and mammals would result in a conservative estimate of oral exposure of amphibians and reptiles.

6208 On the basis of FIR comparison, it can be concluded that the exposure assessment of secondary
 6209 poisoning for earthworm- and fish-eating birds and mammals will cover amphibians and reptiles with a
 6210 similar diet. However, some reptiles are specialised predators and some snake species feed mainly on
 6211 rodents. Snakes can access the burrows where baits are put and where rodents can be found. In
 6212 addition, poisoned rodents may be more prone to predation (Cox & Smith 1992). Therefore it is likely
 6213 that snakes can be exposed *via* secondary poisoning. Snakes can take up in one meal much more
 6214 relative to their body size than a predatory bird or mammal. Therefore it is unlikely that exposure
 6215 estimates for predatory birds and mammals would cover snakes. It is proposed that a risk assessment
 6216 for snakes is conducted for rodenticides.

6217 Comparison of oral exposure of amphibians, reptiles and birds and mammals based on worst case
 6218 assumptions and species specific food intake rates can be found in Appendix F. The calculations
 6219 confirm that birds and mammals have a greater oral exposure than amphibians and reptiles. Hence
 6220 the screening and first-tier exposure assessment for insectivorous and herbivorous birds and
 6221 mammals would most likely cover amphibians and reptiles.

6222 Furthermore the comparisons show that insectivorous lizards have a similar oral exposure to
 6223 insectivorous amphibians and that herbivorous reptiles (tortoise) have a greater oral exposure than
 6224 insectivorous reptiles. The estimated oral exposure of snakes from consumption of an oversprayed
 6225 frog is slightly greater than the oral exposure of insectivorous lizards and amphibians and it is slightly
 6226 below the oral exposure of tortoise.

6227 For the estimation of the dietary human exposure to pesticides from the consumption of different
 6228 commodities where pesticides might be present as residues, the regulated concentration of the
 6229 specific active ingredient in/on the different commodities i.e. the Maximum Residue Level (MRL)
 6230 (details see Annex F) at the time of harvest as established from supervised field trials is considered.
 6231 The MRL together with the consumption for each commodity eaten daily, as identified from dietary
 6232 and nutrition surveys, are used for the estimation of dietary exposure for the specific a.i. For

6233 consumer risk assessment, the estimated dietary exposure for the specific a.i. (mg/Kg Body weight
6234 per day) is compared to the respective toxicological threshold, i.e. the Acceptable Daily Intake (ADI)
6235 for chronic exposure and to the Acute Reference Dose (ARfD) in case of a.i. that pose a risk for toxic
6236 effects after short term exposure.

6237 The principle followed for human risk assessment could be used for the dietary risk assessment of
6238 amphibians and reptiles. The background information that should be needed for amphibians and
6239 reptiles is: 1. the food composition, 2. the food consumption, 3. the residue levels in/on the different
6240 items at the time of consumption (different from MRLs since MRLs are set at a specific time point that
6241 meet the preharvest interval) and the respective toxicological threshold for acute and chronic effects.

6242 An oral-exposure, risk-assessment method for amphibians and reptiles (T-herps) was developed by
6243 the US-EPA. For a detailed review please see Appendix G. The model also includes prey items such as
6244 small mammals (rodents) and amphibians. The T-herps model could be used as a risk assessment tool
6245 after adjusting it to model European species (such as the crested newt or *Natrix natrix*) for adapted
6246 FIR.

6247

6248 **Oral exposure via water uptake**

6249 Exposure *via* drinking water depends on the concentration of pesticidal active substances in the
6250 sources of water and the water demand of the animals. The standard risk assessment for birds and
6251 mammals considers two sources

- 6252 1. Puddles in the field
- 6253 2. Water in leaf axils of crops

6254 The exposure assessment is based on 100% uptake of water from these sources. For details on the
6255 risk assessment for birds and mammals from uptake of contaminated drinking water see Annex E.

6256 It is unlikely that amphibians and reptiles would use water sources with higher concentrations of
6257 pesticidal active substances. Therefore, whether the exposure estimates for birds and mammal cover
6258 also amphibians and reptiles depends on the daily water requirements for amphibians and reptiles.

6259 Amphibians take up water *via* their skin and hence this exposure route will be covered by a dermal
6260 exposure assessment. Reptiles take up water orally to satisfy their water demand. A first-tier
6261 calculation for water uptake was conducted for lizards based on the allometric equation in Friday and
6262 Thompson 2009 (see Appendix F for details). The drinking-water demand for a medium sized lizard
6263 (11g) was calculated as 0.049 L/kg bw/d. This is about 10 times and about 5 times lower than the
6264 drinking water demand of 0.46 L/kg bw/d and 0.24 L/kg bw/d for a small granivorous bird (15.3 g)
6265 and small granivorous mammal (21.7g), which are the bases for calculating drinking-water exposure
6266 in EFSA 2009 (Birds and Mammals GD). Therefore it can be concluded that the estimate for drinking-
6267 water uptake for birds and mammals would cover the water uptake of lizards.

6268 If the drinking-water uptake for birds and mammals is refined in the risk assessment, then the
6269 exposure estimate may not cover lizards any longer. Uncertainty remains with regard to other groups
6270 of reptiles such as snakes and tortoise because no information was available for calculating the water
6271 demands. This should be investigated further in order to draw conclusions on the coverage of snakes
6272 and tortoises by the existing exposure estimates for water uptake in birds and mammals.

6273 **Oral exposure from granular formulations and treated seeds**

6274 Ingestion of granules as food, grit or accidental uptake (mistaken as food) and residues of
6275 applications in food items is evaluated for birds and mammals.

6276 If there is a possibility that granules are mistaken as food items, then the same assessment procedure
6277 applies as for contaminated food items (e.g. from overspray). The same conclusions with regard to
6278 coverage of amphibians and reptiles as above for uptake of contaminated food items can be drawn
6279 (i.e. amphibians and reptiles are covered by existing exposure estimates).

6280 There is no evidence that amphibians and reptiles take up grit intentionally. European amphibians and
6281 reptiles do not have muscular gizzards. Therefore this exposure is most likely not relevant.

6282 There may be accidental ingestion of granules when eating food contaminated with soil. It is unknown
6283 how much soil amphibians and reptiles take up when feeding and whether it is different from birds
6284 and mammals. If the amount of soil taken up per food item is similar, then the exposure estimates
6285 from soil uptake of birds and mammals will cover amphibians and reptiles as well since they have
6286 lower food-intake rates and hence feed less.

6287

6288 **Dermal and inhalation exposure**

6289 The risk from dermal and inhalation exposure is not included in the risk assessment for birds and
6290 mammals in Europe. However, dermal and inhalation exposure models for birds were developed by
6291 the US-EPA (Terrestrial Investigation Model, TIM). Dermal and inhalation exposure models also exist
6292 for human risk assessment. A review of the potential use of different exposure models can be found in
6293 Appendix G.

6294 The dermal exposure model from the US-EPA for birds could provide a basis for suggesting an
6295 exposure model for amphibians and reptiles. However, it would be necessary to use amphibian and
6296 reptile specific factors such as dermal absorption fraction (DAF), the surface area of the animal and
6297 foliar contact rate.

6298 For example, for birds it is assumed that only the feet have direct contact to foliage while for
6299 amphibians and reptiles the full surface area could come in contact with foliar residues. It may be
6300 possible to refine this assumption if data from contact surface of the animal with different crop types
6301 become available e.g. the sides of the animal are in contact with cereals and the ventral side is in
6302 contact with crops where animals can climb (e.g. orchards).

6303 A default factor for foliar contact rate is applied in TIM. This factor would need to be adjusted for
6304 amphibians and reptiles. Such a factor could be derived from information on the speed of movement
6305 and surface area of the animal in contact with foliage during movement.

6306 The dermal route equivalency factor is applied to estimated dermal exposures in order to derive an
6307 estimate of the equivalent oral dose. It is not expected that oral toxicity and dermal toxicity data are
6308 available for amphibians and reptiles. This constitutes a problem for adding up the exposures and
6309 comparing them to one endpoint (either dermal or oral LD50). Whether the dermal route equivalency
6310 factor for mammals or birds could be extrapolated to amphibians and reptiles is highly uncertain.
6311 Because of the specific functions of amphibian skin for gas exchange and water regulation it is
6312 expected that amphibians will be more sensitive to dermal exposure than birds or mammals.

6313 The following is needed in order to address dermal exposure from contact to residues in soil and
6314 plants:

- 6315 1. An estimate of the body surface of amphibians, snakes and tortoise in contact with soil and
6316 plants while moving.
- 6317 2. Dermal absorption factors for amphibians and reptiles
- 6318 3. Estimate of the body surface in contact with the soil when they move.
- 6319 4. Speed of movement
- 6320 5. Time when they are actively moving *vs* resting

6321

6322 A possible method to calculate exposure from overspray is included in Appendix F. The calculations
6323 were conducted with worst case assumptions such as direct overspray of animals and 100% dermal
6324 absorption in both groups amphibians and reptiles. The main differences in dermal exposure are
6325 therefore related to differences in the shape of their bodies.

6326 The dermal exposure from overspray is greater for reptiles than for amphibians with equal weights.
6327 Lizards and snakes have similar dermal exposure because of the similarities in their shape and hence
6328 surface area to volume ratio.

6329 For amphibians the dermal exposure from overspray is comparable to the daily dietary dose while for
6330 lizards and snakes the dermal exposure from overspray is about one order of magnitude greater than
6331 the daily dietary exposure from oral uptake.

6332 The dermal exposure from overspray is lower for amphibians compared to the daily dietary dose of
6333 birds and mammals. However, the dermal exposure for reptiles (lizards and snakes) is in the same
6334 range as the daily dietary dose for birds and mammals.

6335

6336 Ventilation rates and oxygen consumption of different reptile groups and birds and mammals were
6337 compared by Bennett (1973). Higher ventilation rates of homeotherms are principally the result of a
6338 greater ventilation frequency in mammals and a greater tidal volume in birds. The inhaled volume of
6339 air per minute is about 3.6 times and 4.9 times greater in birds and mammals compared to reptiles.
6340 Therefore it is expected that the contribution of inhalation exposure to the total exposure is much less
6341 than oral and dermal exposure and it is not considered necessary to assess inhalation exposure by
6342 default. However an inhalation exposure assessment may be needed if a substance is volatile and very
6343 toxic to reptiles. Inhaled volumes in amphibians are likely to be even less than for reptiles as their skin
6344 has an important function for gas exchange. Therefore it is not considered necessary to conduct an
6345 inhalation exposure assessment for amphibians.

6346

6347 The Potential Dermal Exposure equation, used for the 1st tier potential dermal exposure estimation for
6348 workers could be applied for the PDE estimates of amphibians and reptiles. More specifically, the DFR
6349 values $3\mu\text{g active substance/cm}^2$ of foliage/kg a.s. applied/ha could be used as a 1st tier assessment.
6350 Furthermore, the TC could be estimated on the basis of the fraction of the total body area of the
6351 organism(s) and its activity (contact duration with new surfaces per hour) assuming that it is in
6352 continuous contact with the treated crop for a number of hours (T). The time will depend on the
6353 behavior of the animal and it will be estimated from the time spent in the treated crop or in the
6354 contaminated field. Furthermore, for multiple applications the MAF could be considered. If this
6355 approach is applied, the following parameters need to be identified for the most relevant life stage of
6356 the organism:

6357 - Toxicological endpoint (TEP) and the respective threshold (NOAEL and Acceptable level of
6358 dermal exposure).

6359 - The assessment factor for the conversion of the NOAEL to the toxicological threshold,

6360 - If the TEP will be derived from a study carried out *via* the dermal route of exposure, no
6361 dermal absorption factor is needed. In this case the acceptable dermal exposure (Regulatory
6362 threshold for acceptable exposure = $\text{NOAEL}_{\text{dermal}} / \text{assessment factor}$) can be directly
6363 compared to DE. However, if it is derived from oral exposure (Regulatory threshold for
6364 acceptable exposure = $\text{NOAEL}_{\text{oral}} / \text{assessment factor}$), information on both oral and dermal
6365 absorption is necessary [oral absorption for correction of the oral dose in order to get the
6366 systemic threshold and the dermal absorption for the estimation of the systemic dermal
6367 exposure ($\text{SDE} = \text{DE} \times \text{DA}$) from the dermal exposure (DE)]

6368 - Body surface area in contact with the foliage,

6369 - Behaviour of the animal and time spent in the treated field.

6370

6371

6372

6373 10.4. Conclusions on the coverage by the current risk assessment

6374 10.4.1. Overall conclusions for aquatic life stages by the current risk 6375 assessments in the aquatic risk assessment

6376 Apart from accurately predicting the toxicity and exposure, assessment factors need to be defined in
6377 order to evaluate the risks to amphibians and reptiles from pesticides. The assessment factors should
6378 cover the uncertainties in the risk assessment, which are described in section 7.11.

6379 Based on the above we conclude the following for amphibians and reptiles in their aquatic
6380 environment:

6381

6382 • Endpoints derived in acute toxicity testing with fish are considered sufficiently accurate to
6383 predict the acute toxicity for aquatic life stages of amphibians if an extrapolation factor will be
6384 included to achieve a higher proportion (>70%) of cases covered by the acute endpoint for
6385 fish. The magnitude of an additional assessment factor should be calculated after agreement
6386 on the proportion of amphibian response data to be covered by the fish acute endpoints.
6387 Based on the current data with its limitations regarding number of active substances and
6388 amphibian species tested, an extrapolation factor of at least 100 seems justified. If endpoints
6389 from other surrogate species (aquatic invertebrates or aquatic plants) are considered, a
6390 higher coverage of the sensitivity is achieved. However, this is only applicable for the first-tier
6391 risk assessment.

6392 • In order to assess the chronic toxicity to aquatic life stages of amphibians, an extended AMA
6393 or LAGDA test should be conducted in order to address effects on metamorphosis and
6394 reproduction, which are not adequately addressed by the chronic studies with fish. Sensitive
6395 and relevant endpoints to assess populations in the field considering habits and behaviour of
6396 the biphasic species after multiple exposures need to be defined

6397 • With regards to the exposure assessment by FOCUS step 3, the working group is unable to
6398 predict whether the acute exposure will be conservative for amphibians in their aquatic
6399 environment. Amphibian ponds are smaller in size and shallower than FOCUS ponds, so
6400 concentrations in FOCUS ponds are expected to be non-conservative for amphibian ponds. For
6401 FOCUS streams and ditches, it is not possible to predict *a priori* whether their acute exposure
6402 concentrations will be conservative or not but, due to their high flow-through rate compared
6403 to the one in FOCUS ponds, their chronic exposure concentrations are expected to be non-
6404 conservative.

6405 • The sediment-exposure assessment by the step 3 FOCUS surface-water scenarios is expected
6406 not to represent realistic worst-case exposure situations. The FOCUS surface water step 3
6407 scenarios were designed to represent realistic worst-case exposure situations for the PECs in
6408 water. Due to the partition between water and sediment, high concentrations in the water
6409 layer are associated to low concentrations in the sediment. Moreover, for slowly degrading
6410 and/or sorbing pesticides, accumulation of pesticides over the years is important and the
6411 FOCUS scenarios cannot account for this as they simulate only one year.

6412 • The suitability of the current assessment factors used in the first tier to cover the
6413 uncertainties for amphibians and reptiles need to be evaluated.

6414

6415 10.4.2. Overall conclusions with regard to coverage of amphibians and reptiles by 6416 existing risk assessments for birds, mammals and humans

6417

6418 • No general conclusion can be drawn on whether the acute oral toxicity of pesticidally active
6419 substances is covered by studies with birds and mammals because data sources are scarce. The
6420 main issues that need to be addressed are potential differences in species sensitivity and
6421 relevance of coverage of the different modes of action of pesticidally active substances in

- 6422 respect of the different susceptibility between species. Even greater uncertainty exists regarding
6423 potential coverage of long-term toxicity. For example, detrimental effects on amphibian
6424 metamorphosis are not covered. It might be possible to use effects observed in bird-
6425 reproduction studies (with some modifications of the test protocol e.g. adding histopathological
6426 observations) in the risk assessment for reptiles.
- 6427 • Amphibians and reptiles are poikilothermic and therefore have lower metabolic rates compared
6428 to birds and mammals. Ambient temperature is an important factor modifying toxicity in
6429 poikilothermic animals. The specific mode of action of a pesticidally active substance and the
6430 interplay between metabolic rates and temperature need to be taken into account when using
6431 bird and mammal toxicity data as surrogates.
 - 6432 • Coverage of endocrine effects in amphibians and reptiles by available studies with birds and
6433 mammals is uncertain because sensitive life stages, critical routes of exposure and critical
6434 windows of development may be different. Specific test protocols exist for amphibians but are
6435 lacking for reptiles. It needs to be investigated further if endocrine effects on reptiles are
6436 covered by mammals and birds.
 - 6437 • Dermal toxicity and adsorption data from mammals may be used as surrogates for reptiles but
6438 not for amphibians. Amphibian skin has important functions such as respiration, water
6439 regulation and immune function. The structure of amphibian skin with its mucus layer is very
6440 different from mammalian skin and therefore it is not possible to extrapolate to effects on
6441 amphibians. A test to investigate local effects on amphibian skin is needed. Systemic effects
6442 from dermal exposure also need to be addressed. Ideally such tests should be *in vitro* studies to
6443 avoid animal testing. The WG recommends developing *in vitro* tests with amphibian skin for
6444 inclusion in future risk-assessment schemes.
 - 6445 • The oral exposure estimates from the screening steps in the risk assessment for birds and
6446 mammals may cover the risk to amphibians (depending on the toxicological sensitivity and
6447 assessment factors that are applied).
 - 6448 • The dermal exposure estimates for lizards and snakes are in the same range as the daily dietary
6449 exposure estimates for birds and mammals. The risk from dermal exposure is not assessed for
6450 birds and mammals. Therefore coverage of reptiles by the risk assessment for birds and
6451 mammals is highly uncertain.
 - 6452 • The comparisons of the daily dietary exposure and dermal exposure from overspray give an
6453 indication that both exposure pathways are of high importance and both need to be considered
6454 in the risk assessment for amphibians and reptiles.
 - 6455 • Dermal toxicity endpoints need to be refined for amphibians and reptiles. Specific attention
6456 should be paid to dermal overspray and its direct and indirect effects in amphibians (impact of
6457 exposure on the mucus layer and consequence of the health status of the individual).
 - 6458 • Coverage of Amphibians and reptiles by human risk assessment could be considered as a 1st
6459 step, with appropriate exposure factors (body surface area in contact with foliage, behaviour
6460 and time spent in the treated field). Overall, coverage by human risk assessment may be highly
6461 uncertain because of lack of an appropriate toxicity endpoint.

6462

6463

6464 11. Conclusions

- 6465 • Overall, the Panel concludes that several species of amphibians and reptiles occur in agricultural
6466 landscapes where they are exposed to Plant Protection Products (PPP), and this exposure may
6467 have unacceptable consequences on individuals and populations. Therefore, a specific
6468 environmental risk-assessment scheme is needed for both amphibians and reptiles.

6469 **General risk-assessment considerations related to the biology of amphibians and reptiles.**

- 6470 • Although traditionally studied together under the discipline of herpetology, amphibians and
6471 reptiles present important differences in many of their biological and ecological features. What

6472 differentiates them from birds or mammals is that they are poikilothermic. Sensitivity and
6473 exposure to pesticides, affected by poikilothermy through its influence on physiology, growth,
6474 development, behaviour or reproduction may be shared, but other factors e.g. skin permeability in
6475 amphibians, may also have a large influence on risks associated with PPPs.

6476 • The presence of amphibians and reptiles in agricultural areas, both in-field and on the edge of the
6477 field, is well documented. Potential for overspray, dermal exposure by contact with soils or plants
6478 during or following PPP applications, and oral uptake of pesticides through ingestion of
6479 contaminated materials exist for both groups. Exposure of amphibians and reptiles when
6480 inhabiting a treated area can be prolonged, especially in the case of the most territorial reptile
6481 species or of the amphibian aquatic life stages.

6482 • The potential of surrogate-based risk assessment to cover toxicity of pesticides on amphibians and
6483 reptiles by other vertebrate groups is compromised by some particular biological processes typical
6484 of these animals, including metamorphosis in amphibians or hormonal-dependent sex
6485 determination in both amphibians and reptiles. Also, the peculiarity of the amphibian life cycle
6486 compared to other vertebrate groups has a major influence on potential exposure scenarios,
6487 which is difficult to predict from data generated from other taxa. When compared to fish,
6488 amphibians possess some structures typical of higher vertebrates that do not occur in fish (e.g.
6489 the Müllerian ducts that are precursors of sexual organs). Impacts of pesticides on these
6490 structures cannot be identified through fish-based evaluations and require assessment at specific,
6491 sensitive time windows within the amphibian aquatic development.

6492 • Amphibians and reptiles are two very diverse groups. This diversity has been considered in the
6493 definition of functional groups for assessment, based not only on taxonomic differences but also in
6494 large ecological differences within the same main taxonomic group. From the identified groups,
6495 potential focal species are proposed on the basis of traits related to pesticide exposure and
6496 potential to exert toxicity.

6497 • For individual amphibians and reptiles, exposure to PPPs can take place differentially in space and
6498 time, depending upon the behaviour of the animals coincident with PPP availability in the
6499 environment. Therefore, realistic risk assessments should take spatial behaviour within a season
6500 into account. This is particularly important for migrating amphibians.

6501 • Population structure and spatio-temporal dynamics can have important implications for the
6502 evaluation of impacts of PPP on amphibian and reptile populations. Therefore, for inclusion of
6503 both spatial and temporal implications of PPP usage, and to take the ecological state of the
6504 population before application of PPPs into account, a systems approach is recommended (EFSA
6505 SCER, 2016a).

6506 • Spatially explicit individual-based modelling at landscape scales is an important part of the ERA
6507 toolbox for amphibians and reptiles. It should be used to help set the tolerable magnitude of
6508 effects for Specific Protection Goals (SPG), to translate toxicity data to population modelling
6509 endpoints, and as a higher-tier assessment tool.

6510 • Precise context for application of the models requires careful consideration. The regulatory
6511 scenarios need to consider all factors; in particular, landscape structure and weather have a large
6512 impact on the outcome of the long-term risk assessment.

6513

6514 **Conclusions on protection goals and general risk assessment framework**

6515 • The Panel proposes SPG options to be considered in the risk assessment of amphibians and
6516 reptiles exposed to PPPs. These SPG options were derived based on (i) the legislative
6517 requirements in place for non-target vertebrates, (ii) the need to encompass the endangered
6518 status of a great proportion of amphibian and reptile species, and (iii) the importance of
6519 amphibians and reptiles as drivers of valuable ecosystem services in agricultural landscapes.

6520 • The key drivers (or service providing units, SPUs) identified among amphibians are Anura (frogs
6521 and toads) and Caudata (newts and salamanders); for reptiles, Sauria (lizards, skinks and
6522 geckos), Ophidia (snakes) and - within the Testudines - terrestrial and freshwater turtles.

- 6523 • Amphibians and reptiles are key drivers of the following ecosystem services: provision of genetic
6524 resources and biodiversity, maintenance of cultural services, provision of food and pharmaceutical
6525 resources, support of nutrient cycling and soil structure formation, regulation of pest and disease
6526 outbreak and invasion resistance, and the support of food webs in agricultural landscapes.
- 6527 • The Panel does not consider it appropriate to define SPG options on the basis of functional groups
6528 of amphibians and reptiles. In order to protect these significantly endangered vertebrate groups,
6529 the ecological entities to be protected are the individuals and populations of species.
- 6530 • It is proposed to set SPG options on the individual level for the survival of adult amphibians and
6531 reptiles. In addition, the long term persistence of populations should be considered. Attributes of
6532 population persistence relate to the assessment of abundance/biomass of amphibian and reptile
6533 species and also to the landscape occupancy of these species, and to changes in population
6534 growth rates. Giving the mobility of most amphibian and reptile species, no separate SPG options
6535 are proposed for in-field and off-field areas.
- 6536 • The need to assess the risk for these non-target vertebrate species when exposed to PPPs faces a
6537 paucity of standardized testing methodologies and comprehensive data sets on their toxicological
6538 sensitivity to (active substances in) PPPs. Therefore, the Panel proposes a risk-assessment
6539 scheme that will need, in the short- and mid-term, the provision of ecotoxicological endpoints
6540 regarding the effects of (active substances in) PPPs on amphibian and reptile species. The Panel
6541 proposes to require ecotoxicological data for a limited period of time and to review the evidence
6542 after some time in order to decide on possible waiving of tests in future.
- 6543 • Data are needed on the chronic toxicity of PPPs for amphibians, starting from the exposure in the
6544 aquatic stages up to and including the adult stages. Data are also needed on the effects of PPP on
6545 amphibian terrestrial stages *via* the dermal route of exposure (overspray, contact with plants and
6546 soil).
- 6547 • Toxicological endpoints related to certain aspects of amphibian biology, like metamorphosis or
6548 hormone-dependent sex determination, cannot be predicted from information generated from
6549 surrogate taxa. A specific approach to investigate chronic toxicity leading to effects on these
6550 aspects is required.
- 6551 • Variability in sensitivity throughout the life cycle is also translated in the existence of key moments
6552 at which certain effects are more likely to happen. This must be considered when short-term
6553 toxicity is assessed. For instance, maturation of sexual organs has a tightly defined time window,
6554 and testing reproductive toxicity of pesticides outside this window could lead to wrong
6555 assumptions about lack of effects.
- 6556 • Given the scarcity of data, it is not possible to conclude for the time being whether toxicity data or
6557 existing risk-assessment schemes with other non-target vertebrates or other organism groups
6558 cover the acute and/or chronic risk of intended PPP uses to reptiles. Given the scarcity of
6559 information, it is also not possible for the time being to request specific tests to close these data
6560 gap.
- 6561 • The Panel proposes to follow a tiered approach for the risk assessment of amphibians and reptiles
6562 comprising an evaluation of effects at local and long-term effects at the landscape scale.
- 6563 • At local scale, a risk assessment is required for all relevant environmental compartments in which
6564 different life-stages live. For amphibians, this means that an evaluation of risk in the aquatic and
6565 in the terrestrial compartment is needed. This is not the case for reptiles.
- 6566 • Given the importance of different exposure routes, it will be necessary to assess the impact of
6567 PPPs on amphibians and reptiles resulting from a combination of the main exposure routes. In a
6568 first assessment step, it is suggested to address the outcome of exposure to PPPs through several
6569 routes by assessing the combined risks of the main routes.
- 6570 • After an assessment of effects at local scale, the risks of intended uses of PPP have to be
6571 assessed at landscape scale. This should be performed in a first step using pre-run population
6572 models that address the long-term repercussions of year-on-year PPP use on amphibians and
6573 reptiles.

- 6574 • It will be necessary to satisfy the risk-assessment criteria at both local and landscape scales in
6575 order to conclude that SPGs set for amphibians or reptiles are met.

6576

6577 **Conclusions on exposure considerations**

- 6578 • The use of Exposure Assessment Goals defining the spatial unit with its Ecotoxicologically
6579 Relevant Exposure Quantities (EREQs) and exposure routes allows for an explicit and systematic
6580 methodology to calculate Predicted Exposure Concentrations (PECs) in the field, as well for
6581 amphibians in their aquatic and terrestrial environment as for reptiles.

- 6582 • For amphibians and reptiles, no standard ecotoxicological experiments are required in the current
6583 pesticide-registration procedure. Therefore, it is not yet possible to make a final choice on the
6584 EREQs that enable a coherent linking between exposure in the field and the endpoints of
6585 ecotoxicological experiments. This implies that all EREQs in this opinion are proposals, which may
6586 need to be changed later on.

- 6587 • For amphibians in the aquatic environment, exposure *via* dermal contact with pond water is
6588 judged to be more important than exposure *via* food intake. The main entry routes for pesticides
6589 into ponds in agricultural areas are spray-drift deposition, run-off or drainage. Sediment may
6590 accumulate pesticide residues and in such cases exposure of tadpoles by uptake of sediment may
6591 be an important route. In their terrestrial environment, dermal exposure is an important route for
6592 amphibians, especially by contact, e.g. with recently deposited pesticides on soil or plants, or in
6593 puddles within agricultural field. Overspray or spray-drift deposition might occur. Intake of
6594 residues *via* food is another potential exposure route.

- 6595 • For reptiles, main exposure routes are *via* food intake, contact with residues in soil and plants and
6596 contact of eggs with contaminated soil. As many reptile species have a high site fidelity, dermal
6597 uptake may be important for reptiles living in and near agricultural fields, although their skin is
6598 less permeable than the skin of amphibians.

- 6599 • The analysis of the dimensions of the Spanish and Swiss amphibian ponds and the ponds in the
6600 UK demonstrated that most of them (70-90%) are considerably smaller than the FOCUS ponds,
6601 used at present in the EU registration procedure. Therefore, it is expected that the FOCUS ponds
6602 do not deliver conservative exposure estimates for amphibian ponds. For peak concentrations in
6603 FOCUS ditches and streams, the Working Group was unable to make a general statement on
6604 conservativeness, but for chronic exposure FOCUS ditches and streams are expected to be non-
6605 conservative, due to their relatively rapid flow-through rates.

- 6606 • The step 3 FOCUS surface-water scenarios are expected to result in non-conservative exposure
6607 estimates for the sediment, as on the one hand they were designed to represent realistic worst-
6608 case exposure situations for the PECs in water (generally associated with low sediment
6609 concentrations due to the partition between water and sediment) and on the other hand they do
6610 not account for multi-year accumulation in the sediment due to their simulations lasting only one
6611 year.

6612

6613

6614 **Conclusions on oral, dermal and inhalation exposure for different groups of amphibians** 6615 **and reptiles**

- 6616 • The dermal exposure levels from overspray (assuming that half of the body surface receives the
6617 full application rate and 100% dermal uptake) is estimated to be greater for reptiles than for
6618 amphibians with equal weights and assuming the same skin permeability (which is unlikely).
6619 Lizards and snakes are expected to receive similar levels of dermal exposure under the same
6620 exposure scenario (because of the similarities in their shape and hence surface area to volume
6621 ratio).

- 6622 • For amphibians in the terrestrial environment, the dermal exposure from overspray is comparable
6623 to the daily dietary exposure.

- 6624 • For lizards, the dermal exposure from overspray is about one order of magnitude greater than the
6625 daily dietary exposure – given the same assumption as above on dermal uptake.
- 6626 • The dermal exposure from overspray is lower for amphibians compared to the daily dietary
6627 exposure of birds and mammals. However, the exposure level for reptiles is in the same range as
6628 the daily dietary exposure for birds and mammals.
- 6629
- 6630 **Preliminary conclusions with regard to potential coverage of amphibians and reptiles in**
6631 **current risk assessment schemes for aquatic organisms, birds, mammals and human risk**
6632 **assessment.**
- 6633 • Available studies with fish assess the acute mortality and long term effects on appearance, size,
6634 behaviour and reproduction. Several studies were found in the literature indicating that the acute
6635 endpoints for amphibians (tadpoles) are lower than the acute endpoint for fish (mainly rainbow
6636 trout). This was the case for 30% of the assessed data points. Therefore, if a higher percentage
6637 of all amphibian mortality data points should be covered, an extrapolation factor needs to be
6638 applied on the acute fish endpoint to deliver an appropriate toxicity endpoint for the acute risk
6639 assessment of amphibians. Based on the current data set an extrapolation factor of at least 100 is
6640 suggested.
- 6641 • Two review studies were found comparing chronic endpoints for fish and amphibians. However,
6642 one study showed shortcomings in the methodologies as neither duration of study nor type of
6643 effects nor specification of concentration were comparable. In the other study, amphibians were
6644 more sensitive than fish in around 50% of the cases. Therefore, no conclusion can be drawn for
6645 the coverage of the chronic sensitivity by fish for amphibian species. Furthermore, the chronic fish
6646 studies do not adequately address relevant sublethal endpoints considered relevant for
6647 amphibians, such as effects on metamorphosis, reproduction or immunosuppression. No data
6648 (thus no comparison in toxicity) were found for reptiles in aquatic environments.
- 6649 • Shortcomings of the current toxicity comparisons are that i) for a rather limited number of
6650 substances and life stages only the endpoints and not the slopes of the dose-response curve were
6651 compared, ii) the majority of amphibian studies were conducted with *Xenopus laevis*, for which
6652 limited information is available with regard to its representativeness for European species, and iii)
6653 the variability between amphibian species is unknown, leaving it open which assessment factor
6654 adequately addresses the uncertainties stemming from the effect assessment.
- 6655 • In the aquatic system, amphibians as well as certain reptiles (water dwelling snakes and
6656 terrapins) may be exposed dermally to water and sediment or orally to feed and drinking water.
6657 Furthermore eggs may be exposed by maternal transfer. The studies with fish adequately address
6658 the dermal exposure *via* water and the study with *Lumbriculus* *via* sediment. Therefore, further
6659 data about these habitats in the member states needs to be gathered. There is currently a lack of
6660 chemical monitoring data of aquatic amphibian habitats (especially small and standing water
6661 bodies) in Europe.
- 6662 • The oral exposure estimates from the screening steps in the risk assessment for birds and
6663 mammals may cover the exposure estimate for oral uptake of PPP residues to amphibians.
- 6664 • The dermal exposure estimates for lizards and snakes are in the same range as the daily dietary
6665 exposure estimates for birds and mammals. The risk from dermal exposure is not assessed for
6666 birds and mammals. Therefore coverage of reptiles by the risk assessment for birds and mammals
6667 is highly uncertain.
- 6668 • The comparisons of the daily dietary exposure and dermal exposure from overspray give an
6669 indication that both exposure pathways are of high importance.
- 6670 • Whether the risk to amphibians and reptiles *via* oral uptake of PPP residues with food is covered
6671 by the risk assessment of birds and mammals depends on the differences in toxicological
6672 sensitivity and assessment factors applied.

- 6673 • Differences in sensitivity among life stages, especially within amphibians, because of the
6674 morphological and physiological differences among them, should be considered when determining
6675 the toxicity of pesticides.
- 6676 • Some standard tests are available for amphibians. These are the Larval Amphibian Growth and
6677 Developmental Assay (LAGDA), the Amphibian Metamorphosis Assay (AMA), and the Frog Embryo
6678 Teratogenesis Assay – *Xenopus* (FETAX). These tests do not cover the reproductive phase of
6679 amphibians. A full life-cycle test with amphibians (e.g. with *Xenopus tropicalis*) could be very
6680 useful in a risk-assessment context because it enables the observation of reproductive effects.
- 6681 • No standard test guidelines exist for reptiles. In addition, very few data obtained from non-
6682 standard tests are available. This lack of data makes it very difficult to compare the toxicological
6683 sensitivity among different reptile species and other groups of vertebrates and hence impossible
6684 to propose endpoints from surrogate species (e.g. birds) and assessment factors.
- 6685 • Sources of uncertainties in the current risk assessment have been identified, which need to be
6686 quantified for the calibration of a risk-assessment scheme for amphibians and reptiles.
6687 Uncertainties were identified in the effect as well as exposure assessment.

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6690 12. Recommendations

- 6691 – The choice of potential focal species that can be also suitable to develop population models to
6692 support specific protection goals must be based on traits leading to potential exposure and
6693 sensitivity to pesticides.
- 6694 – Landscape-scale spatially-explicit mechanistic models for the six species identified as potential
6695 focal species for the assessment of amphibians and reptiles need to be developed and tested.
6696 These should include:
- 6697 Mechanistic modelling of dispersal, reproduction and mortality factors for all life-stages;
6698 The potential to introduce a wide range of impacts of PPPs in terms of modes of action, exposure and
6699 regulatory scenarios;
6700 Spatial and temporal representation of resource distributions;
6701 Realistic pesticide-exposure modules including realistic or agreed methods to implement pesticide
6702 concentrations in ponds;
6703 Exposure routes linking environmental concentration to the body-burden of pesticide need to be
6704 defined and included, as well as inclusion of a suitable representation of multiple exposure events for
6705 an individual. Development of TK/TD models for amphibians and reptiles could help to address this
6706 issue.
- 6707 – Population modelling endpoints should include the abundance of the animals, their distribution
6708 and relative change in population growth rate as a result of application of the PPP. The latter
6709 takes into account long-term impacts which can be difficult or impossible to see using other
6710 approaches.
- 6711 – The threshold limits of changes to population level endpoints that correspond to unacceptable
6712 impacts on the SPG need to be identified. This should not be done on an individual end-point
6713 basis but combining abundance, occupancy and changes in growth rate.
- 6714 – There is a need to develop suitable refinement for the combination of exposure routes. If a risk is
6715 identified by a lower tier assessment by the addition of exposure routes then a potential
6716 refinement might be possible if it can be shown that different routes are not additive. However,
6717 this would need to be supported by experimental data.
- 6718 – The Panel recommends the review of available data on amphibians and reptiles after an
6719 appropriate time frame in order to decide whether initial triggers flagging high/low risk for (active
6720 substance in) PPP can be set. The aim is to identify (active substances in) PPP for which

- 6721 requirements on toxicity data on amphibian may be waived. Owing to scarcity of data, this is not
6722 possible for the time being.
- 6723 – For chronic risk assessment of the aquatic stages of amphibians, the current exposure assessment
6724 *via* FOCUS step3 scenarios may not be protective. For the acute risk assessment this needs to be
6725 investigated further.
- 6726 – The FOCUS surface-water scenarios (step 3) were not designed to give a protective exposure in
6727 sediment and hence are not recommended to be used in the risk assessment for sediment
6728 exposure of amphibians.
- 6729 – Chemical monitoring should be encouraged especially in small standing surface waters.
- 6730 – As dermal exposure is a main exposure route for amphibians in their terrestrial environment, the
6731 Panel recommends carrying out experiments that allow for the quantification of the substance
6732 amount taken up by the animals including the identification of differences in skin permeability of
6733 the different body parts of the animal.
- 6734 – Acute toxicity tests with fish are considered sufficiently accurate to predict the acute toxicity for
6735 aquatic life stages of amphibians if an extrapolation factor will be included to achieve a higher
6736 proportion (>70%) of cases covered by the acute endpoint for fish. The magnitude of an
6737 additional assessment factor should be calculated after agreement on the proportion of amphibian
6738 response data to be covered by the fish acute endpoints. Based on the current data with
6739 limitations regarding number of active substances and amphibian species tested, an extrapolation
6740 factor of at least 100 seems justified. If endpoints from other surrogate species (aquatic
6741 invertebrates or aquatic plants) are considered, a higher coverage of the sensitivity is achieved.
6742 However, this is only applicable for the first-tier risk assessment.
- 6743 – To assess the chronic toxicity to aquatic life stages of amphibians, an extended AMA or LAGDA
6744 test should be conducted in order to address effects on metamorphosis and reproduction, which
6745 are not adequately addressed by the chronic studies with fish.
- 6746 – Sensitive and relevant endpoints to assess populations in the field considering habits and
6747 behaviour of the biphasic species after multiple exposures need to be defined.
- 6748 – Sources of uncertainties in the current risk assessment, which have been identified, need to be
6749 addressed to calibrate a risk-assessment scheme for amphibians and reptiles.
- 6750 – Oral and dermal exposure need to be considered in the risk assessment for terrestrial life stages
6751 of amphibians and reptiles. Inhalation exposure seems to be less relevant and hence development
6752 of inhalation-exposure models has lower priority. If further information becomes available
6753 indicating that inhalation exposure is a relevant route of exposure, then it would be an option to
6754 adapt either the human inhalation-exposure approach or the US EPA TIM model for birds to
6755 estimate inhalation exposure for amphibians and reptiles.
- 6756 – Currently, oral exposure in the bird and mammal guidance to assess the risk by intake of
6757 contaminated food relies on focal species and considers herbivores, insectivores and granivores.
6758 The food intake rate of amphibians and reptiles is generally lower. Many amphibian and reptile
6759 species are predators and it is recommended to adjust the models to the food intake rate of the
6760 selected species and include other prey items in the model, such as small mammals like rodents.
- 6761 – The US-EPA T-herps model could be used in a first-tier assessment to address the risk from oral
6762 exposure of amphibians and reptiles, and it is recommended to adjust the model to the food
6763 intake rate of European species. However, appropriate data to estimate the toxicological
6764 sensitivity of amphibians and reptiles are lacking and are needed to calculate the risk quotient.
- 6765 – Dermal exposure from overspray could be evaluated on the basis of the total surface of an animal
6766 divided by two and the applied rate. Allometric equations for body weight and body surface and
6767 example calculations are included in Appendix F.
- 6768 – The exposure model for workers or alternatively the dermal exposure models for birds from US-
6769 EPA TIM could be used to estimate the dermal exposure of amphibians and reptiles from contact
6770 to residues on plants or soil.

6771 – The equations from the US-EPA TIM model would need to be adjusted with amphibian and reptile
6772 specific factors such as dermal absorption fraction (DAF), the surface area of the animal, foliar
6773 contact rate.

6774 – In all models, the NOAEL and the assessment factor for the conversion of NOAEL to Toxicological
6775 Endpoint needs to be addressed. If this endpoint is not derived from a dermal exposure study,
6776 information on both oral and dermal absorption is necessary to estimate the systemic exposure.

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6778 **Recommendations for future research:**

6779 – Amphibian and reptile specific dermal absorption factors are needed to refine dermal exposure
6780 calculations when the animals are in contact with soil and plants. For the time being 100% uptake
6781 of the substances is suggested. It may be possible to refine this value once data on dermal
6782 absorption become available for different active substances.

6783 – Estimates of the body surface in contact with the soil and in contact with plant surfaces when
6784 amphibians and reptiles move and the speed of movement and time when they are actively
6785 moving *versus* resting are needed. This information is a prerequisite to calculate dermal exposure
6786 from contact with soil and plants when amphibians and reptiles move in the treated field or in the
6787 field margins with existing equations (human risk assessment and US-EPA TIM).

6788 – No standard tests exist for reptiles, which compromises the availability of toxicological information
6789 for these animals. Developing standard methods for toxicity testing in reptiles is a necessity
6790 beyond risk-assessment requirements. This is a necessary stage in order to have possibilities in
6791 the future to explore extrapolation from surrogate species.

6792 – An estimate of the body surface area for tortoise is needed in order to calculate dermal exposure
6793 from overspray.

6794 – We recommend collection of data on the presence, distribution, dimensions and hydrological
6795 behaviour of waterbodies hosting amphibians, e.g. by using GIS information coupled to field
6796 observations on amphibians. This could be achieved by setting up and supporting specific
6797 projects, or by nation-wide groups of volunteers gathering the relevant data, comparable to what
6798 was done in Switzerland and Spain, respectively. The panel further recommends combining these
6799 surveys with chemical monitoring, to evaluate the extent of exposure of amphibian populations in
6800 the field. Small surface waters are not routinely monitored and thus the chemical monitoring
6801 should be extended. As dermal exposure is a main exposure route for amphibians and reptiles in
6802 their terrestrial environment, the Working Group recommends performing experiments to analyse
6803 the ecotoxicological effects as well as the compound mass taken up by the animals. This should
6804 be done in close cooperation between exposure experts and ecotoxicological effect experts to
6805 enable a coherent linking in the regulatory risk assessment between the exposure in the field and
6806 the exposure in the experiments that gave the best correlation with the observed effects.

6807 – In-vitro test methods for measuring effects on amphibian skin and skin permeability should be
6808 developed. A research project which generates enough results with in-vitro/in vivo outcomes could
6809 help to replace in-vivo testing. Furthermore, the results of such a research programme could also
6810 be used to set up and test TK/TD models for amphibians.

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6813 **13. Glossary and/or abbreviations**

AF Assessment Factor

DT₅₀ Half-life in a medium due to degradation (transformation) and other processes (such
as volatilisation and leaching)

ECx	Concentration at which x % effect was observed/calculated
EFSA	European Food Safety Authority
ERA	Environmental Risk Assessment
ERC	Ecotoxicologically Relevant Concentration, the exposure concentration (e.g peak, time weighted average over 3 days) that gives the best correlation to the observed effect in an ecotoxicological experiment
EREQ	Ecotoxicologically Relevant Exposure Quantity, the exposure quantity (e.g peak concentration, application rate, daily mass taken in by an individual bee) that gives the best correlation to the observed effect in an ecotoxicological experiment
ES	Ecosystem service
ETP	Ecological Threshold Principle
EU	European Union
FOCUS	FORum for Co-ordination of pesticide fate models and their Use
Formulation	Synonymous for PPP, the product containing ingredients in addition to the pesticidal active substance, formulations differ depending on the types of uses
MMT	Colorimetric assay based on cell metabolic activity reflecting the number of viable cells
NOEC	No observed Effect Concentration
PEC	Predicted Environmental Concentration
PPPs	Plant Protection Products
PPR Panel	EFSA's Scientific Panel on Plant Protection Products and their Residues
SPGs	Specific Protection Goals, an explicit expression of the environmental component that needs protection, the maximum impacts that is predicted or can be tolerated, where and over what time period. In this document, the concept of SPG is consistent with (effect) 'assessment endpoint'
SPU	Service Providing Unit, structural and functional components of ecosystems, including biodiversity, necessary to deliver a given ecosystem service at the level required by service beneficiaries. SPUs refer to functional/taxonomic groups or landscape elements/habitats requiring protection
SSD	Species Sensitivity Distribution
TER	Toxicity exposure ratio (i.e. NOEC/PEC or EC ₁₀ / PEC)

TK/TD Toxicodynamics/toxicokinetic

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6817 **14. References**

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DRAFT

8025 Annex A – **The population-dynamics context to defining SPGs in**
 8026 **Environmental Risk Assessment**

8027

8028 Whatever specific protection goals (SPGs) are defined for amphibians and reptiles, the main features
 8029 of interest will fall into the following categories:

8030 *Distribution* – where do they occur?

8031 *Abundance* – how many are there (in the places where they occur)?

8032 *Condition* – are the individuals in a population in good health or in poor health?

8033 When defining SPGs, it is important to consider the dynamics of populations in nature such as
 8034 changes in abundance and distribution over time. Distribution and abundance are rarely, if ever,
 8035 static. How, then, may SPGs involving distribution and abundance be looked at more dynamically and
 8036 realistically?

8037

8038 **Processes that determine population-level outcomes: the simplest concepts**

8039

8040 **Table 43:** We begin by defining the symbols used in the basic equations:

8041

Symbol	Definition
N_t	Total population size (i.e. numbers of animals in a defined area) at some time t .
ΔN	Change in population size during some time interval (e.g. between time t and time $t+1$)
B	Number of births during some time interval (e.g. between time t and time $t+1$)
D	Number of deaths during some time interval (e.g. t to $t+1$)
I	Number of animals migrating into a population during some time interval (e.g. t to $t+1$)
E	Number of animals migrating out of a population during some time interval (e.g. t to $t+1$)
PGR	Population Growth Rate (or Ratio) per unit time = N_{t+1}/N_t

8042

8043

8044 The *processes* and *parameters* that determine distribution and abundance are:

8045 Fundamental equations of population change

8046
$$N_{t+1} = N_t + \Delta N$$

8047
$$\Delta N = B - D + I - E$$

8048 The basic quantities as defined in the Table 43: are

8049 Numbers of births (B) and deaths (D)

8050 Immigration (I) and emigration (E) numbers (which could be converted into migration rates)

8051 Population Growth Rate, $PGR = N_{t+1}/N_t$

8052

8053 **Population limitation**

8054 Population growth may be limited by:

8055 Feedback processes that lead to an increase or a decrease of vital rates in response to changes in
8056 population size or density

8057 Environmental stochasticity, leading to changes in resources directly or indirectly affecting vital rates

8058 **In order to maintain a specified population (and the associated SPGs) over time, it is**
8059 ***necessary but not sufficient* that $PGR \geq 1$ over a defined period of time for the whole of**
8060 **that specified population.**

8061 PGR may vary over the range of the specified population. It is possible that some patches of
8062 environment have few or no members of the species of interest and that the SPGs are not achieved in
8063 those patches, even though overall $PGR \geq 1$. This is why $PGR \geq 1$ may not be a *sufficient* condition to
8064 achieve some SPGs, but it is a *necessary* condition if SPGs are to be achieved over the longer term.

8065

8066 **Extending the basic theory to age- or stage-structured populations**

8067 In practice, we are interested in the numbers of different age groups or stages and in the differential
8068 effects on them of potential stressors such as PPPs. Different stages will almost always differ both in
8069 their exposure profiles and in the ecotoxicological effects of PPPs. How, then, can we make
8070 predictions about the effects of PPPs on Population Growth Rate? One approach is to use simple
8071 theory to make broad generalisations, recognising that such generalisations may not apply to every
8072 case in the real world and being careful not to make misleading generalisations. Another approach is
8073 to build a specific model using all the data available for a particular species in a particular
8074 environment, recognising that such a model (a systems model) may lack generality (as described in
8075 chapter 4).

8076 Roughgarden et al. (1996) introduced the distinction between minimal models for ideas and
8077 minimal/synthetic models for a system. Models for ideas are developed for exploring general concepts
8078 across systems, such as density dependence, competitive exclusion, competition/dispersal trade-offs,
8079 and stabilizing mechanisms. They have to be as simple as possible, but are not designed for making
8080 specific, testable predictions. In contrast, models for a system are more tailored to specific systems or
8081 classes of systems. Here, the intended potential for making testable predictions is an important
8082 modelling design criterion (Topping et al 2015a). We argue that both approaches have value and that,
8083 where possible, both should be used.

8084 An example of a question that might be posed is as follows:

8085 *"If a PPP has a detrimental effect on reproductive adults, will this have a greater effect in reducing*
8086 *population growth rate than if it had the same effect on juveniles?"*

8087 This question will be looked at later using a very simple model.

8088

8089 *Simple life-history model*

8090 Adults (females) produce **f** offspring (female) each per year.

8091 A proportion **s(a)** of adults survives from one year to the next.

8092 A proportion **s(j)** of juveniles survives to become reproductive adults after one year.

8093 *What is the population growth rate (PGR) and how is it affected by changes in **s(j)**, **s(a)** or **f**?*

8094 Can project or predict population growth by putting these demographic parameters into a population-
8095 projection matrix **L** (known as a Leslie matrix or a Lewis-Leslie matrix):

$$\begin{array}{cc} 8096 & \mathbf{0} & \mathbf{f} \\ 8097 & \mathbf{s(j)} & \mathbf{s(a)} \end{array}$$

8098 Top row of matrix – age-specific fecundities (only the adults have non-zero fecundity **f**)

8099 Sub-diagonal – age-specific survivorship = proportion **s(j)** of juveniles surviving to adulthood

8100 Diagonal terms – proportion of a stage remaining in that stage; **s(a)** is the proportion of adults
8101 surviving from one year to the next

8102 The numbers of juveniles $\mathbf{n(j)}$ and adults $\mathbf{n(a)}$ in year t are listed in a population vector $\mathbf{n_t}$:

8103 $\mathbf{n(j)}$

8104 $\mathbf{n(a)}$

8105 Thus the total population size in year t is $\mathbf{N_t = n(j) + n(a)}$. Changes from year to year are projected
8106 using the matrix equation:

8107 $\mathbf{n_{t+1} = L n_t}$

8108 PGR can be expressed as the finite rate of increase or multiplication factor, $\mathbf{PGR = N_{t+1} / N_t}$ (or the
8109 instantaneous rate of increase $\mathbf{r = \ln R}$). **PGR** can be computed as a mathematical property of the
8110 projection matrix **L**; it is the dominant eigenvalue (or latent root) of the matrix. Some of the
8111 demographic and evolutionary consequences of this formulation are explored by Smith (1991).

8112 For this simplest model, PGR is calculated as:

8113 $\mathbf{PGR = f * s(j) + s(a)}$

8114 Note that the three vital rates **f**, **s(j)** and **s(a)** are, in the simplest case, treated as constants that do
8115 not vary with population size (i.e. not density dependent).

8116 If the data exist, it is possible in principle to make one or more of the vital rates a function of
8117 population size rather than a constant. This would generally lead to population size increasing or
8118 decreasing towards an equilibrium population size although it may be that the dynamic behaviour is
8119 cyclical or chaotic rather than equilibrial.

8120

8121 An ecotoxicological question

8122 *"If a PPP has a detrimental effect on reproductive adults, will this have a greater effect in reducing
8123 population growth rate than if it had the same effect on juveniles?"*

8124 The simple answer to the question is *"it depends"*.

8125 In a stable population, $\mathbf{PGR \sim 1}$ (or $\mathbf{r \sim 0}$). This could be a consequence of density dependence in one
8126 or more of the vital rates. Without knowing anything about density dependence, this provides a trick
8127 that gives a first approximation to an answer to the above question for contrasting life histories. For
8128 illustration, consider two caricatures that we call the Model 1 and the Model 2, representing a range of
8129 demographic variables of the sort that we might find across reptiles and amphibians.

8130

8131 Model 1 Low fecundity, high survival

8132	0	5
8133	0.1	0.5

8134

8135 Model 2 High fecundity, low survival

8136	0	100
8137	0.009	0.1

8138

8139 Note that $\mathbf{PGR = f * s(j) + s(a) = 1}$ in both these caricatures.

8140 There are two standard, demographic approaches that may help to answer the ecotoxicological
8141 question:

8142 *Sensitivity* – the effect on **PGR** of an **absolute** change in a vital rate such as fecundity or survival

8143 *Elasticity* – the effect on **PGR** of a **proportional** change in a vital rate such as fecundity or survival

8144 Elasticity analysis is more appropriate for PPPs where we might characterise a detrimental effect as
8145 reducing survival (or fecundity) by a certain %.

8146

8147 *Elasticity analysis for the caricature models*

8148 Model 1 Low fecundity, high survival

8149 **0** **5**8150 **0.1** **0.5**8151 Reduce each by 10% in turn, i.e. reduce **f** or **s(j)** or **S(a)** x0.9; the consequence of any of these is to
8152 make **PGR = 0.95** (a 5% reduction in PGR)8153 *i.e. Model 1 is equally sensitive (elastic) to changes in any of the three parameters, close to*
8154 *equilibrium where **PGR**~1.*

8155 Model 2 High fecundity, low survival

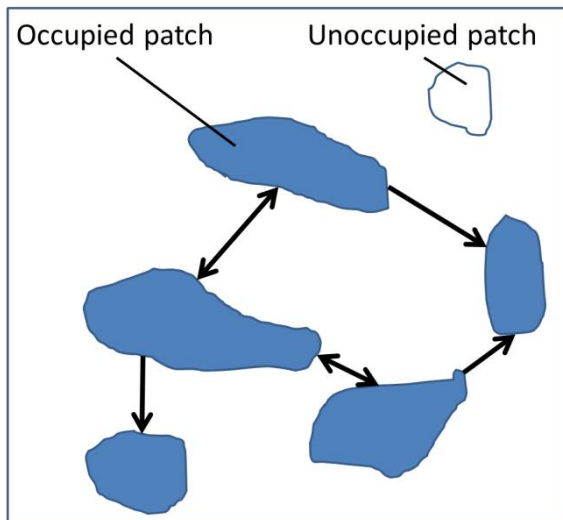
8156 **0** **100**8157 **0.009** **0.1**

8158 Reduce each by 10% in turn:

8159 reduce **f** or **s(j)** x0.9; **PGR = 0.91** (a 9% reduction in PGR)8160 reduce **S(a)** x0.9; **PGR=0.99** (a 1% reduction in PGR)8161 *i.e. Model 2 is most sensitive (elastic) to changes in fecundity **f** or juvenile survival **s(j)** , close to*
8162 *equilibrium where **PGR**~1*8163 Thus, for this equilibrium analysis of Model 2, reducing either fecundity or juvenile survival has a
8164 greater effect on PGR than the same reduction in adult survival.8165 In contrast, for an equilibrium analysis of Model 1, reducing any of the three vital rates by a given
8166 amount had the same effect on PGR whichever rate was reduced.8167 It would be possible, of course, to set up a caricature with even lower fecundity and higher survival
8168 such that PGR was most sensitive (elastic) to changes in adult survival.8169 These simple, two-stage models could be extended to any number of stages to mimic a more realistic
8170 life cycle. The broad conclusions would not change although drastically changing the detail could
8171 throw up some odd results.8172 Note, however, that the above is an *equilibrium* analysis of a single population with no spatial
8173 structure. Modelling what happens away from equilibrium in non-spatially distributed population would
8174 require specific assumptions about the form of density dependence and these could change the
8175 conclusions. Elasticity analysis can be robust, but can also give quite misleading results if interpreted
8176 without a clear under-standing of their assumptions and limitations (Mills, Doak & Wisdom 1999). In
8177 cases where we have spatially heterogenous populations, spatial models may be necessary to capture
8178 the dynamics. This is why we urge caution about the interpretation of simple, demographic models;
8179 *they can be useful for illustrating general concepts but should not generally be used for predictive*
8180 *purposes, e.g. in risk assessment.* For predictive purposes, we need models that include the important
8181 factors and mechanisms that drive population processes at scales and detail commensurate with
8182 SPGs. This involves incorporating greater realism along two related axes, of spatial structuring and
8183 population structuring.

8184

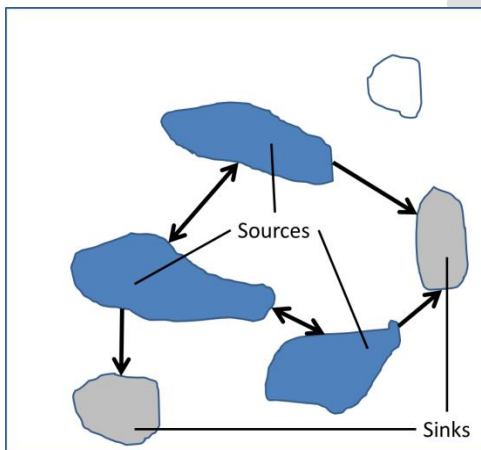
8185 **Spatially structured populations: theoretical considerations**8186 Continuous populations that are well mixed and distributed fairly evenly over the landscape are
8187 generally expected to be the most resilient to local adverse effects. In reality, many species have a
8188 more complex structure and are influenced by heterogeneity in the landscape. Many amphibians in
8189 particular respond to heterogeneity (patchily distributed breeding sites).8190 Here, we describe three types of spatially structured population model, which show the range of
8191 structural modelling typically used. Note, however, that these are points on a continuum illustrative of
8192 approaches.



8193

8194 **Figure 39:** A metapopulation structure

8195 In the 'classic' metapopulation model, populations are not found everywhere across the landscape
 8196 (Figure 39:). Suitable patches of habitat may be either occupied or unoccupied and local extinction is
 8197 a normal event. The key to persistence is recolonisation *via* migration. Thus an adverse effect that is
 8198 synchronised over several local populations is potentially the most damaging, because recolonization
 8199 will be absent or slow.



8200

8201 **Figure 40:** A source-sink population structure

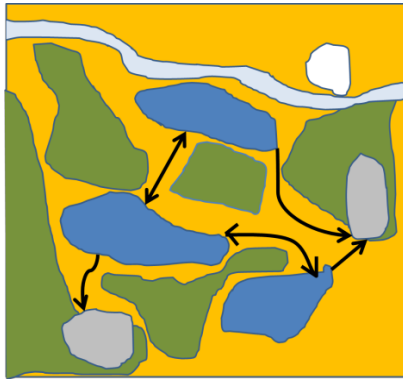
8202

8203 In the source-sink model, most or all patches may be occupied (Figure 40:). The population of
 8204 interest appears to be widespread and may be abundant.

8205 'Sink' populations ($PGR < 1$) occupying low-quality patches, however, are maintained only by migration
 8206 from 'source' populations (where $PGR > 1$).

8207 Reduction of PGR in source populations can be disastrous and lead to rapid population collapse. This
 8208 could be a result of use of a PPP in high-quality patches. Sink populations are no longer topped up
 8209 from source populations and will become extinct.

8210 An example of an apparently successful and widespread species that once collapsed as a result of PPP
 8211 (organochlorine) use is the sparrowhawk in England. Although it is once again widespread and
 8212 breeding in woodlands across eastern England, many of these woodlands are sinks where $PGR < 1$
 8213 (Newton, 1998).



8214

8215 **Figure 41:** A landscape representation

8216

8217

8218 The real world is more complex than either Table 39: , Table 40: or (Figure 41: . There are various
 8219 landscape features such as rivers, hedgerows, and a variety of crops, that may act as either barriers
 8220 or corridors between populations. In addition, populations will often not occur in neat patches but be
 8221 more generally distributed. For these situations landscape simulation is necessary. This may include
 8222 both detailed representation of spatial structure, but can also include dynamic modelling of the spatial
 8223 distribution of habitat quality, environmental conditions and mortality. These approaches are typically
 8224 associated with detailed population structuring (see below) since fine population structure is needed
 8225 to take advantage of this spatial heterogeneity.

8226 **Population structuring – theoretical considerations**

8227 Similar to spatial structure, a population can be considered as a single entity or split into smaller units,
 8228 which in the extreme can be a detailed representation of individuals and their differences.

8229 Like the spatial structuring, this population-structuring axis is a continuum. At one extreme we have
 8230 the single entity exemplified by the two stage model presented above. This population can then be
 8231 divided into smaller units, for example in a stage structured model. Here we can consider different
 8232 life-stages e.g. eggs, larvae, adult and as well as reproduction, the rates of survival from one stage to
 8233 the next. This allows for incorporation of stage-specific information. This approach can be extended to
 8234 spatially distributed populations but models become mathematically complex as more stages and
 8235 processes are included. At some point as more structure is included, an individual-based approach can
 8236 be chosen. This is usually a pragmatic choice to overcome complexity in the system being modelled
 8237 (Grimm 1999). The theoretical advantage of this choice is primarily to incorporate feedback
 8238 mechanisms between heterogeneous individuals and their environment. These individual
 8239 representations can become highly detailed, including individual differences e.g. in behaviour and
 8240 genetics. For example, philopatry in amphibians may be important in their spatial dynamics, but
 8241 requires detailed differences in behaviour of individuals.

8242

8243

8244 **Annex B – Relevant characteristics of ponds hosting amphibians to be** 8245 **able to estimate exposure**

8246 Introduction

8247 A pond is defined as a body of standing water 0.0010 to 2 ha in area, which usually holds
 8248 water for at least four months of the year. We are interested in the distribution of ponds
 8249 across the current EU. This implies:

8250 # “standing water”: slowly moving is still OK (order of few 1000 m/day), but not fast flowing
 8251 streams or rivers.

8252 # “to 2 ha” : the water body should be delimited in size and especially length (so not be a
8253 stream). Sticking to the definition of up to 2 ha and assuming a minimum width of 30 m, the
8254 maximum length could be 667 m.

8255
8256 Our aim is to simulate environmental concentrations of pesticides in ponds for risk
8257 assessment for amphibians. The concentrations may be predicted as a function of time with
8258 the aid of a simple model and some additional assumptions. We would like to obtain a spatio-
8259 temporal statistical distribution of environmental concentrations from which we could select
8260 the pond with the wished percentile worst-casedness in concentration. We would like to be
8261 able to perform a ranking of the ponds across: (i) the entire EU, (ii) the individual EU Member
8262 States (incl. the UK), (iii) regions of ‘similar’ agro-environmental –ecological conditions.

8263
8264 As we are interested in concentrations the water depth is an important characteristic we need
8265 (next to surface area of pond). As the aim is to use the data for pesticide registration a first
8266 criterion for selection of ponds is: they are located in an agricultural area (agriculture within
8267 50 m of pond)

8268
8269
8270 Required characteristics + explanation

8271 The following attributes for the ponds are important:

8272 (N.B. The bold items are most important)

- 8273
8274 1. surface area + time of observation (at least month, year)
8275 2. water depth + time of observation (at least month, year)
8276 3. drawdown
8277 4. seasonality: truly seasonal, permanent or semi-permanent
8278 5. presence of inflow, or outflow, or both
8279 6. soil type in which pond is located
8280 7. land use in surrounding, (e.g. % arable in 0-5 m and 0-100 m perimeter around pond)
8281 8. distance agricultural field - edge of water
8282 9. presence of vegetation on the edge of the pond
8283 10. presence of amphibians ?

8284
8285 If possible also:

- 8286 11. presence of water plants inside pond, percentage surface area covered by plants
8287 12. likely to contain water in spring or not, for at least one month ?

8288
8289 Concerning the location of the pond:

- 8290 13. coordinates plus + EU-Member State name

8291
8292
8293 Ad 1. Surface area + time of observation:

8294 Lower limit: What is the smallest pond size that can be detected ? Our proposal for upper
8295 limit: 2 ha.

8296 Surface area of the ponds should be listed or be classified according to surface area in the
8297 following size classes: < 10 m², 10-25 m², 25-100 m², 100-400 m², 400-1000 m², 1000-
8298 2000 m², 2000 m²-1 ha, 1-2 ha)

8299 N.B. Small ponds (up to ~1000 m²) are an important habitat for amphibians, so it is crucial
8300 that these ponds are included in the analysis.

8301 Considering time of observation: for how many years the data are available ? For reasons of
8302 simplicity a proposal could be to select an “average” meteorological year, resulting in
8303 “average” surface and water depth to be used. Maybe combined with surface area and water
8304 depth for the “last-but-one driest and last-but-one wettest year ?

8305
8306 Ad 2. Water depth + time of observation:

- 8307 An explanation should be added how the water depth has been calculated. What is its level
8308 of detail, which differences in depth have been captured ? Was the water depth obtained by
8309 a calculation method (has this been validated ?) or are measured depths used ? Please state
8310 what the water level represents exactly: the average water depth over the entire pond, or the
8311 maximum water depth over the area of the pond ?
8312
- 8313 Ad 3. Draw down:
8314 The drawdown is a measure of how far water levels drop in summer compared to their bank-
8315 full winter standing water levels
8316
- 8317 Ad 4. Seasonality:
8318 We use the following definitions for seasonality:
8319 Permanent: pond does not dry out
8320 Semi-permanent: pond dries out in drought years
8321 Truly seasonal; pond dries out every year
8322
- 8323 Ad 5. Presence of inflow, outflow or both:
8324 Is there a visible inflow or outflow of water into/out of the pond or both ?
8325
- 8326 Ad 6. Soil type:
8327 Please describe also the used soil type classification system
8328
- 8329 Ad 7. Land use in surrounding:
8330 As precise as possible, preferably crop type. Crucial for pesticide registration.
8331
- 8332 Ad 8. Distance agricultural field-edge of water:
8333 The distance between the nearest agricultural field and the edge-of-water is an important
8334 factor defining the spray drift deposition. Spray drift experts need the distance between the
8335 last row of crops and the edge-of-water to be able to predict the deposition.
8336
- 8337 Ad 9. Presence of vegetation on edge of the pond:
8338 This is also an important factor for the spray drift deposition.
8339
- 8340 Ad 10. Presence of amphibians:
8341 Is it possible to know whether amphibians are present or might be present ? Overlay with
8342 map on presence of amphibians possible ?
8343
- 8344 Ad 11. Macrophytes:
8345 Please state whether floating or emerging water plants are visible in the pond and the
8346 percentage of the surface area they cover.
8347
- 8348 Ad 12. Water in spring or not ?
8349 During the spring, i.e. the breeding season of amphibians the ponds should have water for at
8350 least one month to be able to host amphibians from eggs to metamorphosis to their terrestrial
8351 life stage.
8352
- 8353 Ad 13. Within EU or certain MS:
8354 Easy to allocate
8355
8356
- 8357 Annex C – **Overview on exposure routes for amphibians and reptiles and**
8358 **available exposure models**
8359

8360 **Table 44:** Exposure routes – Amphibians and Reptiles terrestrial phase (other than ingestion)

	medium	Available model*	unit	Description of ecotoxicological exposure quantity
A-Juvenile, adults	Soil	Dermal exposure Residents	mg/m ²	Mass of ai dissolved per mass of soil
A-Juvenile, adults	Plant	Time to re-entry	mg/day	Only for dried residues
A-Juvenile, adults	Air	Bystanders, residents, TIM	mg/m ³	Spray drift
R-Eggs, juveniles, adults	Soil	PEC soil	mg/kg	mass of ai dissolved per mass of soil
R-Juveniles, adults	Plant, stone walls	Worker exposure (dermal), TIM	mg/m ²	Dislodgeable Foliar Residues
R-Juvenile, adults	Air	Resident, By-stander, TIM	mg/m ³	Spray drift
R-Juvenile, adults	Air	Overspray+ Crop interception	mg/m ²	Direct overspray

8361

8362

 8363 **Annex D – Overview on existing risk assessment for birds and mammals**

8364

 8365 **Risk assessment for oral uptake:**

 8366 The TER values are calculated for generic focal species in the first tier assessments according to the
 8367 following formula:

 8368 $TER = \text{toxicity endpoint} / DDD$ (daily dietary dose)

 8369 $DDD = SV * \text{application rate}$

 8370 $SV = \text{shortcut value} = FIR/bw * RUD$

 8371 $FIR = \text{daily food intake rate}$

 8372 $bw = \text{body weight}$

8373 RUD = residue per unit dose (residues for different food items e.g. insects, plants, seeds), mean RUD
8374 values are used for the reproductive risk assessment and 90%tile RUD values are used for acute risk
8375 assessment.

8376 The RUD values are generic residue values per kg a.s. applied per ha, the unit is mg a.s./kg food item

8377 Conservative assumptions in the first tier risk assessment for birds and mammals:

8378 In the first tier risk assessment it is assumed that a bird obtains 100% of its food from the treated
8379 field.

8380

8381 **Secondary poisoning:**

8382 For active substances with a log Pow > 3, an assessment of the risk posed by bioconcentration of the
8383 substance in the prey of birds and mammals shall be provided.

8384 The risk assessment of secondary poisoning of earthworm eating birds and mammals is described in
8385 the EFSA birds and mammals GD (EFSA 2009) on p. 71-73.

8386 The bioconcentration factor (BCF) for earthworms is calculated and based on the BCF the
8387 concentration in earthworms is calculated.

8388 The daily dietary dose (DDD) is calculated by multiplying the concentration in earthworms by 1.28 for
8389 mammals (10g mammal eating 12.8 g worms per day) and by 1.05 for birds (100 g bird eating 104.6
8390 g worms per day)

8391 The TER is calculated with the long-term NOAEL ($TER = NOAEL/DDD$). The trigger value is 5.

8392 The risk assessment of secondary poisoning of fish eating birds and mammals is presented in the
8393 EFSA birds and mammals GD on p.74.

8394

8395 The BCF for fish is available from the studies in the dossier and can be used to calculate the
8396 concentration in fish.

8397 The daily dietary dose (DDD) is calculated by multiplication of the concentration in fish by 0.142 for
8398 mammals and by 0.159 for birds.

8399 The TER is calculated with the long-term NOAEL ($TER = NOAEL/DDD$). The trigger value is 5.

8400

8401 Conservative assumptions in the risk assessment of secondary poisoning for birds and mammals:

8402 Only one food item is considered. 100% of the food is contaminated with the compound. The
8403 concentrations in earthworms are based on the full application rate and related soil or pore water
8404 concentrations in soil. For the concentrations in fish the regulatory acceptable concentration is used
8405 (maximum concentration in surface water for which the risk to water organisms is considered to
8406 meet the protection goals).

8407 Overall it is assumed that the risk to amphibians and reptiles is covered by the assessment of the risk
8408 to birds and mammals for the food items earthworms and fish – this needs checking – look at the
8409 uptake rates for earthworm/fish eating amphibians and reptiles. Exposure via other prey items such
8410 as insects, amphibians and small mammals should be considered. Direct overspray of insects could
8411 be considered as a first tier approach and a worst-case scenario for amphibians and reptiles.

8412

8413 Assessment of biomagnification in terrestrial food chains (p. 75 EFSA birds and mammals GD):

8414 If information from the toxicology section (Absorption Distribution Metabolism Excretion [ADME]
8415 studies) indicates low potential of bioaccumulation then this assessment is not required.

8416 Bioaccumulation factor (BAF) should be less than 1. It is calculated according to the following
8417 formula:

$$8418 \text{ BAF} = \alpha * \text{FIR} / k_2$$

8419 α = fraction from ingested dose that is absorbed

8420 k_2 = rate constant for depuration, $k_2 = \ln(2) * T_{1/2}$ (T = elimination half-life)

8421 FIR = food intake rate relative to body weight

8422

8423 Uptake of contaminated water by drinking:

8424 Two scenarios are assessed:

8425 1. Daily drinking water demand is satisfied from drinking from puddles in leaf axils (= pool
8426 scenario) (applied rate / 5) or

8427 2. drinking from puddles in the field (= puddle scenario) on the bare soil.

8428 The drinking water uptake is calculated for a small granivorous bird (bw = 15.3 g, daily water uptake
8429 of 0.46 L/kg bw) and a small granivorous mammal (bw = 21.7 g, daily water uptake of 0.24 L/kg bw)

8430 For the leaf axil scenario only an acute risk assessment is conducted while for the puddle scenario
8431 also a long-term risk assessment is conducted.

8432 Conservative assumptions in the first tier risk assessment for birds and mammals:

8433 In the first tier risk assessment it is assumed that a bird obtains 100% of its drinking water from the
8434 treated field.

8435 Risk assessment for granular formulations (EFSA birds and mammals GD p.43-56)

8436 The following oral exposure routes are listed for evaluation:

8437 Ingestion of granules as food

8438 Birds may ingest granules as grit

8439 Birds may mistake granules for small seed

8440 Birds and mammals may consume food contaminated with residues resulting from granular
8441 applications.

8442 Birds and mammals may ingest granules when they eat food contaminated with soil.

8443

8444 Annex E – **Endpoints available in dossiers from standard birds and**
8445 **mammal studies**

8446

8447 **Birds:**

8448 For all avian and mammalian feeding studies, average achieved dose shall be reported, including
8449 where possible the dose in mg substance/kg body weight. The following endpoints are available in the
8450 dossiers as standard requirements.

8451

8452 **Acute oral toxicity**8453 Guidelines: **OECD 223**

8454 Exposure: oral, single dose via gavage

8455 Observation period: 14 days

8456 Effects: mortality, LD50, The lethal threshold dose, time courses of response and recovery, the LD 10
8457 and LD 20 shall be reported together with the no observed effect level (NOEL) and gross pathological
8458 findings.

8459

8460 **Short term dietary toxicity** This test is only required if there is an indication from the mode of
8461 action or from the mammalian studies that the short-term dietary test could result in a lower LD50
8462 than the acute short term test.

8463 Guidelines: **OECD 205**

8464 Exposure: oral over 5 d, ad libitum food uptake

8465 Observation period: at least for 9 days

8466 Effects: mortality, lowest lethal concentration (LLC), where possible, NOEC values, time courses of
8467 response and recovery and pathological findings shall be reported in such study.

8468

8469 **Reproductive toxicity**8470 Guidelines: **OECD 206**

8471 Exposure: oral, exposure of adults 10 weeks before egg-laying until egg-laying is finished, food uptake
8472 ad libitum, eggs are removed and artificially incubated, chicks are not exposed

8473 Observation period: from 10 weeks before egg-laying until at least 8 weeks after egg laying is
8474 finished.

8475 Effects: EC10, EC20 and NOEC in mg a.s./kgbw/d for the following:

8476 Adult body weight and food consumption

8477 Number of eggs laid per hen

8478 The mean eggshell thickness

8479 The proportion of eggs set

8480 The proportion of fertile eggs with viable embryos

8481 The proportion of eggs that hatch and produce chicks

8482 The survival of chicks at 1d and 14d of age

8483

8484 **Mammals:**

8485 For all avian and mammalian feeding studies, average achieved dose shall be reported, including
 8486 where possible the dose in mg substance/kg body weight. The following endpoints are available in the
 8487 dossiers as standard requirements.

8488

8489 **Acute oral toxicity to mammals**

8490 Guidelines: **OECD 420, OECD 423, OECD 425**

8491 Exposure: oral, single dose by gavage

8492 Observation period: 14 days

8493 Effects: mortality, LD50, unless otherwise needed, only female rats will be used, overall food
 8494 consumption on the day of exposure as well as the time of onset and disappearance of overt clinical
 8495 signs should be monitored.

8496

8497 **Long term reproductive toxicity to mammals**

8498 Guidelines: **OECD 416**, the following tests may also be available: **OECD 414, 407, 408**

8499 Exposure: oral, ad libitum via spiked food (the Guideline allows also gavage or exposure via drinking
 8500 water) over the whole test duration starting with the first generation (during growth for at least one
 8501 full spermatogenic cycle) and F1 until weaning of the F2 generation.

8502 Observation period: From parental generation to weaning of F2

8503 Effects:

8504 The most sensitive and ecotoxicologically relevant mammalian long-term toxicological endpoint
 8505 (NOAEL) expressed as mg substance/kg bw/day shall be reported. Where EC 10 and EC 20 cannot be
 8506 estimated an explanation shall be provided.

8507 Typical observations /primary endpoints are: fertility, litter size and survival, growth, development and
 8508 sexual maturation (of F1 generation).

8509 In addition there are developmental studies with rabbits and rats (such as **OECD 414**).

8510

8511 The EFSA birds and mammals GD considers the following endpoints as relevant for reproductive
 8512 performance:

8513 NOAEL for body weight change, behaviour effects and systemic toxicity

8514 NOAEL for indices of gestation, litter size, pup and litter weight

8515 NOAEL for indices of viability, pre- and post-implantation loss

8516 NOAEL for embryo/foetal toxicity including teratogenic effects

8517 NOAEL for number abortions and number early delivery

8518 NOAEL for systemic toxicity and effects on adult body weight

8519 NOAEL for indices of post-natal growth, indices of lactation and data on physical landmarks

8520 NOAEL for survival and general toxicity up to sexual maturity

8521

8522 **Table 45:** Overview on available endpoints

Juveniles	Mortality	Oral	LD50 acute	
-----------	-----------	------	------------	--

			(b+m) LD50 short term feeding (b)	
	Mortality	Dermal	LD50 dermal in rodents	
	Growth	Oral	NOEL (m)	
		Dermal	No endpoints	
	Behaviour	Oral	No specific studies only observation of unusual behaviour in studies with b+m	
		Dermal	No specific studies only observation of unusual behaviour in studies with b+m	
	Lesions (external and internal)	Oral	Endpoints available from mammal studies	
		Dermal	Endpoints available from mammal studies	
	Malformati	Oral		

	on			
		Dermal		
Adult	Mortality Reproducti on Behaviour Lesions	High	unknown Less sensitive than juveniles?	Water Soil Food Plants Air (possibly low) Overspray

8523

8524 **Annex F – Coverage of the risk to amphibians and reptiles by the human**
8525 **risk assessment**

8526 Human exposure to plant protection products and the respective risk assessment is related to two
8527 main categories of exposure, the dietary and the non-dietary exposure. The dietary exposure is
8528 relevant for the consumers of the agricultural products where pesticides can be present as residues
8529 on/in the different commodities. The non-dietary exposure is relevant for the operators, workers,
8530 bystanders and residents of the rural areas.

8531 For both dietary and non-dietary risk assessment, the exposure levels, measured or estimated using
8532 mathematical models, are compared to the appropriate toxicological threshold values.

8533 The toxicological threshold used for the dietary risk assessment is the Acceptable Daily Intake (ADI).
8534 The basis for ADI setting is the chronic/long term toxicity studies, from which the highest dose that
8535 does not produce any adverse effects on the experimental animals is identified (WHO, 1987). The No
8536 Observed Adverse Effect Level (NOAEL) is divided by the appropriate assessment factor (usually 100)
8537 for extrapolation from animals to humans taking into consideration toxicokinetic and toxicodynamic
8538 variability as well as the human variability. In addition, for compounds that may produce adverse
8539 effects following subacute/acute exposure the Acute Reference Dose (ARfD) is also established from
8540 the respective study(ies) with the application of the assessment factor (EC, 2001). Both the ADI and
8541 ARfD are considered to be “external” doses since they do not reflect the absorbed amount of the
8542 substance through the gastrointestinal tract but the highest amount that can be ingested without
8543 any adverse effect on human health.

8544 The toxicological threshold used for the non-dietary risk assessment is the Acceptable Operator
8545 Exposure Level (AOEL). The basis for the AOEL are the repeated dose toxicity studies considering the
8546 most relevant toxicological end-point in the most sensitive species. From these studies, the highest
8547 dose that does not produce any adverse effects on the experimental animals is identified. The NOAEL
8548 is then divided by the appropriate assessment factor (usually 100) for extrapolation from animals to
8549 humans as with other reference values. Usually, the studies that are used for the AOEL setting are

8550 oral exposure studies. For substances that may produce detrimental effects after a single day of
8551 exposure the Acute Acceptable Operator Exposure Level (AAOEL) is to be established. Considering
8552 the new EFSA Guidance on the assessment of exposure of operators, workers, residents and
8553 bystanders in risk assessment for plant protection products, as noted by the European Commission
8554 (SANTE-10832-2015, 29 May 2015) the AAOEL is required especially for the risk assessment on
8555 residents and bystanders. However, there is still no guidance for the derivation of the AAOEL. For the
8556 estimation of the AOELsystemic, the oral AOEL is corrected by the oral absorption factor when the
8557 extent of oral absorption is lower than 80% of the ingested amount, as identified in relevant ADME
8558 studies.

8559 The units for both the ADI and AOEL values are mg/kg bw/day, whereas for ARfD it is mg/kg bw.

8560 As far as it relates to dietary exposure, consumer exposure estimations [based on Maximum Residue
8561 Levels in/on the different commodities and food consumption factor], toxicological thresholds (ADI
8562 and ARfD) and risk assessment, are not considered to provide any useful information either for
8563 exposure estimations or risk assessment for amphibians and reptiles. The non-dietary human risk
8564 assessment, as a whole, is not applicable to A&R risk assessment, since the exposure scenarios and
8565 the hazard thresholds are specific to humans. However, some of the parameters and principles used
8566 in human risk assessment could be applicable in the case of A&R risk assessment as well.

8567

Appendix A – Species list

8568 List of amphibian and reptile species in the European Union (excluding overseas,
 8569 Macaronesian and northern African territories), classified by assessment groups suggested
 8570 for further identification of focal species (see section 2.5 for details).

8571 **Table 46:** Temporary title 1

Assessment group	Family	Species	Distributed in zones			Present in arable land
			South	Centre	North	
Caudates	Plethodontidae	<i>Speleomantes ambrosii</i>	X			
		<i>Speleomantes flavus</i>	X			
		<i>Speleomantes genei</i>	X			
		<i>Speleomantes imperialis</i>	X			
		<i>Speleomantes italicus</i>	X			
		<i>Speleomantes sarrabusensis</i>	X			
		<i>Speleomantes strinatii</i>	X			
		<i>Speleomantes supramontis</i>	X			
	Proteidae	<i>Proteus anguinus</i>	X			
	Salamandridae	<i>Calotriton arnoldi</i>	X			
		<i>Calotriton asper</i>	X			
		<i>Chioglossa lusitanica</i>	X			
		<i>Euproctus montanus</i>	X			
		<i>Euproctus platycephalus</i>	X			
		<i>Ichthyosaura alpestris</i>	X	X	X	
		<i>Lissotriton boscai</i>	X			X
		<i>Lissotriton helveticus</i>	X	X		X
		<i>Lissotriton italicus</i>	X			X
		<i>Lissotriton montandoni</i>	X	X		X
		<i>Lissotriton vulgaris</i>	X	X	X	X
		<i>Lyciasalamandra helverseni</i>	X			
		<i>Lyciasalamandra luschani</i>	X			
		<i>Pleurodeles waltl</i>	X			
		<i>Salamandra algira</i>	X			
		<i>Salamandra atra</i>	X	X		
		<i>Salamandra corsica</i>	X			
		<i>Salamandra lanzai</i>	X			
		<i>Salamandra salamandra</i>	X	X		X
		<i>Salamandrina perspicillata</i>	X			
		<i>Salamandrina terdigitata</i>	X			
		<i>Triturus carnifex</i>	X	X		
		<i>Triturus cristatus</i>	X	X	X	X
		<i>Triturus dobrogicus</i>	X	X		X
<i>Triturus karelinii</i>		X			X	
<i>Triturus marmoratus</i>	X			X		
<i>Triturus pygmaeus</i>	X			X		

Anurans	Alytidae	<i>Alytes cisternasii</i>	X			
		<i>Alytes dickhilleni</i>	X			
		<i>Alytes muletensis</i>	X			
		<i>Alytes obstetricans</i>	X	X		X
		<i>Discoglossus galganoi</i>	X			X
		<i>Discoglossus jeanneae</i>	X			
		<i>Discoglossus montalentii</i>	X			
		<i>Discoglossus pictus</i>	X			X
		<i>Discoglossus sardus</i>	X			
	Bombinatoridae	<i>Bombina bombina</i>	X	X	X	X
		<i>Bombina variegata</i>	X	X		X
		<i>Bombina pachypus</i>	X			X
	Pelobatidae	<i>Pelobates fuscus</i>	X	X	X	X
		<i>Pelobates cultripes</i>	X			
		<i>Pelobates syriacus</i>	X	X		X
	Pelodytidae	<i>Pelodytes ibericus</i>	X			X
		<i>Pelodytes punctatus</i>	X			
	Hylidae	<i>Hyla arborea</i>	X	X	X	X
		<i>Hyla intermedia</i>	X			X
		<i>Hyla meridionalis</i>	X			
		<i>Hyla sarda</i>	X			
		<i>Hyla savignyi</i>	X			X
	Bufonidae	<i>Bufo bufo</i>	X	X	X	X
		<i>Bufotes balearicus</i>	X			X
		<i>Bufotes boulengeri</i>	X			X
		<i>Bufotes siculus</i>	X			X
		<i>Bufotes variabilis</i>	X	X	X	
		<i>Bufotes viridis</i>	X	X	X	X
		<i>Epidalea calamita</i>	X	X	X	X
	Ranidae	<i>Pelophylax bedriagae</i>	X			X
		<i>Pelophylax bergeri</i>	X			
		<i>Pelophylax cerigensis</i>	X			X
		<i>Pelophylax cretensis</i>	X			
		<i>Pelophylax epeiroticus</i>	X			
		<i>Pelophylax esculentus</i>	X	X	X	X
		<i>Pelophylax hispanicus</i>	X			
		<i>Pelophylax kurtmuelleri</i>	X			
		<i>Pelophylax lessonae</i>	X	X	X	X
		<i>Pelophylax perezi</i>	X			X
		<i>Pelophylax ridibundus</i>	X	X	X	X
<i>Rana arvalis</i>		X	X	X	X	
<i>Rana dalmatina</i>		X	X			
<i>Rana graeca</i>		X				
<i>Rana iberica</i>		X				
<i>Rana italica</i>	X					

		<i>Rana latastei</i>	X			
		<i>Rana pyrenaica</i>	X			
		<i>Rana temporaria</i>	X	X	X	X
Tortoises	Testudinidae	<i>Testudo graeca</i>	X	X		
		<i>Testudo hermanni</i>	X	X		
		<i>Testudo marginata</i>	X			
Terrapins	Emydidae	<i>Emys orbicularis</i>	X	X	X	
		<i>Emys trinacris</i>	X			
	Geoemydidae	<i>Mauremys leprosa</i>	X			
		<i>Mauremys rivulata</i>	X			
Saurians	Agamidae	<i>Stellagama stellio</i>	X			X
	Anguidae	<i>Anguis cephalonnica</i>	X			X
		<i>Anguis fragilis</i>	X	X	X	
		<i>Pseudopus apodus</i>	X			
	Chamaeleonidae	<i>Chamaeleo chamaeleon</i>	X			
	Arycidae	<i>Eryx jaculus</i>	X	X		
	Gekkonidae	<i>Hemidactylus turcicus</i>	X			
		<i>Mediodactylus kotschy</i>	X			X
	Lacertidae	<i>Acanthodactylus erythrurus</i>	X			X
		<i>Acanthodactylus schreiberi</i>	X			
		<i>Algyroides fitzingeri</i>	X			X
		<i>Algyroides marchi</i>	X			
		<i>Algyroides moreoticus</i>	X			
		<i>Algyroides nigropunctatus</i>	X			X
		<i>Anatololacerta anatolica</i>	X			
		<i>Anatololacerta oertzeni</i>	X			
		<i>Archaeolacerta bedriagae</i>	X			
		<i>Dalmatolacerta oxycephala</i>	X			
		<i>Darevskia praticola</i>	X	X		
		<i>Dinarolacerta mosorensis</i>	X			
		<i>Eremias arguta</i>		X		
		<i>Hellenolacerta graeca</i>	X			
		<i>Iberolacerta aranica</i>	X			
		<i>Iberolacerta aurelioi</i>	X			
		<i>Iberolacerta bonnali</i>	X			
		<i>Iberolacerta cyreni</i>	X			
		<i>Iberolacerta galani</i>	X			
<i>Iberolacerta horvathi</i>		X	X			
<i>Iberolacerta martinezricai</i>	X					
<i>Iberolacerta monticola</i>	X					
<i>Lacerta agilis</i>	X	X	X	X		
<i>Lacerta bilineata</i>	X	X		X		
<i>Lacerta schreiberi</i>	X					
<i>Lacerta trilineata</i>	X	X		X		
<i>Lacerta viridis</i>	X	X		X		

		<i>Ophisops elegans</i>		X		
		<i>Phoenicolacerta troodica</i>	X			
		<i>Podarcis bocagei</i>	X			
		<i>Podarcis carbonelli</i>	X			
		<i>Podarcis cretensis</i>	X			
		<i>Podarcis erhardii</i>	X			
		<i>Podarcis filfolensis</i>	X			X
		<i>Podarcis gaigeae</i>	X			
		<i>Podarcis hispanicus</i>	X			
		<i>Podarcis levendis</i>	X			
		<i>Podarcis lilfordi</i>	X			
		<i>Podarcis melisellensis</i>	X			
		<i>Podarcis milensis</i>	X			X
		<i>Podarcis muralis</i>	X	X		X
		<i>Podarcis peloponnesiacus</i>	X			X
		<i>Podarcis pityusensis</i>	X			X
		<i>Podarcis raffonei</i>	X			
		<i>Podarcis siculus</i>	X			X
		<i>Podarcis tauricus</i>	X	X		X
		<i>Podarcis tiliguerta</i>	X			X
		<i>Podarcis vaucheri</i>	X			
		<i>Podarcis waglerianus</i>	X			X
		<i>Psammodromus algirus</i>	X			X
		<i>Psammodromus hispanicus</i>	X			X
		<i>Psammodromus jeanneae</i>	X			
		<i>Psammodromus manuelae</i>	X			
		<i>Timon lepidus</i>	X			X
		<i>Zootoca vivipara</i>	X	X	X	
	Phyllodactylidae	<i>Tarentola mauritanica</i>	X			X
	Scincidae	<i>Ablepharus budaki</i>	X			
		<i>Ablepharus kitaibelii</i>	X	X		
		<i>Chalcides bedriagai</i>	X			X
		<i>Chalcides chalcides</i>	X			
		<i>Chalcides ocellatus</i>	X			
		<i>Chalcides striatus</i>	X			X
		<i>Ophiomorus punctatissimus</i>	X			
		<i>Trachylepis aurata</i>	X			X
		<i>Trachylepis vittata</i>	X			X
		Sphaerodactylidae	<i>Euleptes europaea</i>	X		
	<i>Saurodactylus mauritanicus</i>		X			
	Typhlopidae	<i>Typhlops vermicularis</i>	X	X		
Blind snakes	Amphisbaenidae	<i>Blanus cinereus</i>	X			X
		<i>Blanus strauchi</i>	X			X
Fully terrestrial	Colubridae	<i>Coronella austriaca</i>	X	X	X	
		<i>Coronella girondica</i>	X			

snakes		<i>Dolichophis caspius</i>	X	X			
		<i>Dolichophis jugularis</i>	X			X	
		<i>Eirenis modestus</i>	X			X	
		<i>Elaphe quatuorlineata</i>	X			X	
		<i>Elaphe sauromates</i>	X	X			
		<i>Hemorrhois nummifer</i>	X				
		<i>Hemorrhois algirus</i>	X			X	
		<i>Hemorrhois hippocrepsis</i>	X			X	
		<i>Hierophis cypriensis</i>	X				
		<i>Hierophis gemonensis</i>	X				
		<i>Hierophis viridiflavus</i>	X			X	
		<i>Macroprotodon brevis</i>	X				
		<i>Macroprotodon cucullatus</i>	X			X	
		<i>Platyceps collaris</i>	X			X	
		<i>Platyceps najadum</i>	X			X	
		<i>Rhinechis scalaris</i>	X			X	
		<i>Telescopus fallax</i>	X			X	
		<i>Zamenis lineatus</i>	X			X	
		<i>Zamenis longissimus</i>	X	X		X	
		<i>Zamenis situla</i>	X				
		Psammophidae	<i>Malpolon monspessulanus</i>	X			X
			<i>Malpolon insignitus</i>	X			
		Viperidae	<i>Vipera ammodytes</i>	X	X		X
		<i>Vipera berus</i>	X	X	X	X	
		<i>Vipera aspis</i>	X	X		X	
		<i>Montivipera xanthina</i>	X			X	
		<i>Vipera seoanei</i>	X			X	
		<i>Vipera latastei</i>	X				
		<i>Vipera ursinii</i>	X	X			
		<i>Macrovipera schweizeri</i>	X			X	
Water snakes	Natricidae	<i>Natrix natrix</i>	X	X	X	X	
		<i>Natrix tessellata</i>	X	X			
		<i>Natrix maura</i>	X	X	X		

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8573

Appendix B – Consequences of choices made by risk managers concerning the effects of intended PPP use on amphibians and reptiles

8574 **Table 47:** Temporary table title 2

amphibians and reptiles as key drivers of	Consequences of option choice regarding the effects of intended PPP use on amphibians and reptiles		
	Option: below the limit of operation	Option: Limit of operation	if above limit of operation

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amphibians and reptiles as key drivers of	Consequences of option choice regarding the effects of intended PPP use on amphibians and reptiles		
	Option: below the limit of operation	Option: Limit of operation	if above limit of operation
Provisioning services: Biodiversity Genetic resources Cultural services Food and pharmaceutical resources	<ul style="list-style-type: none"> - The upper level of the normal operating range for amphibians and reptiles in agricultural landscapes is sustained. Species-specific interactions, food-web structure and ecosystem processes are unaffected by the intended PPP use. - General protection goal ‘no unacceptable effect on biodiversity and the ecosystem’ set out in Regulation (EC) No. 1107/2009⁷ is fully achieved. - Support of the target “<u>Increase the contribution of agriculture to maintaining and enhancing biodiversity</u>” (3a) of the EU 2020 Biodiversity Strategy¹², which has shown no significant progress so far. - This Option contributes to Action 10 of the EU 2020 Biodiversity Strategy¹²: “The Commission and Member States will encourage the uptake of agri-environmental measures <u>to support genetic diversity in agriculture and explore the scope for developing a strategy for the conservation of genetic diversity</u>”. - The aims of Council Directive 92/43/EEC⁸ on the <u>conservation of natural habitats and of wild fauna and flora</u> are achieved. 	<ul style="list-style-type: none"> - The limit of operation identified in the SPG tables indicate a tipping point for the normal operating range of amphibian and reptile key drivers delivering genetic resources and cultural services and supporting all ecosystem services. - Reduction in species diversity reduces the efficiency with which ecological communities capture biologically essential resources, control pests, produce biomass, decompose and recycle biologically essential nutrients. - Biodiversity is supported to a degree that insures the long term functioning of agricultural system, even if sensitive species are affected in the short term and species-specific interactions might be disrupted. - General protection goal ‘no unacceptable effect on biodiversity and the ecosystem’ set out in Regulation (EC) No. 1107/2009 is still achieved if unsprayed areas of pertinent size in a diversified landscape sustain the upper level of biodiversity normal operating range. 	<ul style="list-style-type: none"> - Species loss above a tipping point may force ecosystems to move to a different (locally) stable state or to collapse. - Loss of biodiversity will weaken the ability of agricultural ecosystems to respond to external changes such as climate change (loss of stability and resilience). - Biodiversity losses will lead to disruption of valuable ecosystem functions thereby reducing delivered services. Cultural services will be reduced if vulnerable species decline or disappear. - General protection goal ‘no unacceptable effect on biodiversity and the ecosystem’ set out in Regulation (EC) No. 1107/2009 is not achieved. - The target “Increase the contribution of agriculture to maintaining and enhancing biodiversity” (3a) of the EU 2020 Biodiversity Strategy¹² will most probably not be met. - The aim of halting of biodiversity loss by 2020 is not achieved: ‘Halting biodiversity loss constitutes the absolute minimum level of ambition to be realised by 2020’ (2009/2108(INI)⁹ and 2011/2307(INI)¹⁰. - UN sustainable development goals (SDG) ¹¹ Sustainable Goals 2.4 and 15.5 are jeopardized. These goals are: <ul style="list-style-type: none"> ▪ “By 2030, ensure sustainable food production systems and implement resilient agricultural practices that increase productivity and production, that help maintain ecosystems, that strengthen capacity for adaptation to climate change, extreme weather, drought, flooding and other disasters and that progressively improve land and soil quality” and ▪ “Take urgent and significant action to reduce the degradation of natural habitats, halt the loss of biodiversity and, by 2020, protect and prevent the extinction of threatened species”

⁷ European Union: Regulation (EC) No. 1107/2009 of the European Parliament and of the Council concerning the placing of plant protection products on the market and repealing Council Directives 79/117/EEC and 91/414/EEC. 21 October 2009. Official Journal of the European Union L 309, 24 November 2009, 50 pp.

⁸ Council Directive 92 /43 /EEC of 21 May 1992 on the conservation of natural habitats and of wild fauna and flora.

⁹ 2009/2108(INI) Report on the implementation of EU legislation aiming at the conservation of biodiversity.

¹⁰ 2011/244(INI) Communication: on our life insurance, our natural capital: an EU biodiversity strategy to 2020 Committee on the Environment, Public Health and Food Safety.

amphibians and reptiles as key drivers of	Consequences of option choice regarding the effects of intended PPP use on amphibians and reptiles		
	Option: below the limit of operation	Option: Limit of operation	if above limit of operation
continued: Biodiversity Genetic resources Cultural services Food and pharmaceutical resources	<ul style="list-style-type: none"> - The aims of Council Directive 92/43/EEC¹² on the <u>conservation of natural habitats and of wild fauna and flora</u> are achieved, especially regarding <ul style="list-style-type: none"> ▪ species and sub- species listed in Annex IV, for which a strict protection regime must be applied across their entire natural range within the EU, both within and outside Natura 2000 sites - UN <u>sustainable development goals</u> (SDG) Error! Bookmark not defined. Sustainable Goals and 2.4 and 12.2 are supported These goals are: <ul style="list-style-type: none"> ▪ "By 2030, ensure sustainable food production systems and implement resilient agricultural practices that increase productivity and production, that help maintain ecosystems, that strengthen capacity for adaptation to climate change, extreme weather, drought, flooding and other disasters and that progressively improve land and soil quality" and ▪ "By 2030, achieve the sustainable management and efficient use of natural resources 	<ul style="list-style-type: none"> - Member States are still supported in the measures they need to take to maintain or restore the species in Annex II and IV list at a '<u>favourable conservation status</u>' in the EU (cf Article 2). <ul style="list-style-type: none"> ▪ populations are maintaining themselves over the long term and are no longer showing signs of continuing decline; their natural range is not being reduced; ▪ there is, and will probably continue to be, a sufficiently <u>favourable</u> large habitat to maintain its populations on a longterm basis. 	<ul style="list-style-type: none"> - The aims of Council Directive 92/43/EEC¹³ on the <u>conservation of natural habitats and of wild fauna and flora</u> are not achieved. - Member States are not compliant with obligations arising from the Habitats directive, and do not take the necessary measures to ensure the conservation of amphibian and reptile species protected and listed under Annexes II and IV - Member State do not take the requisite measures to establish a system of strict protection for Annex II and IV species. <ul style="list-style-type: none"> ▪ animal killing / destruction of eggs in the wild ▪ deterioration of breeding sites or resting places; as consequence of PPP intended uses will take place / at a rate considered unacceptable to maintain their conservation status
Supporting services as Nutrient	- Upper limit of the normal operating range of amphibians and reptiles as drivers of supporting ecosystem services is ensured.	- This limit of functioning marks the lower threshold of the normal operating range for amphibians and	- Vulnerable species at higher trophic level might decline further and may become extinct. Disruption of trophic networks can occur, impairing the ecological equilibrium of the system.

¹¹ United Nations General Assembly (2015): Resolution adopted by the General Assembly on 25 September 2015. Transforming our world: the 2030 Agenda for Sustainable Development. Distr. General, 21 October 2015. Seventieth session, Agenda items 15 and 116, A/RES/70/1, 35pp.

¹² Council Directive 92 /43 /EEC of 21 May 1992 on the conservation of natural habitats and of wild fauna and flora.

¹³ Council Directive 92 /43 /EEC of 21 May 1992 on the conservation of natural habitats and of wild fauna and flora.

amphibians and reptiles as key drivers of	Consequences of option choice regarding the effects of intended PPP use on amphibians and reptiles		
	Option: below the limit of operation	Option: Limit of operation	if above limit of operation
cycling Soil structure formation Food-web support	<ul style="list-style-type: none"> - The aims of the EU thematic strategy for soil protection¹⁴ to “<u>protect soil and to preserve its capacity to perform its functions in environmental, economic, social and cultural terms</u>” are fully supported. - UN <u>sustainable development goals</u> (SDG) Error! Bookmark not defined. Sustainable Goals and 2.4 and 12.2 are supported These goals are: <ul style="list-style-type: none"> ▪ “By 2030, ensure sustainable food production systems and implement resilient agricultural practices...” and ▪ “By 2030, achieve the sustainable management and efficient use of natural resources - Structure and functioning of the terrestrial food web in agricultural landscape is fully preserved and <u>the support of above-ground terrestrial food webs</u> is achieved. Vulnerable species at higher trophic level supported. - The aim of halting of biodiversity loss by 2020 is fully supported: ‘Whereas the <u>disappearance of species may break the food chain that is key to the survival of other animal and plant species of vital importance for food production, adaptation to climatic conditions, resistance to external agents and the preservation of genetic values</u>’ (e.g. 2009/2108(INI) and 2011/2307(INI)) 	<ul style="list-style-type: none"> reptiles in supporting services - Disruption of trophic networks can occur when vulnerable species are affected by PPP intended uses in the short term, impairing the ecological equilibrium of the system - The General Protection Goal ‘no unacceptable effect on biodiversity and the ecosystem’ of Regulation (EC) No. 1107/2009 and the aims of Council Directive 79/409/EEC¹⁵ on the conservation of wild birds and of Council Directive 92/43/EEC on the conservation of natural habitats and of wild fauna and flora are still achieved in the long term - as long as unsprayed areas of pertinent size in a diversified landscape deliver the upper level of biodiversity normal operating range, in order to sustain recovery of vulnerable amphibian and reptile species 	<ul style="list-style-type: none"> - General protection goal ‘no unacceptable effect on biodiversity and the ecosystem’ set out in Regulation (EC) No. 1107/2009 is not achieved. - Aims of Council Directive 79/409/EEC on the conservation of wild birds and of Council Directive 92/43/EEC on the conservation of natural habitats and of wild fauna and flora are not achieved. - The aim of halting of biodiversity loss by 2020 is not achieved: ‘Whereas the disappearance of species may break the food chain that is key to the survival of other animal and plant species of vital importance for food production, adaptation to climatic conditions, resistance to external agents and the preservation of genetic values’ (e.g. 2009/2108(INI) and 2011/2307(INI)) - The aims of the EU thematic strategy for soil protection¹⁵ to “protect soil and to preserve its capacity to perform its functions in environmental, economic, social and cultural terms” may not be met. - UN sustainable development goals (SDG) Error! Bookmark not defined. 2.4 and 12.2 are jeopardized. These goals are: <ul style="list-style-type: none"> ▪ “By 2030, ensure sustainable food production systems and implement resilient agricultural practices that increase productivity and production, that help maintain ecosystems, that strengthen capacity for adaptation to climate change, extreme weather, drought, flooding and other disasters and that progressively improve land and soil quality” and - “By 2030, achieve the sustainable management and efficient use of natural resources

¹⁴ COM/2006/0232 final (2006): Proposal for a Directive of the European Parliament and of the Council establishing a framework for the protection of soil and amending Directive 2004/35/EC.

¹⁵ Council Directive 79/409/EEC of 2 April 1979 on the conservation of wild birds

amphibians and reptiles as key drivers of	Consequences of option choice regarding the effects of intended PPP use on amphibians and reptiles		
	Option: below the limit of operation	Option: Limit of operation	if above limit of operation
Regulating services as Pest and pathogen control Invasion resistance	<ul style="list-style-type: none"> - Control of specific pest and pathogens by amphibians and reptiles is at the upper level of the normal operating range for agricultural soils. - Aims of Directive 2009/128/16 for achieving a <u>sustainable use of pesticides</u> are fully supported: 'Member States shall establish or support the <u>establishment of necessary conditions for the implementation of integrated pest management</u>. In <u>protection and enhancement of important beneficial organisms</u>, e.g. by adequate plant protection measures' 	<ul style="list-style-type: none"> - Resilient organisms will still deliver the service of pest and pathogen control in agricultural soils. - However, control of specific pathogens by vulnerable key drivers might be reduced in the short term. - The General Protection Goal 'no unacceptable effect on biodiversity and the ecosystem' of Regulation (EC) No. 1107/2009 and the aims of Directive 2009/128/¹⁶ for achieving a sustainable use of pesticides are still implemented, as long as unsprayed areas of pertinent size in a diversified landscape deliver the upper level of biodiversity normal operating range, in order to sustain recovery of vulnerable amphibian and reptile species in the middle and long term. 	<ul style="list-style-type: none"> - Enhanced proliferation of pest and pathogens through the disruption of intra- and inter species interaction within terrestrial community (competition, predation, and parasitism) might finally lead to reduced plant productivity. - Pests and pathogens may increase both numerically and in geographical spread, leading to greater reliance on chemical pesticides and further reduction of biodiversity. - Aims of Directive 2009/128/¹⁶ for achieving a sustainable use of pesticides are not implemented: <ul style="list-style-type: none"> ▪ "Member States shall establish or support the establishment of necessary conditions for the implementation of integrated pest management. In protection and enhancement of important beneficial organisms, e.g. by adequate plant protection measures".

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¹⁶ Directive 2009/128/EC of the European Parliament and of the Council of 21 October 2009 establishing a framework for Community action to achieve the sustainable use of pesticides

8576

Appendix C – Dimensions and surrounding land use of ponds in Spain, United Kingdom of Great Britain and Switzerland and comparison with FOCUS water bodies

8577

8578 The working group gathered some data on the presence and size of ponds in a number of Member
8579 States. The databases for Spain and the Aargau canton in Switzerland contain only water bodies in
8580 which amphibians have been observed, while the database for the UK is much larger. Below we
8581 present an analysis of the information contained in these databases with respect to (i) water surface
8582 area, water depth and water volume, and (ii) surrounding land use. The aim of this analysis was to
8583 establish sizes of ponds (and other water bodies) that serve or may serve as aquatic habitat for
8584 amphibians and in addition, establish whether ponds (or other water bodies) that serve as aquatic
8585 habitats for amphibians, are associated to agricultural land use in their surroundings. In this way we
8586 are able to evaluate whether the amphibians are likely to be exposed to pesticides residues in their
8587 aquatic habitat.

8588 A second aim was to compare the amphibian ponds to the so-called FOCUS surface water bodies that
8589 are currently used in the risk assessment for the aquatic ecosystem at EU level: a pond, ditch and
8590 stream. The FOCUS pond measures 30* 30 m and has a water depth of approximately 1 m and a
8591 surrounding, pesticide-treated area of 4500 m² contributes its runoff or drainage water to the pond.
8592 The FOCUS ditch and stream are 100 m long, 1 m wide (rectangular cross-section) and a 1 ha-treated
8593 agricultural field delivers its runoff or drainage to the ditch or stream. Upstream of the ditch two ha of
8594 untreated agricultural fields deliver drainage flows, while the stream is fed by a 100-ha upstream
8595 catchment of which 20 ha are treated with pesticides. The three water bodies receive spray drift
8596 deposition at the moment pesticides are applied on the adjacent field.

8597

8598 Spain

8599 The database of the Spanish ponds was built with the data of the program SARE (monitoring of
8600 Spanish amphibians and reptiles) developed by the Spanish Herpetological Society. Details on the
8601 program can be found at <http://siare.herpetologica.es/sare> (available in Spanish only). The Spanish
8602 Herpetological Society kindly provided the entire database of ponds.

8603 The SARE monitoring is carried out by volunteers who select one or several cells of the 10 x 10 km
8604 UTM grid. Within each cell, the person responsible for the monitoring must design the sampling, as
8605 part of which at least three water points have to be selected for sampling aquatic amphibians. When
8606 designing the monitoring, the volunteers record some information about the water points, including
8607 dimensions like the surface area (for ponds, for which surface shape is assumed to be elliptical,
8608 lengths of the major and minor axes are collected) and maximum depth of the water column, and
8609 characteristics of the surrounding habitat. Once the sampling strategy has been designed, the
8610 volunteer sends it to a regional coordinator who validates it. Then, the full sampling of each cell
8611 (including all water points and transects in terrestrial habitats in between) is repeated several times
8612 per year and the number of observed specimens is recorded. Since the objective of the program is to
8613 have long-term trends in amphibian and reptile populations at the national level, it is expected that
8614 volunteers repeat the process year after year as long as they can. With this purpose, the program
8615 coordinators encourage volunteers to select cells that are easily reachable (i.e. located close to where
8616 they live, work or spend time regularly). It may happen that a volunteer must, at some point,
8617 redesign the sampling strategy because something has changed in the sampled habitat (e.g. a water
8618 body has desiccated). It also may happen that significant changes are observed in the characteristics
8619 of the water bodies, which means that the dimensions have to be recorded again for the same point.
8620 For this reason, the same pond may appear more than once in the database.

8621 The first data of the program were recorded in 2010. More data are available for the first years of the
8622 program (2010 and 2011) because ponds are characterized when volunteers incorporate to the
8623 program, and this happened mostly during the initial years; afterwards, the number of volunteers
8624 contributing to the program dropped.

8625 The database of ponds sampled as part of the SARE program provides a very good overview of
8626 amphibian breeding habitats. Contrarily to other pond inventories, these ponds are included here
8627 precisely because they constitute amphibian breeding habitats, and with such purpose have been
8628 selected by volunteer herpetologists (either amateur or professional) who have a good knowledge on
8629 the habitats they are sampling. It is true that a bias may exist in the election of ponds towards those
8630 harbouring a higher number of species, and this may exclude other amphibian breeding habitats that
8631 do not attract so many species. These under-represented habitats can be, on the one hand, very
8632 permanent waters that, because of the frequent presence of fish, are not often used by most
8633 amphibians, and also that are difficult to sample because of their dimensions. On the other hand,
8634 shallow waters like puddles, that can also be used as breeding habitat by some species, are also
8635 under-represented because of the difficulties that their unpredictability supposes for a continued
8636 monitoring over time.

8637 Upon our request the presence of arable land in the immediate surrounding of the amphibian ponds
8638 was assessed by the volunteers for 151 the 794 water bodies in the period September- November
8639 2016. This was done to be able to evaluate whether the ponds were liable to be contaminated by the
8640 agricultural use of pesticides.

8641 The SARE database contained 794 unique records for water bodies. The water bodies have been
8642 classified as ponds (421), artificial pool (152), dam/reservoir (66), lagoon/lake (21), river (30), stream
8643 (85) and wetland/marsh (19). Water depth, water surface area and water volume were analysed of all
8644 water bodies. First, the database was corrected, as some water depths (e.g. 100 m) were not
8645 plausible. In records of ponds, artificial pools, and streams in which the water depth was more than
8646 10 m and the minor axis was less than or equal to 10 m, it was assumed that the depth was reported
8647 in cm instead of m, and the depth was converted to m. This was done for 23 ponds.

8648

8649 Water depth

8650 For 10 waterbodies the depth was not reported. The frequency and cumulative frequency distribution
8651 of the remaining 784 water bodies is given in Figure 42: which shows that 18% of the water bodies
8652 has a water depth of 30 cm or less, i.e. the minimum water depth of the FOCUS ditches and streams.
8653 In total 70% of the water bodies is less than or equal to 1 m deep, i.e. the depth of the FOCUS pond.

8654

8655 Water surface area

8656 The water surface area of the 794 water bodies was calculated from the major and minor axis lengths
8657 according to the surface area of an ellipse: $\pi * a * b$ (with a and b being the major and minor axis
8658 length). The frequency distributions are given for all 794 records. Fig. 43 shows that 59% of the water
8659 bodies has a water surface area of less than or equal to 100 m² (the surface area of the 100 m * 1 m
8660 FOCUS ditches and streams) and 87% an area of less than 900 m² (the surface area of the 30 m * 30
8661 m FOCUS ponds).

8662

8663 Water volume

8664 The water volume of the water bodies was calculated by volume = depth * area. As the recorded
8665 depth is the maximum depth in the ponds, the water volume will be somewhat overestimated. The
8666 frequency distributions are given for 784 records, for which a water depth was available. Fig. 44
8667 shows that 41% of the water bodies have a volume of less than 25 m³, 52% less than 50 m³ (the
8668 minimum water volume of FOCUS ditches and streams is 30 m³) and that 84% of the water bodies
8669 have a water volume of less than 900 m³ (the water volume of the FOCUS ponds).

8670

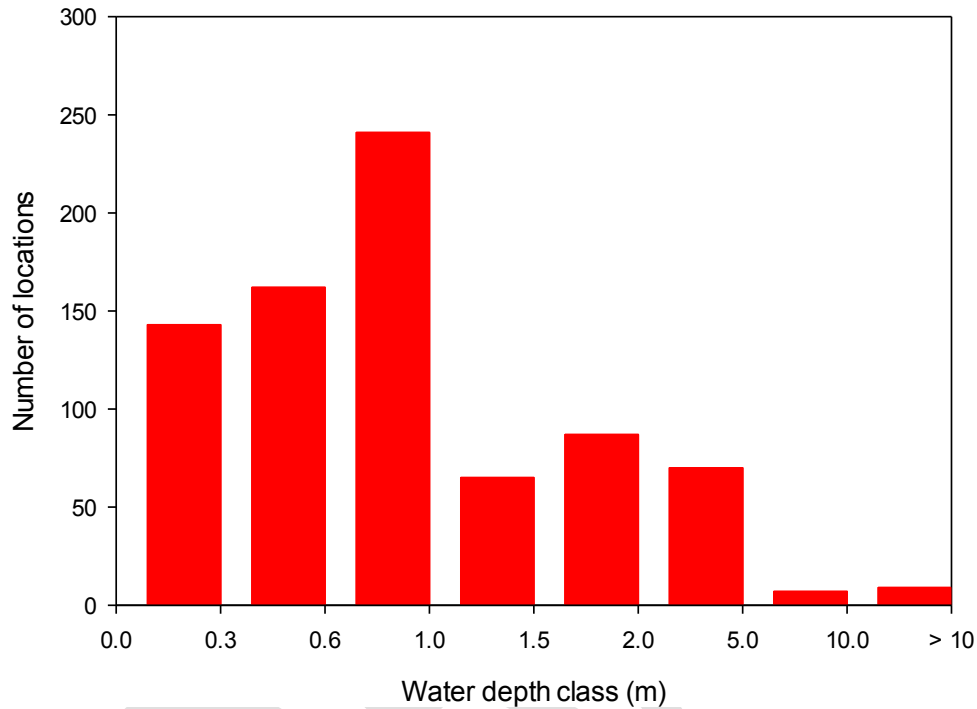
8671 Land use

8672 The data on land use in the immediate surroundings (<100 m) of 151 Spanish ponds indicate that for
8673 81 of these of the ponds the surrounding land use is non-agricultural. For the remaining 70 ponds
8674 agricultural fields are nearby, for 32 ponds the distance edge-of-field to pond water is 0-10 m, for 21
8675 ponds the distance is 10-20 m and for 17 ponds 20-100 m. Of the 70 ponds with agricultural fields

8676 nearby 13 ponds are completely surrounded by the agricultural fields. Figure 45: presents the land
8677 use as function of the surface water area class of the ponds. It demonstrates that for all size classes
8678 arable and non-arable land are approximately equally present, except for the ponds of less than 5 m²
8679 area, which are predominantly surrounded by non-arable land. So, these data demonstrate that a
8680 non-negligible part of the Spanish ponds in which amphibians live, are likely to receive pesticides
8681 residues.

8682

A



B

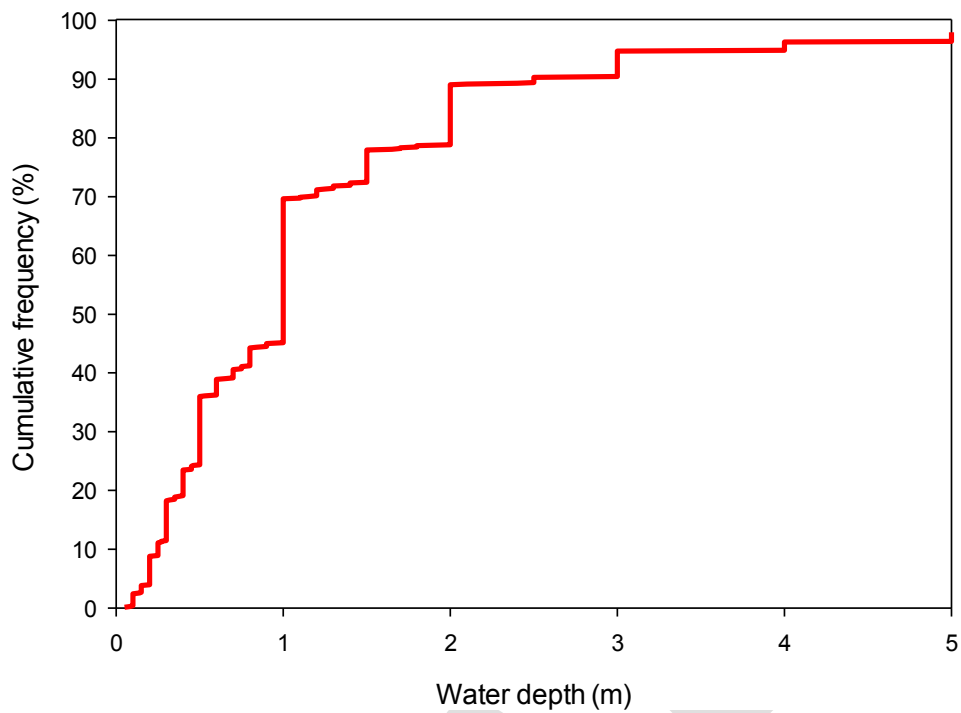
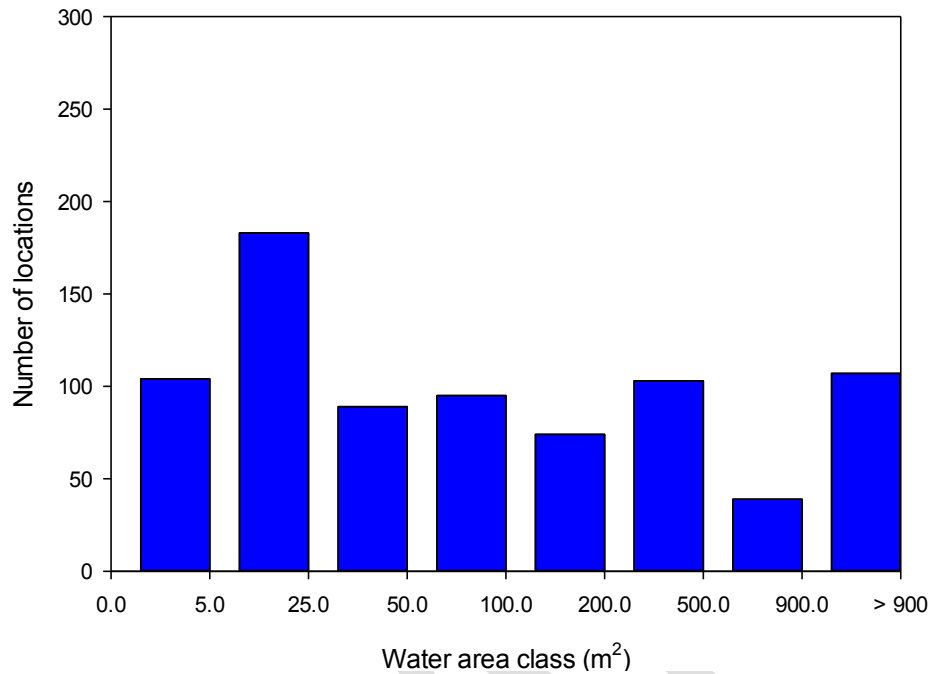


Figure 42: Frequency (A) and cumulative frequency (B) distribution of water depth of 784 water bodies in Spain. Note that in graph 1A the x -axis is not linear and that all class boundaries are indicated. In graph 1B water bodies with a depth greater than 5 m have not been included.

8683

8684

A



B

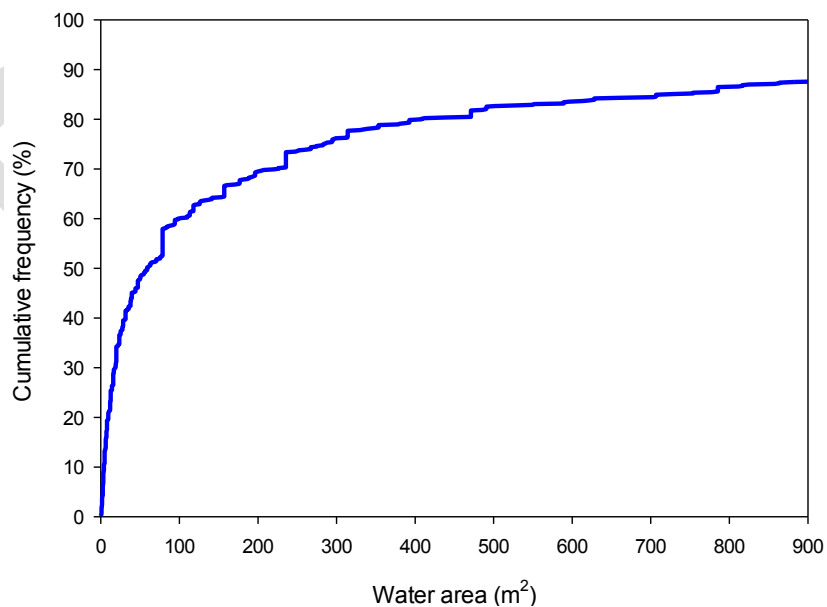
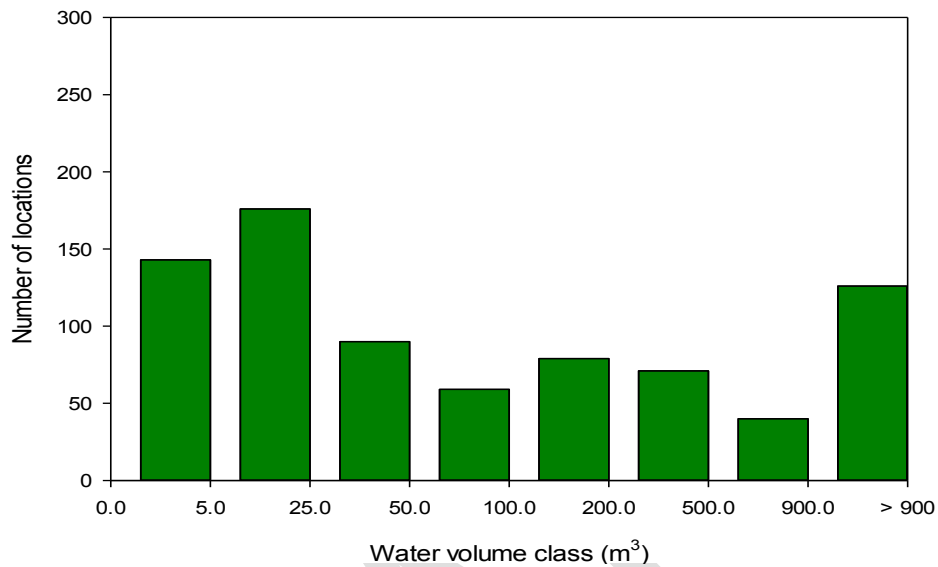


Figure 43: Frequency (A) and cumulative frequency distribution (B) of water surface area of 794 water bodies in Spain. Note that in graph A the x-axis is not linear and that all class boundaries are indicated. In graph B water bodies with an area greater than 900 m² have not been included.

A



B

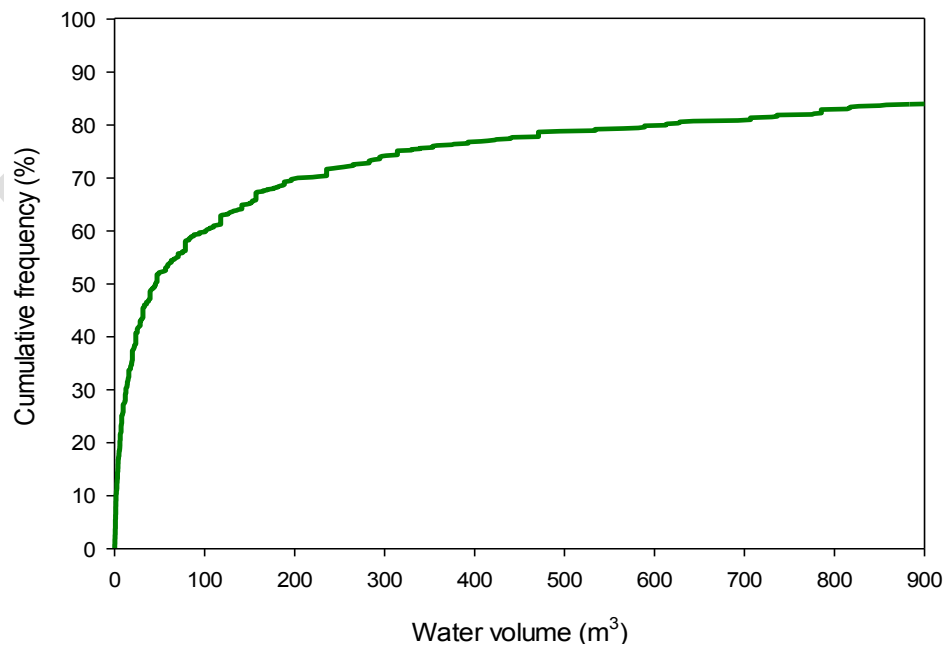


Figure 44: Frequency (A) and cumulative frequency distribution (B) of water volume of 784 water bodies in Spain. Note that in graph A the x-axis is not linear and that all class boundaries are indicated. In graph B water bodies with a volume greater than 900 m^3 have not been included.

8685

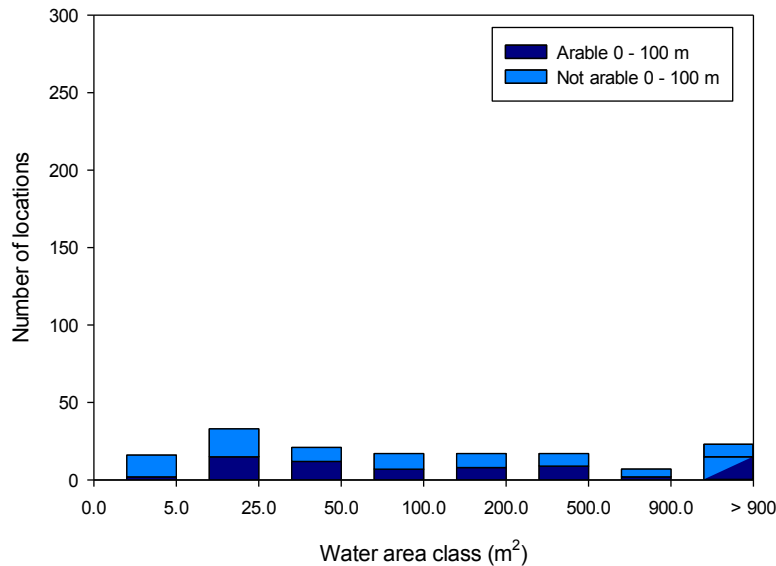


Figure 45: Frequency distribution of water surface area of the 70 ponds in Spain where a arable land use was observed in the 0 - 100 m distance zone from the perimeter of the pond, plus the remaining 81 ponds.

8686

8687

8688 **Switzerland**

8689 The pond data from Switzerland stem from the amphibian monitoring program in canton Aargau in
 8690 the north of Switzerland which has been conducted since 2006 (Ref: Kanton Aargau, Abteilung
 8691 Landschaft und Gewässer, Amphibienmonitoring Aargau). The purpose of the program is to survey
 8692 eight amphibian populations. Therefore the selection of ponds is driven by the occurrence of one
 8693 these eight species (Tree Frog, Natterjack Toad, Midwife Toad, yellow-bellied toad, marsh frog, water
 8694 frogs (all other types), great crested newt, common newt), which mainly occur in open areas. Ponds
 8695 or streams in forests preferred by early breeding species such as common toad, common frog and fire
 8696 salamander are poorly covered. The ponds are monitored three times per year by volunteers
 8697 according to a standardized method. Next to counting the amphibian population, the surface area of
 8698 the ponds (estimated size of all fragmented ponds in a pit combined), the water level (fluctuating,
 8699 stable, unknown), exposure to sunlight and vegetation are estimated. Neither the depth nor the
 8700 surrounding area are recorded. The data regarding the pond surface is estimated by volunteers during
 8701 the period mid-June-end July (with exceptions between March and September).

8702 Aargau can be described as a canton with intensive agriculture. Some of the monitored ponds were
 8703 created by the farmers as ecological compensation in agricultural areas to obtain subsidies. For these
 8704 two reasons the data will include ponds close to fields (in Switzerland a buffer strip of at least 3 m to
 8705 surface waters needs to be adhered to) and further afield.

8706 The Swiss database on Aargau contained 754 unique records for water bodies. The water surface area
 8707 was analysed. Water depth and water volume were not recorded.

8708

8709 Water surface area

8710 For 25 water bodies the water surface area was not recorded, hence, for 729 water bodies the water
8711 surface area is plotted in frequency graphs in Figure 46: . The figure shows that 52% of the water
8712 bodies has a surface area of less than or 100 m² (the area of the FOCUS ditches and streams) and
8713 89% an area of less than 900 m² (the area of the FOCUS ponds).

8714

8715 Land use

8716 There are no data for the Swiss canton Aargau ponds that specify the land use in their immediate
8717 surroundings. So, we are not able to evaluate on a quantitative basis whether the amphibians in these
8718 ponds are likely to be exposed to pesticides residues in their aquatic habitat. However, the Aargau
8719 canton being a canton with intensive agriculture and a good distribution of amphibian populations, a
8720 number of the ponds in the database are likely to represent amphibian habitats that may receive
8721 pesticide residues.

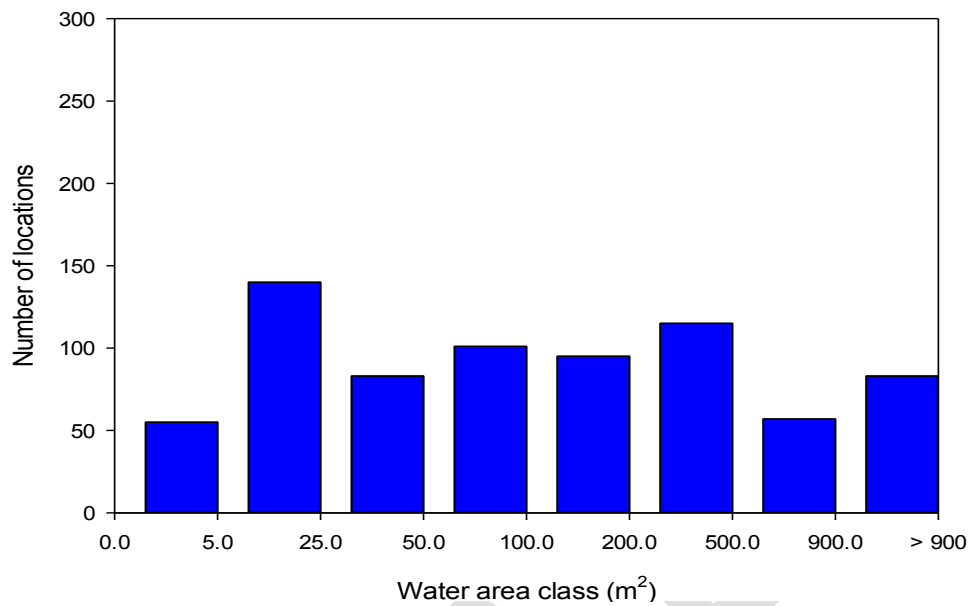
8722

8723

DRAFT

8724

A



B

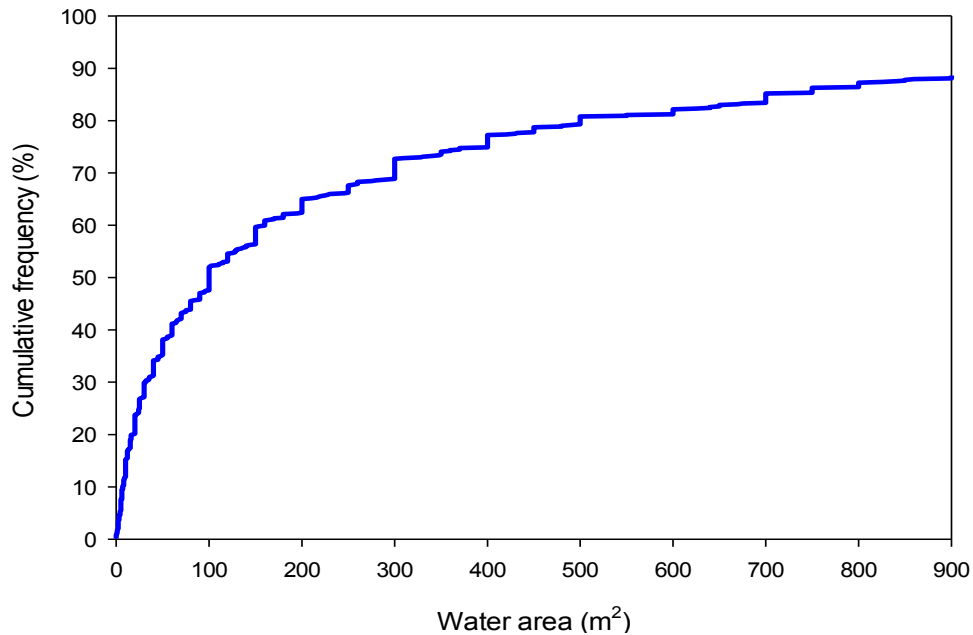


Figure 46: Frequency (A) and cumulative frequency distribution (B) of water surface area of 729 water bodies in canton Aargau, Switzerland. Note that in graph 4A the x-axis is not linear and that all class boundaries are indicated. In graph 4B water bodies with a volume greater than 900 m³ have not been included.

8725

8726 United Kingdom

8727 In the United Kingdom the current state of ponds was described by the Countryside Survey of 2007
8728 (Williams et al, 2010). The survey covered a total of 591 1x1 km square samples spread across
8729 England, Scotland and Wales. A pond was defined as a body of standing water of 25 m² to 2 ha, in
8730 area, which usually holds water for at least four months of the year. So, in principle ponds smaller
8731 than 25 m² were not recorded, while they might have been present. The survey made an inventory of
8732 pond number within the 1x1 km square and of many properties of the ponds, such as biodiversity and
8733 ecological quality determined by e.g. plant species present, physical and chemical condition of ponds,
8734 such as nutrient status, hydrological properties, e.g. inflow and outflow, drawdown (= water level
8735 drop in summer compared to the bank-full winter water level) and water surface area and adjacent
8736 land use. E.g. the survey demonstrated that almost two-thirds (63%) of ponds were directly linked to
8737 the stream network and that a third of these ponds had an inflow, but no outflow, suggesting that
8738 many ponds intercept and retain drainage water. In our analysis we especially focussed on the water
8739 surface area, water depth, surrounding land use and presence of amphibians in the ponds.

8740 The database from CountrySide Survey (2007) contains 259 records with ponds for which the water
8741 surface area has been measured (POND_WATER_QUALITY csv file). Water depth has been recorded,
8742 but only for a limited number of ponds as the time and equipment lacked to measure the maximum
8743 depth in ponds, where it was too deep to wade (pers. comm. J. Biggs, 11 Nov 2016).

8744

8745 Water depth

8746 For 109 ponds the depth had been measured. The frequency distribution of these 109 ponds is given
8747 in Figure 47: as a function of the mean water depth of the pond. The figure shows that 69 of the 109
8748 ponds for which the water depth was measured have a water depth below 0.3 m. As the maximum
8749 depth was not measured in the remaining 150 ponds where it was too deep to wade, the data on
8750 water depth is clearly biased with maximum water depth over 1 m being not represented.

8751

8752 Water surface area

8753 For two ponds the surface area was not recorded, hence we plotted the areas for 257 ponds (Figure
8754 48:). The size of the surface areas has been estimated at the time of the survey, often in the period
8755 May-October 2007. In total 23% of the ponds has a water surface area of less than or equal to 100
8756 m² (the surface area of FOCUS ditches and streams) and 79% an area of less than 900 m² (the
8757 surface area of the FOCUS ponds). Small ponds with areas below 25 m² were not included in the
8758 survey given the pond definition (required surface area of 25 m² to 2 ha), although they are widely
8759 present in the UK landscape. This implies that the percentages given above represent an
8760 underestimation compared to reality.

8761

8762 Water volume

8763 The water volume of the ponds was calculated by volume = mean depth * area. The frequency
8764 distributions are given for 109 records, for which a water depth was available. Figure 49: shows that
8765 50 of the 109 ponds have a volume of less than 25 m³, 58 ponds a volume of less than 50 m³ (30 m³
8766 is the minimum volume of the FOCUS ditches and streams) and that of the 109 water bodies has a
8767 water volume of less than 900 m³ (the water volume of the FOCUS ponds). However these numbers
8768 are largely biased, because for ponds with a mean water depth greater than 1 m, the water depths
8769 were not determined. Moreover, ponds with surface areas were not included in the Countryside
8770 Survey.

8771

8772 Surrounding land use

8773 To obtain an idea about the surrounding land use of the ponds the data base was analysed in two
8774 ways:

8775 (i) the recorded land use classification system according to the Institute of Terrestrial Ecology (Bunce
8776 *et al.*, 1996a and b) was used to identify whether the pond was situated in a land use class in which
8777 arable land is well represented. Table 48: lists the ITE Land Class Number (2007) that we selected as
8778 containing significant areas of arable land use;

8779 (ii) recorded surrounding land use percentage in two distance zones from the perimeter of the pond,
8780 0-5 m and 0-100 m. Arable land use is one of the possible, listed categories (Murphy and Weatherby,
8781 2008).

8782

8783 On the basis of our classification of the ITE Land Class Number (Table 48:) 115 of the 259 ponds
8784 were classified as being located in land-use classes with significant arable land use. Of these 115
8785 ponds the water surface area was not recorded for two ponds. We made a frequency distribution for
8786 the remaining 113 ponds (Figure 50:), which shows that all pond size classes have a comparable
8787 proportion of ponds with significant arable land use nearby. Small ponds with areas below 25 m² were
8788 not included in the survey given the pond definition (required surface area of 25 m² to 2 ha), the few
8789 that have been recorded appear to be predominantly situated in areas with non-arable land use. The
8790 second way of land use classification resulted in 22 ponds that have a percentage of arable land in
8791 their 0-5 m distance zone and 59 ponds that have a percentage of arable land in their 0-100 m
8792 distance zone from the perimeter of the pond (17 of the 59 ponds were already included in the 22
8793 ponds, having arable land in their 0-5 m distance zone). We made a frequency distribution for the 59
8794 ponds (Figure 51:), which shows that all pond size classes have a comparable proportion of ponds
8795 with arable land use in their 0-100 m perimeter. The percentages arable land use vary between 2 and
8796 98%, for 3 of the 59 ponds the percentage was missing.

8797

8798 As expected, comparison of Figure 50: and Figure 51: shows that the more strict classification of
8799 arable land use present in the 0-100 m perimeter around the pond results in a smaller number of
8800 ponds with arable land use, than the regionally-based land use classification using the ITE Land Class
8801 Number. The latter classification resulted in nearly 50% of the ponds being located in land use classes
8802 with significant arable land use (115 of the 259 ponds), while this was only approximately 20% for the
8803 former classification (59 of the 259 ponds).

8804

8805 Amphibians observed in ponds

8806 The presence of amphibians was one of the recorded properties of the surveyed ponds. We expect
8807 the recorded number of ponds to represent an underestimation of the number of ponds hosting
8808 amphibians in reality, as the observation of amphibians will depend on the expertise on amphibians of
8809 the surveyor as well as on the time of the year of the survey (often between April/May to
8810 October/November).

8811 In 49 ponds of the 259 sampled ponds the presence of amphibians (e.g. tadpoles, frogs, newts) was
8812 observed. Except in the few sampled small ponds with areas below 25 m², they were observed in
8813 ponds of all area classes (Figure 52:). Of the 49 ponds with amphibians 7 ponds have a percentage
8814 of arable land in their 0-5 m distance zone and 9 ponds have a percentage of arable land in their 0-
8815 100 m distance zone from the perimeter of the pond. In total 19 of the 49 ponds were included in the
8816 115 ponds which were classified as having significant arable land use according to the ITE Land Class
8817 Number. This indicates that a number of ponds having amphibians may probably receive pesticide
8818 residues via either spray drift deposition or runoff or drainage entries, Moreover part of the ponds
8819 may have an inflow of water that may also carry pesticides into the ponds.

8820

8821

8822 **Table 48:** Land Class Number (without the England, Scotland or Wales indication) according to the
 8823 ITE Land Class Number (2007) classification system with their description (Benefield et al.,
 8824 1982) and our classification in arable land use or not.

8825

Land class	Description of land use	Arable: yes/no
1	cereals, good grasslands and limited native vegetation	yes
2	mainly good grassland, but extensive cereals and built up area	no
3	cereals, other corps and short term grassland	yes
4	arable, with cereals and other crops, good grassland and urban	yes
5	mixed farmland although predominantly good grass; much urban	no
6	mainly good grassland but with some barley	no
7	mainly pasture with some arable and good grass	no
8	mainly pasture with some arable, extensive mudflats and urban development	no
9	mixture of good grass and arable with many urban areas	yes
10	mainly arable but with good grassland and pasture also widespread	yes
11	arable predominates particularly wheat with good grassland and urban	yes
12	arable, mainly wheat with limited food grassland and urban	yes
13	usually mixtures of arable and good grassland but also a variety of other uses	yes
14	mainly arable but also good grassland and much urban	yes
15	mainly pasture mixed with good land and arable	no
16	varied with mixtures of arable pasture and good grassland	yes
17	mainly pastures with some good grassland	no
18	predominantly rough grazing with some limited pasture land	no
19	mainly rough grazing or forest, but some pasture	no
20	much pasture, but some good grassland and occasional crops	no

21	open range grazing or forest	no
22	mainly rough grazing, but also woodland and occasional crops	no
23	limited open range grazing	no
24	limited open range grazing	no
25	mainly barley, but with much good grassland	yes
26	mainly good grassland, but also much barley and pasture	no
27	arable, particularly barley, but also much pasture and good grassland	yes
28	pasture or rough grazing predominate, but some good grasslands also	no
29	mainly open range grazing, but also some crofting	no
30	open range grazing and crofting	no
31	mainly rough grazing, but some good grassland and pasture with crofting	no
32	mainly open range grazing, but some pasture	no

8826

8827

8828

A

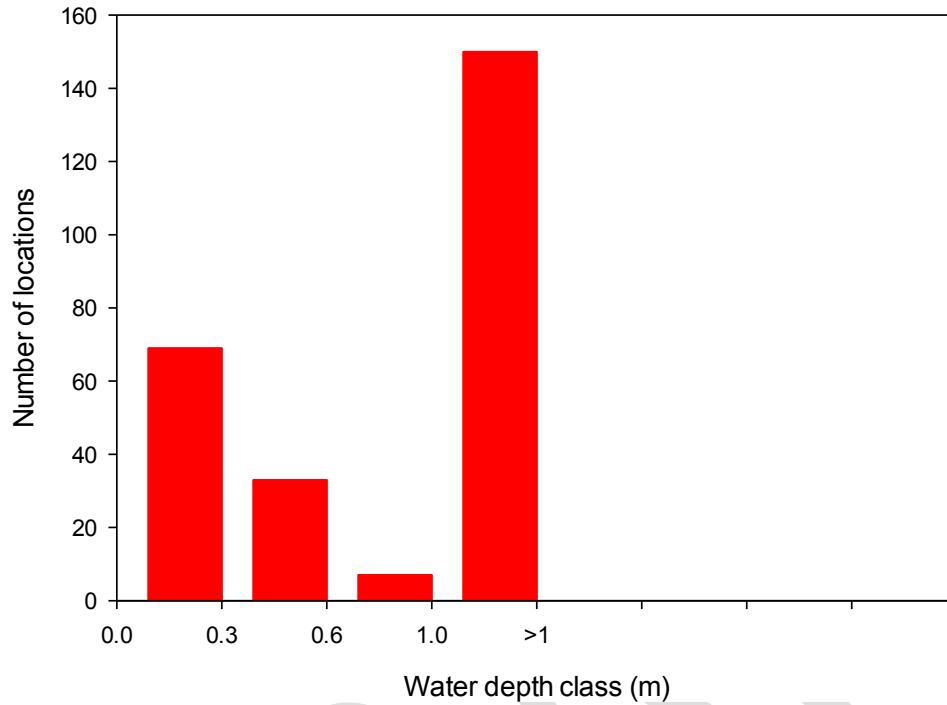


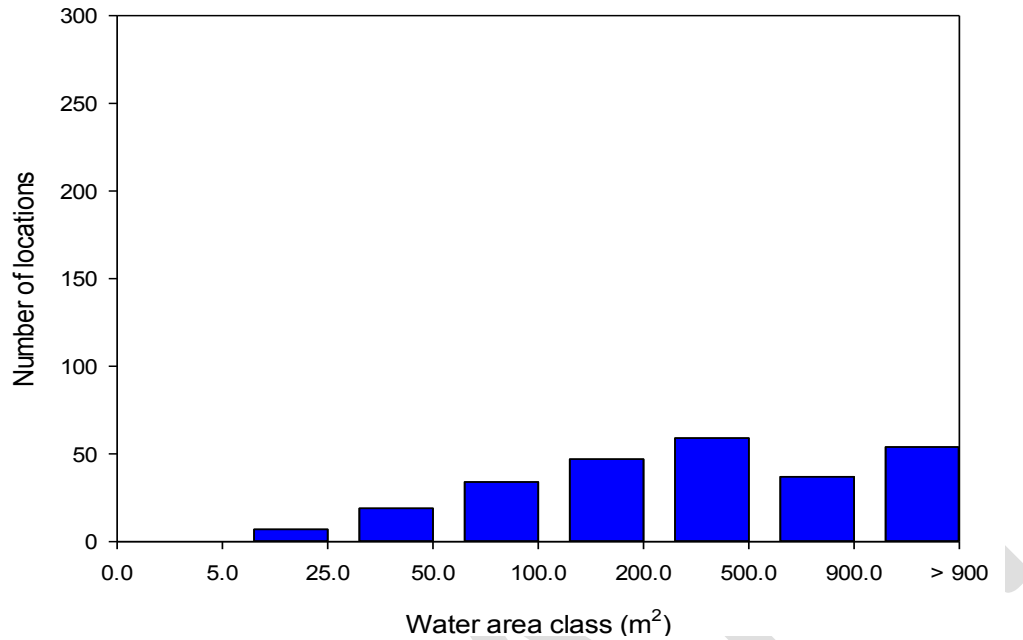
Figure 47: Frequency (A) distribution of water depth of 259 water bodies in the UK. For 150 ponds the mean water depth was not measured because they were too deep to wade (indicated in the class with depth > 1 m). Note that the x-axis is not linear and that all class boundaries are indicated.

8829

8830

8831

A



B

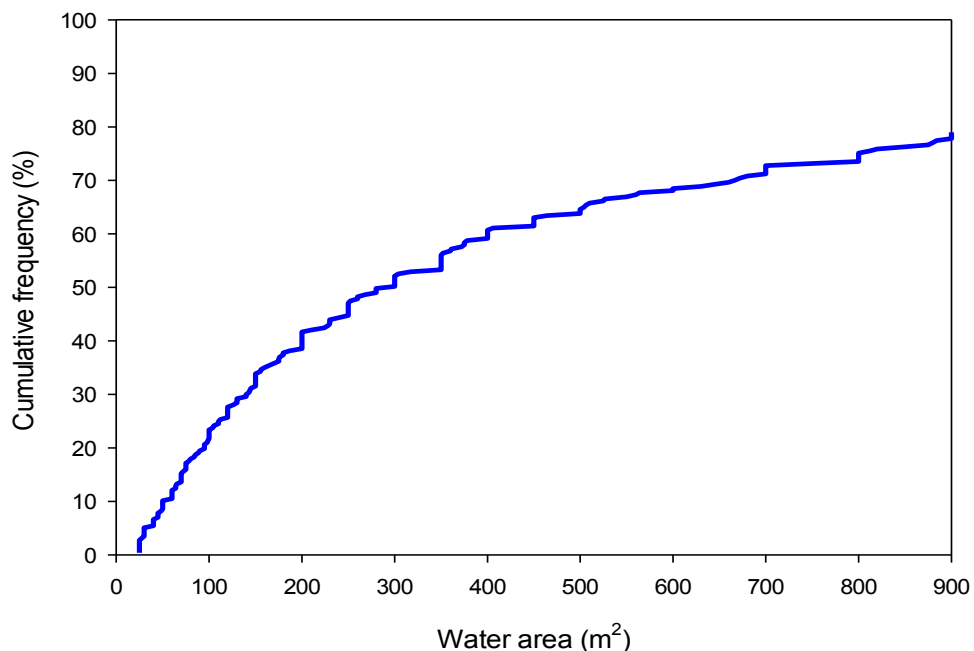
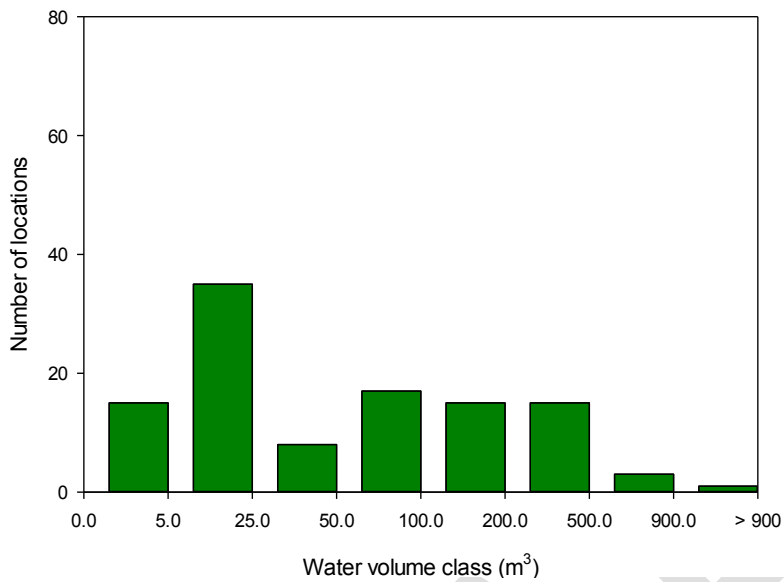


Figure 48: Frequency (A) and cumulative frequency distribution (B) of water surface area of 257 ponds in UK. Note that in graph 7A the x-axis is not linear and that all class boundaries are indicated. In graph 7B ponds with a surface area greater than 900 m² have not been included.

8832

8833

A



B

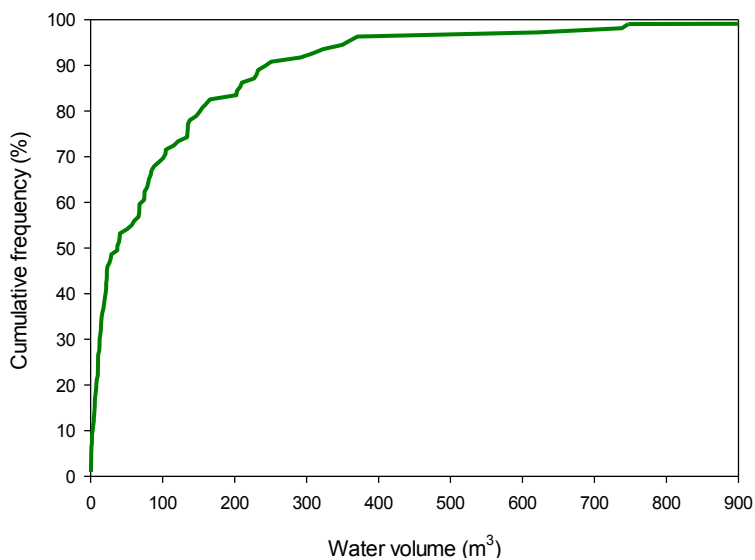
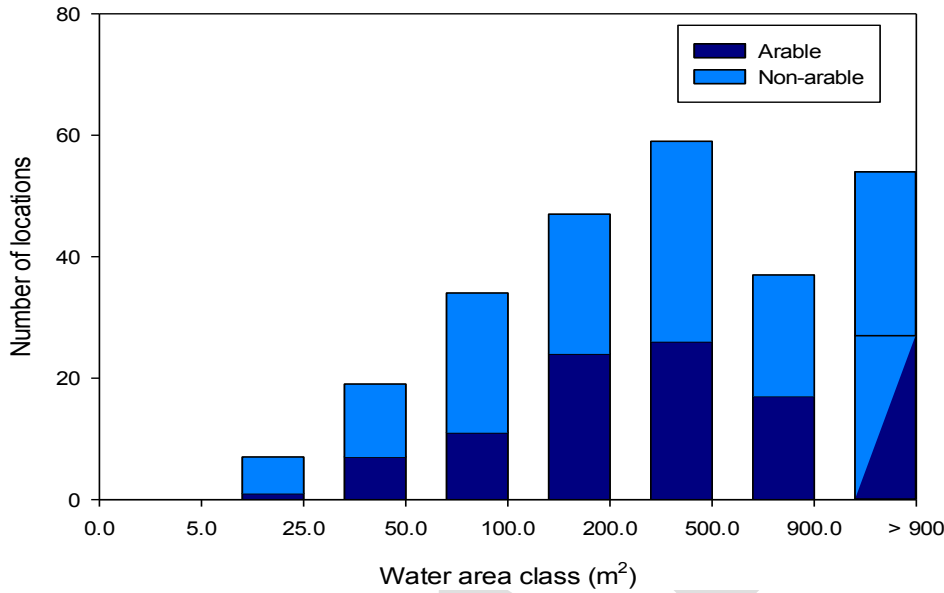


Figure 49: Frequency (A) and cumulative frequency (B) of water volume of 109 water bodies in the UK for which the water depth was determined, i.e. water bodies with depth < 1 m. Note that in graph A the x-axis is not linear and that all class boundaries are indicated. In graph B water bodies with a volume greater than 900 m³ have not been included.

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8835

A



B

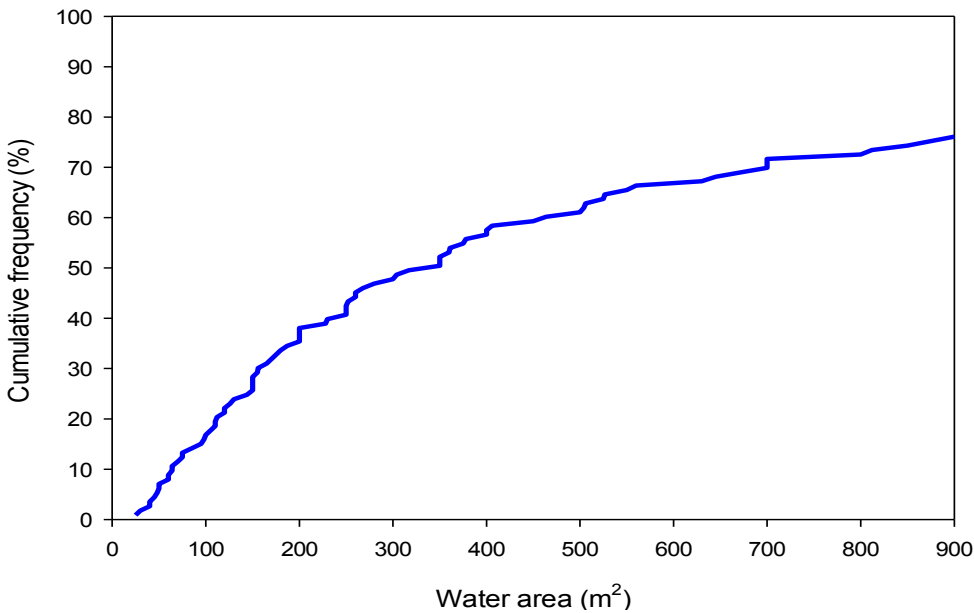


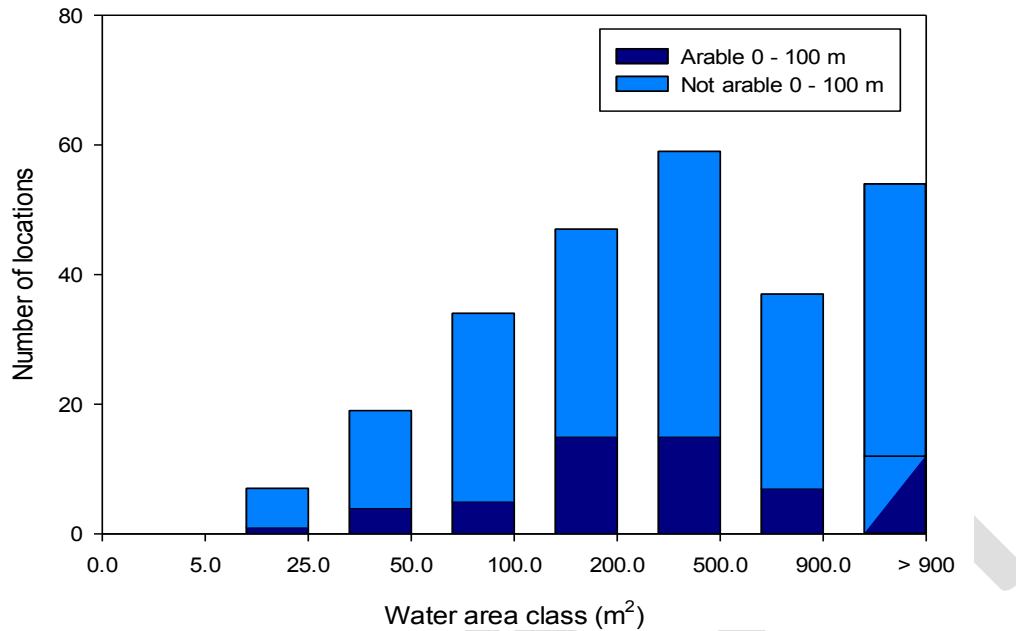
Figure 50: Frequency distribution (A) of water surface area of the 113 ponds in land use classes containing arable land use according to the ITE Land Class Number plus the 144 remaining ponds in the UK. Cumulative frequency distribution (B) is for the 113 ponds.

8836

8837

8838

A



B

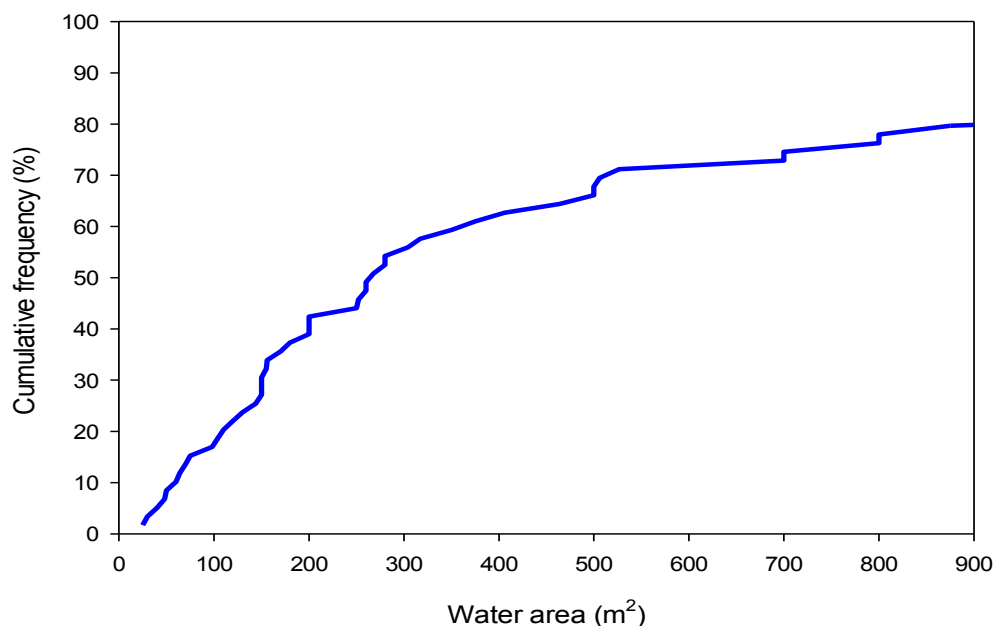
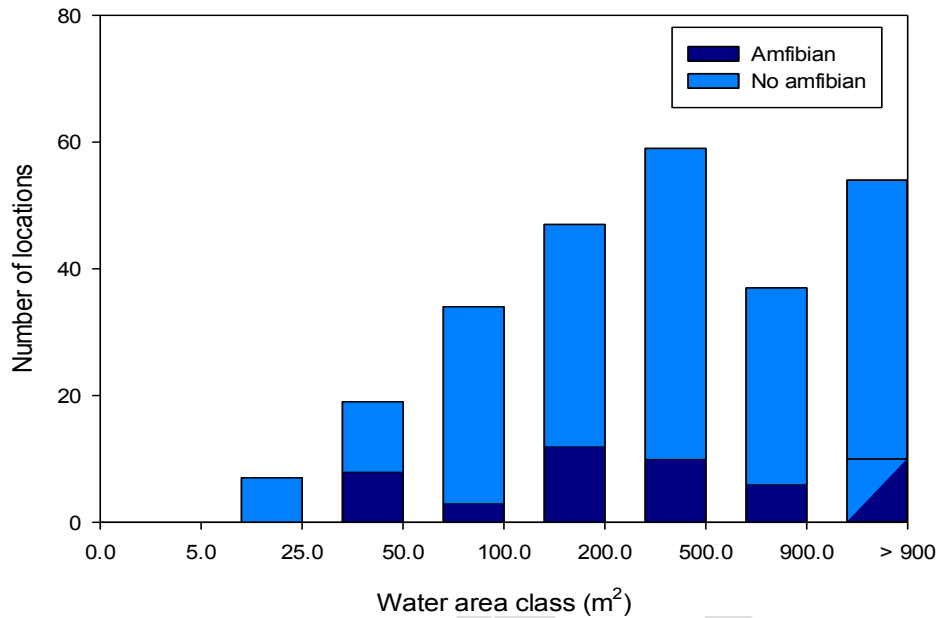


Figure 51: Frequency distribution (A) of water surface area of the 59 ponds in UK where a percentage of arable land use was observed in the 0 - 100 m distance zone from the perimeter of the pond, plus the remaining 198 sampled ponds. Cumulative frequency distribution (B) is for the 59 ponds.

8839

8840

A



B

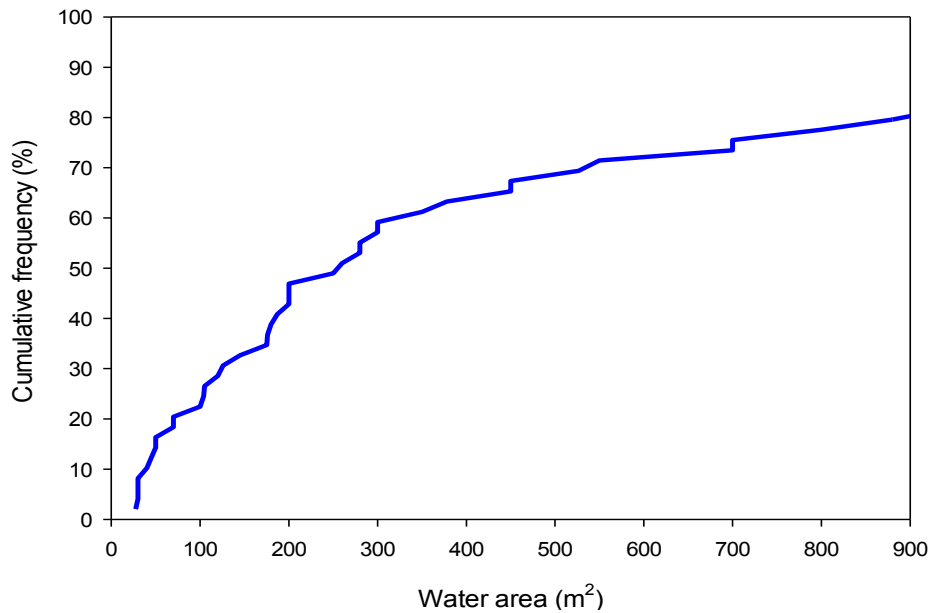


Figure 52: Frequency distribution (A) of water surface area of the 49 ponds in UK where amphibians were observed, plus the remaining 208 sampled ponds. Cumulative frequency distribution (B) is for the 49 ponds where amphibians were observed.

8841

8842

8843

8844 Comparison of analysed ponds and FOCUS water bodies

8845 The analysis of water depth, surface area and volume of the Spanish and Swiss amphibian ponds and
8846 the CountrySide Survey ponds in the UK demonstrates that the large majority (70% - 90%) of them is
8847 considerably smaller than the FOCUS ponds. The smaller water depth and often smaller width imply
8848 that peak concentrations due to spray drift depositions will be higher in the large majority of the
8849 analysed ponds than in the FOCUS pond (assuming similar spray drift deposition). The smaller water
8850 volumes imply that peak concentrations due to drainage or runoff entries will be higher in the
8851 analysed ponds than in the FOCUS ponds (assuming similar drainage or runoff entries per m² treated
8852 area and similar land:water ratios). So, we do not expect peak concentrations in FOCUS ponds to be
8853 conservative estimates for those in the analysed ponds.

8854 Comparing the peak concentrations in the analysed ponds to those in FOCUS ditches and streams is
8855 more complicated, therefore we are unable to make a statement on the conservativeness of peak
8856 concentrations in FOCUS ditches and streams for the analysed ponds. For spray drift-related peaks
8857 we have to consider that spray drift depositions on the FOCUS ditch and stream are expected to be
8858 considerably higher than the deposition on the analysed ponds, because of the relatively short
8859 distances crop-edge of water and the narrow 1-m wide water surface areas for FOCUS ditches and
8860 streams. However, water depths in the analysed ponds may be lower than the minimum water depth
8861 of 0.30 m in FOCUS ditches and streams, so this might countervail the higher deposited areic
8862 pesticide mass, although probably not to the same extent as the two factors mentioned above. So,
8863 spray-drift related peaks might be higher in FOCUS ditches and streams. For runoff-related or
8864 drainage-related peaks the initial water volume in the analysed ponds versus the volume of the
8865 FOCUS ditches and streams does not show clear differences, while the land:water ratio of the
8866 analysed ponds is unknown and the role of the upstream located fields (2 ha untreated for FOCUS
8867 ditch and 100 ha of which 20 ha treated for FOCUS stream) complicates further the comparison.

8868 However, in view of the higher flow-through rates in the FOCUS ditches and streams the pesticide
8869 concentrations are expected to remain for considerable longer periods in the analysed ponds than in
8870 FOCUS ditches and streams and thus the chronic exposure in the analysed ponds is probably
8871 underestimated. So, for the chronic risk assessment we expect that the FOCUS ditches and streams
8872 are not conservative.

8873

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- 8887

Appendix D – Adequacy of Step 3 FOCUS surface-water scenarios and models to predict exposure in the aquatic environment for amphibians:

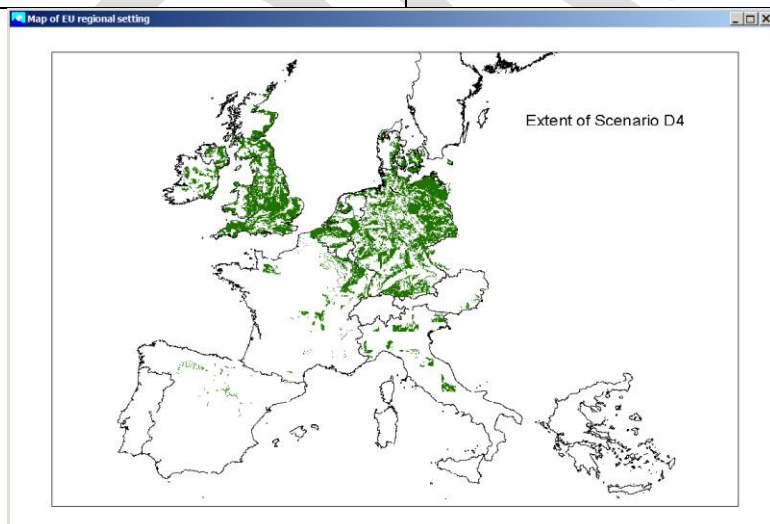
- 8888 In order to predict the exposure to PPP in surface waters different models may be used. The exposure
8889 assessment for the aquatic environment in the EU is currently based on the FOCUS methodology
8890 (FOCUS, 2001). It is a step-by-step procedure for the calculation of PECs in surface water. The
8891 procedure consists of four steps. In step 1 and 2 the water body is static with a depth of 30 cm and a
8892 5 cm deep sediment layer with 5% organic carbon. The width of the water body is not relevant in
8893 steps 1 and 2. In step 3 the water body is either a ditch, pond or stream adjacent to a single pesticide
8894 treated field. The step 3 waterbodies receive spray drift deposition, entries via runoff or eroded soil
8895 particles and entries by drain pipes. The pesticide input in a water body by spray drift deposition is
8896 determined by its distance to the treated field. For pesticide entries via runoff/erosion the size of the
8897 contributing area is relevant and the same holds for drainage entries. FOCUS ditches, streams as well
8898 as ponds may be fed by runoff/erosion or drainage. Streams are fed by a small pesticide-free base
8899 flow plus discharge from an upstream located catchment of 100 ha, of which 20 ha are assumed to be
8900 treated with pesticide. Ditches are fed by a small pesticide-free base flow plus the discharge of two
8901 upstream fields of 1 ha each, assumed to be not-treated. Ponds are fed by a small pesticide-free base
8902 flow plus the discharge of a surrounding area of 4500 m², which is treated (and spray drift deposition
8903 coming in from one 30-m long side). All ditches and streams are assumed to have a length of 100 m,
8904 a width of 1 m and a variable, but minimum depth of 30 cm, flow rates are up to 3100 m³/d for ditches
8905 and 28800 m³/d for streams
- 8906 Ponds are defined by surface water areas of 30 x 30 m together with a depth of 100 cm with
8907 generally low discharges of approximately 0.025 to 0.1 L/s, raising up to 0.4 to 1.6 L/s after runoff
8908 entries (FOCUS, 2001). This methodology was developed to predict a realistic worst-case exposure of
8909 fish, aquatic invertebrates and algae.
- 8910 At present the main limitation of the FOCUS surface water scenarios is that they consider only one
8911 year, which may result in unreliable estimates of 90th percentile exposure concentrations, leading to
8912 an underestimation of long term risks for the aquatic ecosystem. Considering exposure in FOCUS
8913 ponds, a limitation is that evaporation of the water in the pond is not taken into account; especially in
8914 shallow temporary water bodies, a preferred habitat for amphibians, this may be relevant, because
8915 without accounting for evaporation concentrations may not lower as expected or even increase over
8916 time.
- 8917 The FOCUS surface water scenarios were developed as a third step in a stepwise approach to
8918 calculate Predicted Environmental Concentrations (PEC_{sw}) in ten 'realistic worst case' scenarios
8919 (FOCUS, 2001). The scenarios cover a realistic range of surface water bodies, topography, climate,

8920 crops, soil types and agricultural management practices in the major agricultural areas of the
 8921 European Union. Their worst-caseness is mainly based upon the assessment of worst-caseness of
 8922 the pesticide entry routes, statistical methods following the criteria mentioned for the Exposure
 8923 Assessment Goals of Chapter 9 were not yet developed. The scenarios intend to represent 'realistic
 8924 worst case' scenarios for the PECs (dissolved concentration) in the water layer and not for the PECs in
 8925 sediment. Ponds figure in only three of the ten FOCUS scenarios (Table 49: and Figures 53, 54 and
 8926 55) and thus, they do not cover large parts of the European Union, especially in southern Europe.
 8927 Moreover, as the FOCUS surface water scenarios have been designed in the late nineties of the former
 8928 century, the newer MS, including many eastern European MS were not considered in the scenario
 8929 development procedure and thus these are not covered as well. Generally speaking, for an active
 8930 substance to pass the risk assessment the pond as well as the stream and ditch scenario needs to be
 8931 passed.

8932

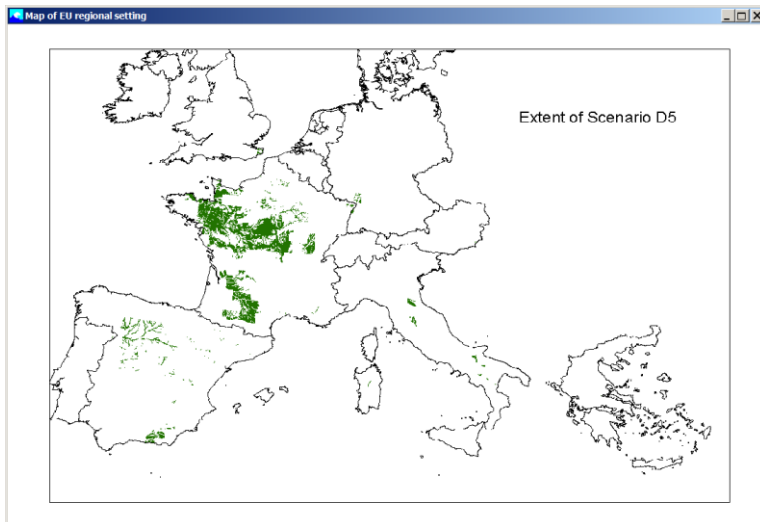
8933 **Table 49:** Overview of FOCUS surface water scenarios with their code D or R (indicating that either
 8934 drainage or runoff+erosion is the main entry route for pesticides, next to spray drift
 8935 deposition), their associated water body types and meteorological station.

Scenario code	Type of water bodies	Meteorological station
D1	Ditch, stream	Lanna, Sweden
D2	Ditch, stream	Brimstone, UK
D3	Ditch	Vredepeel, Netherlands
D4	Pond, stream	Skousbo, Denmark
D5	Pond, stream	La Jaillièrre, France
D6	Ditch	Thiva, Greece
R1	Pond, stream	Weiherbach, Germany
R2	Stream	Porto, Portugal
R3	Stream	Bologna, Italy
R4	Stream	Roujan, France



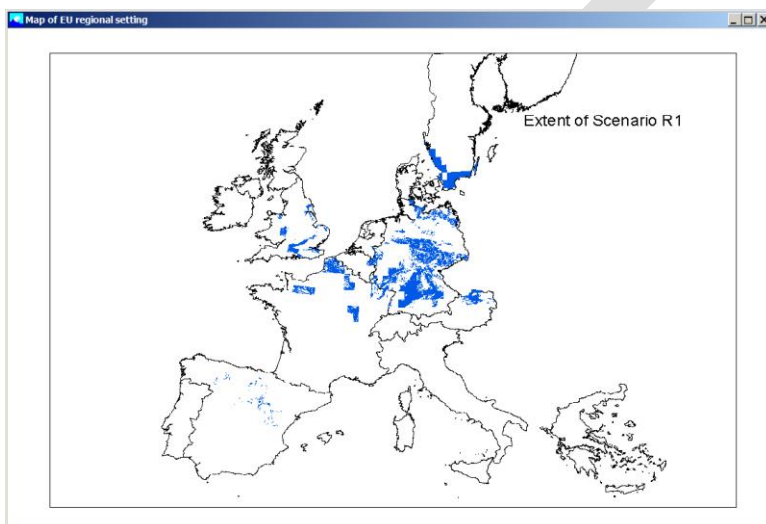
8936

8937 **Figure 53:** Fig. P1. Extent of FOCUS surface water scenario D4 in the EU15, that includes ponds
 8938 (source: FOCUS SWASH 5.3).



8939

8940 **Figure 54:** Fig. P2. Extent of FOCUS surface water scenario D5 in the EU15, that includes ponds
 8941 (source: FOCUS SWASH 5.3).



8942

8943 **Figure 55:** Fig. P3. Extent of FOCUS surface water scenario R1 in the EU15, that includes ponds
 8944 (source: FOCUS SWASH 5.3).

8945

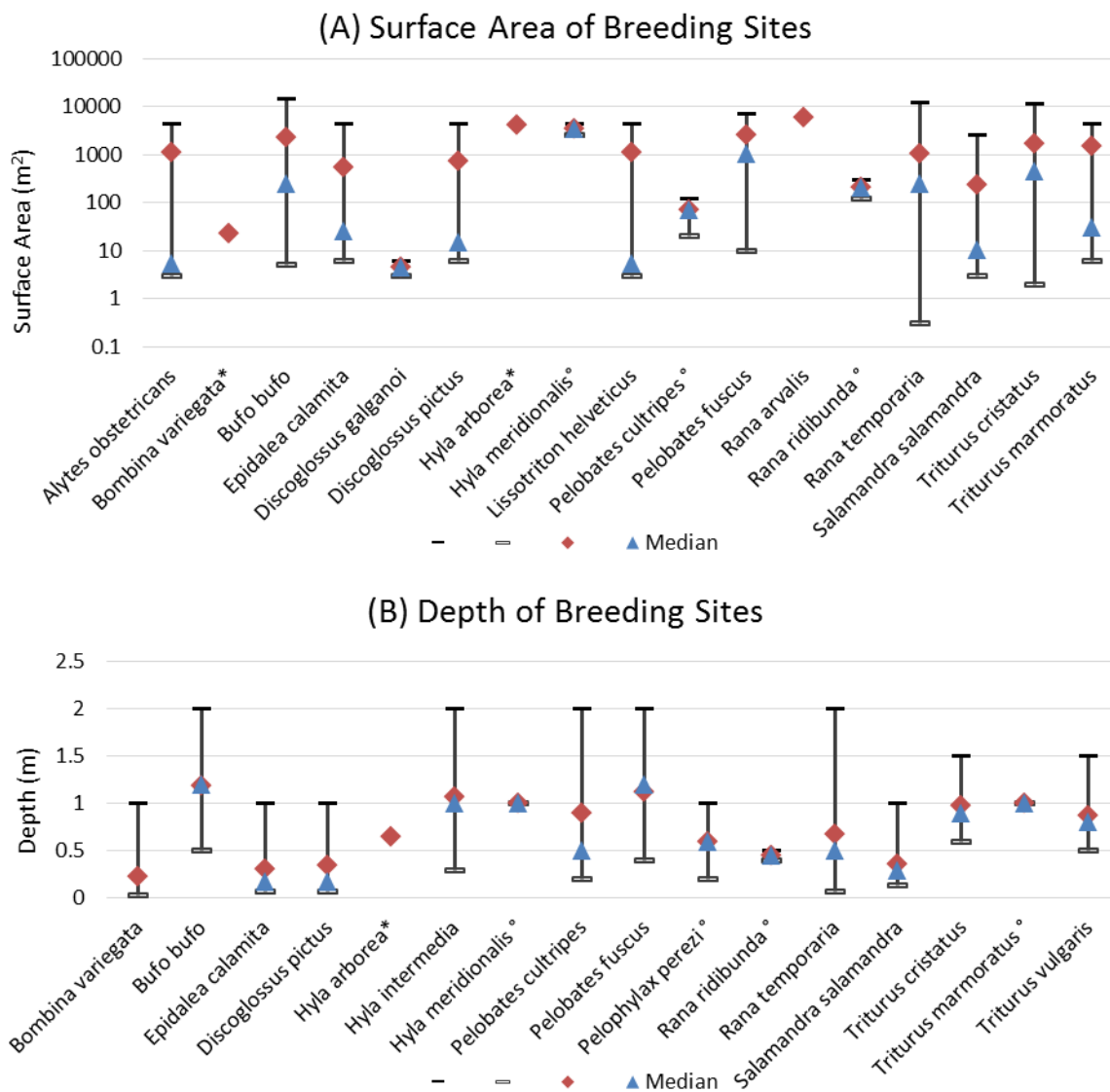
Appendix E – Type and size of water body preferred for breeding by different amphibian species

8946 Surface area of breeding sites

8947 From a summary of data from literature data on breeding sites was extracted and used for descriptive
 8948 statistics. Minimum and maximum values as well as means and medians for different amphibian
 8949 species were determined and displayed in Figure 56: . Data was retrieved from studies, where it was
 8950 explicitly specified that ponds were used as breeding-sites or wherever the presence of juveniles,
 8951 tadpoles, or eggs were reported.

8952 Surface size measurements for sites, in which the use as breeding site was not explicitly stated or only
 8953 the presence of adults was reported, were not considered for this graph but were listed separately
 8954 within the data evaluation excel sheet. Exploratory Analysis on Pond Surface Data of Breeding Sites,
 8955 please refer to Figure 56: .

8956 The retrieved data consisted out of measurements of single ponds and mean values for several ponds
 8957 described in one study. If for one species, both mean values and single values were reported in
 8958 several studies, means were treated as single measurements for descriptive statistics when
 8959 summarizing all studies. Then, for calculation of means, the reported mean values were not weighted
 8960 by the number of ponds but treated as single values since sites were not randomly chosen in the
 8961 studies. This was done to not falsely inflate the descriptive statistics by giving too much power to the
 8962 mean values of selected sites. Instead, they were treated as single pond measurements and used for
 8963 calculations of arithmetic means. If only one mean value was reported for a species, the mean value
 8964 was displayed and species were marked with a * in Figure 56: .



8965

8966

8967 **Figure 56:** Ranges of surface area (A) and depth measurements (B) of breeding sites reported in
 8968 literature for different amphibian species. Medians (blue triangle) and means (red diamond) were
 8969 calculated from literature values for $n \geq 2$. Species for which only 2 data points were available are
 8970 marked with a °; species for which only a single mean value was reported are marked with a *.

8971 Surface area and depth data could be evaluated for 17 and 14 species, respectively. Median surface
 8972 areas ranged from 4.50 m² to 3500 m² for *Discoglossus galganoi* (n=3) and *Hyla meridionalis* (n=2),
 8973 respectively (Garcia-Gonzalez & Garcia-Vazquez 2012; Ruhi et al., 2012). The smallest median depth
 8974 was reported for *Discoglossus pictus* (n=10) with 0.18 m (Ruhi et al., 2012; Sebastian et al., 2015).
 8975 Maximum median depth values were reported for *Bufo bufo* (n=6) and *Pelobates fuscus* (n=23) with
 8976 1.20 m, respectively (Eggert & Guyétant 1999; Nystrom et al., 2007; Ruhi et al., 2012; Sebasti &

8977 Carpaneto 2004; Sztatecsny & Holdl 2009). The compiled data can aid to add certainty to the
8978 characterization of breeding habitats. The data gives a rough estimate of the ranges of breeding pond
8979 sizes within which species occurred, but does not rule out that habitats of different sizes might also be
8980 suitable for the respective species.

8981

8982 **List of Publications that were used to answer the research question and from which data**
8983 **was retrieved**

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Appendix F – Toxicity studies and available endpoints for fish and sediment dwellers

9203 Acute toxicity to fish

9204 Acute Toxicity Test (OECD 203, exposure for 96 hours)

9205 Mortalities are recorded at 24, 48, 72 and 96 hours and the concentrations, which kill 50 per cent of
9206 the fish (LC50), are determined where possible.

9207 Long-term and chronic toxicity to fish

9208 Prolonged Toxicity Test (OECD 204, exposure for 14 days)

9209 Threshold levels of lethal and other observed effects and NOEC are determined at intervals during the
9210 test period, which is at least fourteen days. Observed effects other than lethal effects are on the
9211 appearance, size and behaviour of the fish, e.g. different swimming behaviour, different reaction to
9212 external stimuli, reduction or cessation of food intake.

9213 Fish early life stage toxicity test (OECD 210, exposure from fertilised egg to free-feeding)

- 9214 Tests with the early-life stages of fish are intended to define the lethal and sub-lethal effects of
9215 chemicals on the stages and species tested. Observed effects are cumulative mortality, numbers of
9216 healthy fish at end of test, time to start of hatching and end of hatching, numbers of larvae hatching
9217 each day, length and weight of surviving animals, numbers of deformed larvae, numbers of fish
9218 exhibiting abnormal behaviour. Reproduction is not measured in this test.
- 9219 Juvenile Growth Test (OECD 215, exposure 28 days)
- 9220 This test is designed to assess the effects of prolonged exposure to chemicals on the growth of
9221 juvenile fish. Observed effects are external abnormalities (such as hemorrhage, discoloration),
9222 abnormal behaviour, weight and mortality.
- 9223 Short Term Reproduction Assay (OECD 229, exposure 21 days)
- 9224 A fish assay capable of detecting endocrine active substances. Vitellogenin and secondary sexual
9225 characteristics are the two biomarkers, which are measured in addition to an evaluation of
9226 quantitative egg production (fecundity) and a performance of gonadal histopathology. Additionally
9227 abnormal behaviour (such as hyperventilation, uncoordinated swimming, loss of equilibrium, and
9228 atypical quiescence or feeding), external abnormalities (such as haemorrhage, discoloration),
9229 territorial aggressiveness, appearance of the fish and mortality are noted.
- 9230 Fish full life cycle test (EPA)
- 9231 In the Fish Life Cycle Toxicity Test, fish are cultured in the presence of the test substance from one
9232 stage of the life cycle to at least the same stage of the next generation (e.g. egg to egg) this leads to
9233 study durations of 100-190 days, depending on the selected fish species. The test covers the hatching
9234 of larvae, a growth phase of juvenile fish and reproduction. Once the fish are mature and start
9235 spawning, the egg number and fertilization rate is documented. The hatching success and survival of
9236 the F1 generation is evaluated. During the test period, the fish of the parental and F1 generation are
9237 observed daily for survival, hatching, abnormal appearance and behaviour. Length, weight and sex
9238 ratio are evaluated at the end of the test and of those fish being removed from the test. During the
9239 reproductive phase, coagulated and fertilized eggs are counted. Additionally, at the end of the test the
9240 vitellogenin level, which is an egg yolk precursor produced in the liver as response of circulation
9241 endogenous oestrogen (of blood or tissue sample), is measured. Furthermore, the sex ratio is
9242 analysed and a histopathology is performed.
- 9243 Bioconcentration in fish (OECD 305)
- 9244 The test consists of two phases: the exposure (uptake) and post-exposure (depuration) phases. The
9245 uptake rate constant, the depuration (loss) rate constant (or constants, where more complex models
9246 are involved), the bioconcentration factor, and where possible, the confidence limits of each of these
9247 parameters are calculated from the model that best describes the measured concentrations of test
9248 substance in fish and water.
- 9249 **Long-term and chronic toxicity to sediment dwelling invertebrates**
- 9250 Sediment-Water Chironomid Toxicity Test using spiked sediment (OECD 218, exposure for 28, resp.
9251 65 days)
- 9252 First instar chironomid larvae are exposed in a water-sediment system to spiked sediment. Chironomid
9253 emergence and development rate is measured at the end of the test. The exposure of the chironomid
9254 larvae is expected to mainly occur via the pore water.
- 9255 Sediment-Water Chironomid Toxicity Test Using Spiked Water (OECD 219, exposure for 28, resp. 65
9256 days)
- 9257 First instar chironomid larvae are exposed in a water-sediment system to spiked water. The measured
9258 endpoints are the total number of adults emerged and the time to emergence.
- 9259 Sediment-Water Lumbriculus Toxicity Test using spiked sediment (OECD 225, exposure 28 days)
- 9260 *Lumbriculus variegatus* burrows in the spiked sediment and ingests sediment particles below the
9261 sediment surface. This ensures exposure of the test organisms to the test substance via all possible
9262 uptake routes (e.g. contact with, and ingestion of contaminated sediment particles, but also via

9263 porewater and overlying water). Effects on reproduction and the biomass of the test organisms are
9264 recorded.
9265

Appendix G – Oral and dermal exposure calculations

9266 Introduction

9267 Worst case exposure calculations were conducted in order to:

- 9268 • compare the relative importance of oral and dermal exposure pathways
- 9269 • identify the groups of amphibians and reptiles with the greatest oral and dermal exposure
- 9270 • investigate whether the exposure estimates in first tier risk assessment for birds and
9271 mammals cover amphibians and reptiles (only for oral exposure)

9272 The results should help to focus the efforts to the most important exposure pathways.

9273 The oral exposure was calculated for small, medium and large sized amphibians and reptiles
9274 weighting 1.4 g, 11 g and 100 g in order to find out which of the groups of organisms are most. For
9275 tortoises oral exposure was calculated for animals of 11 g, 100 g and 1000 g of weight. The weight of
9276 tortoise at hatching ranges from 9.6 to 12.7 g (Bertolero et al. 2011) and hence it was considered not
9277 meaningful to calculate oral exposure of a tortoise weighting only 1.4 g.

9278 An application rate of 1kg active substance/ha is assumed in the oral and dermal exposure
9279 calculations for easy comparisons between the different orders of vertebrates and routes of
9280 exposure.

9281 Oral exposure via food uptake

9282 The oral exposure is calculated with generic residues values (RUD) for different food items from the
9283 EFSA birds and mammals guidance (EFSA 2009), Appendix G, table1
9284 (<http://onlinelibrary.wiley.com/doi/10.2903/j.efsa.2009.1438/full>)

9285 The 90th percentile RUD values were used. These are the standard RUDs used in the first tier acute
9286 risk assessment for birds and mammals.

9287 Amphibians

9288 The estimated theoretical exposure (ETE) was calculated based on the power function for food intake
9289 rate (FI) and body weight (BW) from the T/herps model ([https://www.epa.gov/pesticide-science-and-
9290 assessing-pesticide-risks/t-herps-version-10-users-guide-risk-amphibians-and](https://www.epa.gov/pesticide-science-and-assessing-pesticide-risks/t-herps-version-10-users-guide-risk-amphibians-and)):

$$9291 \text{ FI} = 0.013(\text{BW})^{0.773}$$

9292 The body weight for a small frog of 1.4 g was taken from T-herps. The medium and large frogs weight
9293 was chosen in order to be comparable to lizard and birds. T-herps suggests a different food
9294 composition for medium and large frogs as they eat also other amphibians, reptiles and small
9295 mammals. However, in order to be better comparable to the exposure of insect feeding lizards and
9296 birds the food items were left the same as for the small frog (100% insects). The assumption of

9297 uptake of 100% insects is a worst case assumption and may need to be refined in a more realistic oral
 9298 exposure estimate for medium and large frogs.

9299 **Table 50:** Food intake rates frogs

	Body Weight (g)	Ingestion (Fdry) (g dw/day)	water content of food items	Ingestion (Fwet) (g ww/day)	FI (kg-diet/day)
Small frog	1.4	0.01686166	68.8	0.054043782	0.0000540
Medium	11	0.082973593	68.8	0.265941004	0.000265941
Large	100	0.457028573	68.8	1.464835169	0.001464835

9300

9301

9302 **Table 51:** The ETE (in mg/kg bw/d) was calculated for a small, medium and large frog

	RUD (mg/kg) 90%	Small frog (1.4 g)	Medium frog (11 g)	Large frog (100 g)
Food type		ETE mg/kg bw/d (application rate = 1kg/ha)		
insects foliar	54.1	2.088	1.308	0.793
ground dwelling with interception	9.7	0.374	0.235	0.142
ground dwelling without interception	13.8	0.532717	0.334	0.202

9303

9304

9305 Reptiles

9306 The ETE was calculated analogue to the equation in the birds and mammals GD. The estimated daily
 9307 exposure, i.e. the uptake of a compound via a single food item is given by the following equation:

$$ETE = \frac{FIR}{bw} \times C \times PT \quad [mg/kg \text{ bw/d}] \quad (4)$$

9308

9309

9310 In which:

9311 ETE = Estimated theoretical exposure (mg/kg bw/d)

9312 FIR = Food intake rate of indicator species (g fresh weight /d)

9313 bw = Body weight (g)

9314 C = Concentration of compound in fresh diet (mg/kg)

9315 PT = Fraction of diet obtained in treated area (number between 0 and 1)

9316

9317

$$\text{FIR} = \left(\frac{\text{DEE}}{\text{FE} * \left(1 - \frac{\text{MC}}{100}\right) * \left(\frac{\text{AE}}{100}\right)} \right) \quad [\text{g fresh weight/d}] \quad (5)$$

9318

9319 In which:

9320 DEE = Daily energy expenditure of the indicator species (kJ/d)

9321 FE = Food energy (kJ/dry g)

9322 MC = Moisture content (%)

9323 AE = Assimilation efficiency (%)

9324

9325 The daily energy expenditure is calculated according to the following formula:

$$\log \text{DEE} = \log a + b \times \log \text{bw}$$

9326

9327

9328 Log a and b are taken from the EFSA supporting publication Fryday and Thompson 2009)

9329 <http://onlinelibrary.wiley.com/doi/10.2903/sp.efsa.2009.EN-13/pdf>

9330 The calculation is based on the allometric equation for daily energy expenditure of non-desert lizards

9331 (allometric equation in table 9) and the assimilation efficiency of 0.71 for frillneck lizards feeding on

9332 insects

9333 Food energy content and moisture content for arthropod food was taken from birds and mammals

9334 GD (EFSA 2009), Appendix G, table 3.

9335 (https://www.efsa.europa.eu/sites/default/files/scientific_output/files/main_documents/1438.pdf)

9336 **Table 52:** Food intake rate calculation for lizards

	body weight (g)	DEE (kJ/d)	Food Energy (kJ/g dw)	water content	Assimilation efficiency	FI (kg diet/d)
Small lizard	1.4	0.229	22.7	68.8	0.71	0.000045626
Medium	11	1.503	22.7	68.8	0.71	0.00029896

lizard						
Large lizard	100	11.251	22.7	68.8	0.71	0.00223749

9337

9338 **Table 53:** Estimated theoretical exposure calculation for lizards

		Small lizard (1.4 g)	Medium lizard (11 g)	Large lizard (100 g)
	RUD (mg/kg)	ETE mg/kg bw/d (application rate = 1kg)		
insects foliar	54.1	1.763	1.47	1.21
ground dwelling with interception	9.7	0.316	0.264	0.217
ground dwelling without interception	13.8	0.45	0.375	0.309

9339

9340

9341 **Tortoise**

9342 The FIR was calculated based on data for food uptake data from 10 tortoises (4 *T. graeca*, 6 *T. hermanni*) in the weight range of 520 - 1720 g published in Franz et al. 2010

9344 Based on these 10 data points the following allometric equation was derived for the food intake rate:
9345 $y = -3.958\ln(x) + 5.0486$, $R^2 = 0.6987$

9346

9347 **Testudo sp.**

9348 **Table 54:** Estimated theoretical exposure calculation for tortoise

body weight (g)	Ingestion Fdry (g dw/day)	water content of food items	Ingestion (Fwet) (g ww/day)	FI (kg-diet/day)	RUD (mg/kg) 90%	ETE mg/kg bw/d (application rate = 1kg)
11	22.899	88.1	192.425	0.192	70.3	13.528
100	14.162	88.1	119.010	0.119	70.3	8.366
1000	5.049	88.1	42.425	0.042	70.3	2.982

9349

9350 **Snakes:**

9351 It was considered to reflect the oral exposure of a snake better if the oral uptake is calculated for one
9352 feeding event instead of calculating a daily average exposure based on daily energy demand.

9353 In the calculations it is assumed that the snake feeds on a freshly oversprayed frog and that all the
9354 residues on the frog are taken up. For calculations of residues in frogs see below the section on
9355 dermal exposure from overspray. The formula for all frogs ($S_{\text{skin}}(\text{cm}^2) = 1.131 W_t^{0.579}$ (g)) from the
9356 publication of Hutchinson et al.1968 was used for estimating the frog surface.

9357 The oral exposure estimate is based on the average prey item weight expressed in terms of
 9358 percentage of snake body weight. The underlying data on snake body size and prey size are from a
 9359 study of Reading and Davis (1996). The mean prey size of male and female N.atrix was estimated as
 9360 40.25% and 27.6% of their body weight.

9361 **Table 55:** Oral exposure calculation for snakes

Snake body weight (g)	Prey weight in g (40.25 % of snake bw)	Surface of prey (cm ²)	applied rate (mg/cm ²)	Prey dermal dose(mg/kg bw)	Food intake (kg ww/kg bw)	Oral exposure of snake (application rate = 1kg)
1.4	0.5635	0.8114	0.01	7.199565195	0.4025	2.898
2.87	1.155175	1.2295	0.01	5.321791963	0.4025	2.142
11	4.4275	2.6766	0.01	3.022733445	0.4025	1.217
100	40.25	9.6078	0.01	1.193510301	0.4025	0.48
1000	402.5	36.4437	0.01	0.452716342	0.4025	0.182

9362

9363

9364 **For comparison – Birds and Mammals**

9365 **Table 56:** Shortcut values and daily dietary dose calculation for indicator species according to EFSA
 9366 birds and mammals GD (EFSA 2009)

		body weight (g)	Shortcut value (90%tile RUD)	application rate (kg/ha)	Daily dietary dose (mg/kg bw/d)	Scenario
Birds						
Small insectivorous	Blue tit	13.3	46.8	1	46.8	Screening
Large herbivorous	Goose	2645	30.5	1	30.5	Screening
Mammals						
Small insectivorous	Common Shrew	9.7	7.6	1	7.6	1 st tier (cereals)
Herbivorous small	Vole	25	136.4	1	136.4	Screening
Herbivorous large	Rabbit	1543	42.1	1	42.1	1 st tier (cereals)

9367

9368 **Water uptake calculations for reptiles**

9369 The calculation was based on the allometric equation for water flux in non-desert Lacertidae in
 9370 Friday and Thompson 2009:

9371 $\text{Log water flux} = \log - 0.8562 + 0.725 * (\log \text{ body weight})$

9372 Metabolic water (ml) = DEE (kJ) * 0.0278 (ml/kJ)

9373 Water content in food items = 68.8%

9374 The DEE and the water content are identical with the ones above in the calculations for food uptake
9375 in sand lizards.

9376 In order to calculate the drinking water demand the metabolic water and the water content in food
9377 items was deducted from the water flux (see **Error! Reference source not found.** below):

9378 **Table 57:** Water uptake calculation for lizards

Lizard	body weight (g)	water flux (mL/d)	metabolic water (mL)	water content in food items	Drinking water demand	
					mL	L/kgbw/d
Small	1.4	0.177722669	0.00638	0.031390902	0.13995	0.09997
Medium	11	0.792154141	0.04179	0.205681688	0.54468	0.04952
Large	100	3.924641574	0.31278	1.539393464	2.07246	0.02072

9379

9380 The resulting drinking water demand of 0.049 L/kg bw/d for a medium sized lizard (11g) is about 10
9381 times lower than the drinking water demand for a small granivorous bird (15.3 g) of 0.46 L/kg bw/d
9382 which is the basis for calculating drinking water exposure in the birds and mammals GD. Therefore it
9383 can be concluded that the estimate for drinking water uptake for birds would cover the water uptake
9384 of lizards.

9385

9386

9387 **Conclusions for oral exposure estimates**

- 9388 1. Insectivorous lizards have a similar oral exposure than insectivorous amphibians.
- 9389 2. Herbivorous reptiles (tortoise) have a greater oral exposure than insectivorous reptiles.
- 9390 3. The estimated oral exposure of snakes from consumption of an oversprayed frog is slightly
9391 greater than the oral exposure of insectivorous lizards and amphibians and it is slightly below
9392 the oral exposure of tortoise.
- 9393 4. Birds and mammals have a greater oral exposure than amphibians and reptiles. Hence the
9394 screening and first tier exposure assessment for insectivorous and herbivorous birds and
9395 mammals would most likely cover amphibians and reptiles.

9396 5. The estimated drinking water uptake is about 10 times lower in lizards than the estimated
 9397 drinking water uptake used in the birds and mammals GD suggesting that the first tier water
 9398 exposure assessment for birds covers lizards.

9399

9400 **Dermal exposure**

9401 For the calculation of the dermal dose it was assumed that the animal is oversprayed in field at the
 9402 full rate, only upper side exposed (half of its surface) and that 100% is absorbed. The application rate
 9403 was assumed to be 1kg/ha (as for oral uptake).

9404

9405 **Amphibians**

9406 In Wildlife exposure factors handbook (USEPA) equations are provided to calculate the surface with a
 9407 power function of the animals` weight (p3-14 or 514/572):

9408 - $S_{\text{skin}} (\text{cm}^2) = 1.131 W^{0.579}$ (g) (all frogs) (less protective when compared with 2 other
 9409 models)

9410 - $S_{\text{skin}} (\text{cm}^2) = 0.953 W^{0.725}$ (g) bull frog)

9411 - $S_{\text{skin}} (\text{cm}^2) = 0.997 W^{0.712}$ (g) green frog)

9412 - $S_{\text{skin}} (\text{cm}^2) = 8.42 W^{0.694}$ (g) salamanders

9413

9414 The allometric equations for body surface area from the US EPA exposure handbook are identical
 9415 with the ones from Hutchinson et al 1968

9416

9417 The formula for *Hyla arborea* from Hutchinson et al 1968 was added to the species from the Wildlife
 9418 exposure handbook.

9419 $SA = 0.905 * W^{0.823}$

9420 SA = surface area in cm^2

9421 W = body weight in g

9422 **Table 58:** Dermal exposure calculation from overspray for different groups of amphibians

Amphibians	Body weight (g)	Surface (cm ²)	Dermal absorption %	applied rate (kg/ha)	applied rate (mg/cm ²)	Dermal dose (mg/kg)
Green frog	85	23.5744	100	1	0.01	1.387
Bull frog	500	86.2673	100	1	0.01	0.863
All frogs	100	16.2728	100	1	0.01	0.814
Hyla	1.4	1.1937	100	1	0.01	4.263

arborea						
Hyla arborea	11	6.5120	100	1	0.01	2.96
Salamander	50	127.1737	100	1	0.01	12.717

9423

9424

9425 **Reptiles**

9426 Lizards

9427 The surface to body weight equation for *Lacerta agilis* from Fryday and Thompson 2009 (p. 52,
9428 below **Error! Reference source not found.**) was used. ()

9429 $S = 11.6 * W^{0.68}$

9430 SA = surface area in cm²

9431 W = body weight in g

9432 **Table 59:** Dermal exposure calculation from overspray for lizards

	Body weight (g)	Surface (cm2)	Dermal absorption %	applied rate (kg/ha)	applied rate (mg/cm2)	Dermal Dose (mg/kg bw)
Small lizard	1.4	14.582	100	1	0.01	52.08
Medium lizard	11	59.239	100	1	0.01	26.927
Large lizard	100*	265.741	100	1	0.01	13.287

9433 *A body weight of 100 g is outside of the range of adult body weights. It was included only for purpose of comparison
9434 with other groups.

9435

9436 Snakes

9437 The body surface was calculated for 46 *Coronellea austriaca* individuals based on total length and
9438 weight data (Brown et al. 2014). A power function was fitted to weight and body surface resulting in
9439 the following formula (SA = Surface area in cm2, W = body weight in g):

9440 $SA = 12.688 * W^{0.6812}, (R^2 = 0.9742)$

9441 **Table 60:** Dermal exposure calculation from overspray for snakes

	Body weight (g)	Surface (cm2)	Dermal absorption %	applied rate (kg/ha)	applied rate (mg/cm2)	Dermal Dose (mg/kg bw)
Coronella austriaca	1.4	15.9564	100	1	0.01	56.987
Coronella austriaca	2.87	26.0197	100	1	0.01	45.331
Coronella austriaca	11	64.9815	100	1	0.01	29.537
Coronella austriaca	100*	292.2760	100	1	0.01	14.614

9442 * A body weight of 1.4 g is below the range of hatchling body weight and 100 g is outside of the range of adult body
9443 weights. Both weights were included only for purpose of comparison with other groups.

9444

9445 **Conclusions for dermal exposure from overspray:**

- 9446 1. The dermal exposure from overspray is greater for reptiles than for amphibians with equal
9447 weights. Lizards and snakes have similar dermal exposure (probably because the similarities
9448 in their shape and hence surface to volume ratio).
- 9449 2. For amphibians the dermal exposure from overspray is comparable to the daily dietary dose.
- 9450 3. For lizards and snakes the dermal exposure from overspray is about one order of magnitude
9451 greater than the daily dietary dose.
- 9452 4. The dermal exposure from overspray is lower for amphibians compared to the daily dietary
9453 dose of birds and mammals. However, the dermal exposure for reptiles is in the same range
9454 as the daily dietary dose for birds and mammals.
- 9455

9456 **Overall conclusions with regard to coverage of amphibians and reptiles:**

- 9457 1. The oral exposure estimates from the screening steps in the risk assessment for birds and
9458 mammals may cover the risk to amphibians (depending on the toxicological sensitivity and
9459 assessment factors which are applied).
- 9460 2. The dermal exposure estimates for lizards and snakes are in the same range as the daily
9461 dietary exposure estimates for birds and mammals. The risk from dermal exposure is not
9462 assessed for birds and mammals. Therefore coverage of reptiles by the risk assessment for
9463 birds and mammals is highly uncertain.
- 9464 3. The comparisons of the daily dietary exposure and dermal exposure from overspray give an
9465 indication that both exposure pathways are of high importance and both need to be
9466 considered in the risk assessment for amphibians and reptiles.
- 9467

9468 The following is needed in order to address dermal exposure from contact to residues in soil and
9469 plants:

- 9470 1. An estimate of the body surface of amphibians, snakes and tortoise in contact with soil and
9471 plants while moving.
- 9472 2. Dermal absorption factors for amphibians and reptiles
- 9473 3. Estimate of the body surface in contact with the soil when they move.
- 9474 4. Speed of movement
- 9475 5. Time of when they are actively moving vs resting

9476

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9501

Appendix H – Review of existing exposure models and suggestions for development of oral and dermal exposure models for amphibians and reptiles.

9502

9503 Dermal Exposure

9504 Dermal exposure models used in human risk assessment

9505 1) Dermal exposure of worker

9506 2) Dermal exposure of resident

9507 3) Dermal exposure of bystander

9508

9509 Dermal exposure of worker

9510 The worker dermal exposure may take place from contact with residues on foliage and is estimated
9511 as the product of the dislodgeable foliar residue (DFR), the transfer coefficient (TC) and the task
9512 duration (T):

9513

$$\text{Potential dermal exposure (PDE) in mg a.s./day} = (\text{DFR } [\mu\text{g}/\text{cm}^2] \times \text{TC } [\text{cm}^2/\text{h}] \times \text{T } [\text{h}/\text{day}]) / 1\,000$$

(Equation. 1)

9514

9515

9516

9517 The default value for exposure duration is eight hours for harvesting and maintenance type activities
9518 and two hours for crop inspection and irrigation-type activities.

9519 To convert estimated dermal exposures to corresponding systemic exposures, dermal exposure
9520 should be multiplied by a dermal absorption factor, as derived from the toxicological assessment.

9521

9522 Dislodgeable foliar residue (DFR)

9523 The amount of residue on foliage depends on the application rate, application efficiency (how much
9524 reaches and is retained on the target), crop type and the amount of foliage (leaf area index).

9525 Dissipation of residues on crop foliage over time depends on the physical and chemical properties of
9526 the applied PPP, and also on environmental conditions. Where experimentally determined DFR data
9527 are not available, the initial DFR (DFR₀ is the DFR just after application, it assumes that no dissipation
9528 will take place and that everything is dislodgeable) in a first tier assessment should assume **3 µg**
9529 **active substance/cm² of foliage/kg a.s. applied/ha**; which is about the 90th-percentile of the

9530 distribution, thus the provided value was regarded as highly conservative (EUROPOEM II, Re-entry
9531 report 2002¹⁷ and EFSA Guidance 2014).

9532 It is allowed to refine the assessment for dissipation (decay) of the active substance on the foliage if
9533 the exact nature of the dissipation over time is known. If no data are available on the degree of
9534 dissipation, it may be assumed that active substances which are organic chemicals, and for which
9535 there is evidence of breakdown e.g. by photolysis or hydrolysis in soil or water, or decline of
9536 concentration due to plant growth will dissipate with a half-life of 30 days. For other categories of
9537 active substance DFR0 is used in the respective calculations.

9538 For PPPs with multiple treatments sought, the assessment should consider the potential
9539 accumulation of DFR from successive treatments. If no experimental data are available, and an active
9540 substance is assumed to dissipate with a half-life of 30 days [this value differs from that proposed in
9541 the birds and mammals opinion (EFSA PPR, 2008) because it was decided to follow a more
9542 conservative approach based on the available data indicating possible DT50 values up to and
9543 exceeding 30 days for some active substances], the dissipation should be taken into account by
9544 application of an appropriate multiple application factor (MAF), examples of which are given in Table
9545 61: .

9546 For new active substances, it will be possible to consider any new experimental data in the exposure
9547 calculator; refined calculations with specific values are not considered necessary when exposure
9548 estimates in the first tier are below the established trigger.

9549

9550 **Table 61:** Multiple application factors, assuming a default dissipation half-life of 30 days (EFSA PPR
9551 Panel, 2010, EFSA Guidance, 2014)

9552

Days*	Number of applications											
	1	2	3	4	5	6	7	8	9	10	11	12
7	1.0	1.9	2.6	3.2	3.7	4.2	4.5	4.9	5.1	5.4	5.6	5.7
10	1.0	1.8	2.4	2.9	3.3	3.6	3.9	4.1	4.2	4.4	4.5	4.5
14	1.0	1.7	2.2	2.6	2.9	3.1	3.2	3.3	3.4	3.5	3.5	3.5
21	1.0	1.6	2.0	2.2	2.4	2.5	2.5	2.6	2.6	2.6	2.6	2.6

9553 * Interval between applications

9554

¹⁷ POST-APPLICATION EXPOSURE OF WORKERS TO PESTICIDES IN AGRICULTURE, EUROPOEM II PROJECT, FAIR3-CT96-1406, 2002

9555 Transfer coefficient (TC)

9556 The transfer of residues from the plant surface to the clothes or skin of the worker is taken into
 9557 account, regardless of the product applied. The level of exposure depends on the intensity,
 9558 frequency and duration of contact with the foliage. This is determined by the nature and duration of
 9559 the activity during re-entry to the treated crop. Therefore, it is possible to group various crop
 9560 habitats and re-entry activities.

9561

$$TC \text{ (cm}^2\text{/h)} = PDE \text{ (mg/h)}/DFR \text{ (mg/cm}^2\text{)} \text{ (equation 2)}$$

9562

9563

9564 The indicative TC values in Table 62: are based on and modified from EUROPOEM II (2002) and in
 9565 consideration of US EPA values and apply to both outdoor and indoor scenarios. These values are
 9566 used in first tier assessments of potential dermal exposure for the scenarios specified. Three sets of
 9567 TC values are given, according to whether or not it can be assumed that the worker will wear clothing
 9568 that covers the arms, body and legs. It is assumed that harvesting is performed with bare hands or
 9569 with gloves, and that dermal exposure to the body is reduced 10-fold by clothing covering the arms,
 9570 body and legs. When no PPE and no workwear are worn, exposures may be higher than these
 9571 estimates and potential exposure should be estimated using the values in the fourth column of Table
 9572 62: .

9573 These TC values may be extrapolated to other re-entry scenarios, where the intensity and duration of
 9574 contact with the foliage is judged to be similar.

9575

9576 **Table 62:** Transfer coefficients (TCs) (modified from EUROPOEM II (2002) considering US EPA,
 9577 2012; for both outdoor and indoor scenarios)

Crop	Nature of task (a)	Main body parts in contact with foliage	TC (cm ² /h), total potential exposure	TC (cm ² /h) assuming arms, body and legs covered (workwear; bare hands)	TC (cm ² /h), covered body (workwear) and gloves (PPE)	Applicable for the following crops
Vegetables	Reach/pick	Hand and body	5 800	2 500	580	Brassica vegetables, fruiting vegetables, leaf vegetables and fresh herbs, legume vegetables,

						bulb vegetables
Tree fruits	Search/reach/ pick	Hand and body	22 500	4 500	2 250	Citrus, cane fruits, oilfruit s, pome fruits, stone fruits, tree nuts
Grapes (b)	Harvesting and other er acti viti es (e.g : leaf pull ing and tyin g)	Hand and body	30 000	10 100	No justified prop osal poss ible (dat a missi ng)	n.a.
Strawberries	Reach/pick	Hand and for ear m	5 800 (c)	3 000	750	Berries and other small fruit, low
Ornamentals	Cut/sort/ bundle/ carry	Hand and body	14 000	5 000	1 400	Ornamentals and nursery
Golf course,	Maintenance	Hand and body	5 800	2 500	580	n.a.

turf or oth er spo rts law ns						
General (c)	Inspection, irrigation	Hand and body	12 500 (d) 7 500 (e)	1 400 (d)	No justified proposal possible	Cereals, grassland and lawns, hops, oilseeds, root and tuber vegetables, sugar beets, etc.

9578

9579
9580

For reptiles and amphibians the TC could be assumed to be equal to the part of the total surface area of their body in contact with soil or plants and the frequency (times of contact) with the contaminated crop.

9581

9582 According to the EFSA Guidance, the following points are noted:

9583 $SDE = (DFR \times MAF \times AR \times TC \times T / 1\,000 \times DA) / BW$

9584

9585 Dermal exposure for resident

9586 The dermal exposure for resident can take place as a result of exposure to drift, via contact with
 9587 surface deposits and entry into treated fields.

9588 **Table 63:** Overview on parameters for dermal exposure calculation

SDE	systemic dermal exposure	mg a.s./kg b.w/day	To be calculated
DFR	dislodgeable foliar residue	$\mu\text{g}/\text{cm}^2$	$3 \mu\text{g}/\text{cm}^2$ (default; EFSA, 2014)
MAF	multiple application factor	-	EFSA Guidance (2014) - Table 12 (Table 61:)
AR	application rate	kg a.s./ha	List of intended uses (GAP)
TC	transfer coefficient	cm^2/h	EFSA Guidance (2014) - Table 13 Table 62:)
T	duration of exposure	h/day	- 2 h/day for crop inspection or irrigation activities - 8 h/day for activities such as harvesting, cutting, sorting, etc. (defaults; EFSA, 2014)
DA	dermal absorption	-	the higher of the values for the product and for the in-use dilution
BW	body weight of worker	kg	60 kg

9589

9590

9591

9592 Spray drift:

9593 The exposures from spray drift should be calculated using the following equation:

9594

9595
$$\text{Dermal exposure} \times \text{dermal absorption percentage} + \text{inhalation exposure}$$

9596

9597 As concluded in the EFSA WoG and presented in the respective EFSA Guidance (2014) "Guidance on
 9598 the assessment of exposure of operators, workers, residents and bystanders in risk assessment for
 9599 plant protection products", the dermal and inhalation exposures (75th percentile and mean values)
 9600 for residents are as shown in Table 64: and Table 65: .

9601

9602 For arable crops, it was agreed that BREAM data provide a better estimate of exposure and are more
 9603 representative of modern practices. The BREAM results do not provide values for upwards spraying.

9604

9605 For orchard crops and vines, the most appropriate dataset out of the three presented is the dataset
 9606 for conventional nozzles (no drift reduction technologies) applying 470 L/ha from a report by Lloyd et
 9607 al. (1987) for an 8-m distance downwind from the middle of the tree trunk. This dataset gave the
 9608 highest drift exposures in that report and the respective values are considered to be suitable for a
 9609 resident located about 5 m from the edge of a field, assuming the space from the tree trunk to the
 9610 edge of the field is at least 3 m.

9611

9612 **Table 64:** Dermal exposures for residents (75th percentile from data on potential dermal exposures)
 9613 (adapted and amended from EFSA PPR Panel, 2010)

Method of application (distance from sprayer)		
Dermal (mL spray dilution/person)		
	Adults	Children
Arable/ground boom sprayer (from BREAM)		
2 m	0.47	0.33
5 m	0.24	0.22
10 m	0.20	0.18
Orchard/broadcast air assisted applications (Lloyd <i>et al.</i> 1987) (a)		
2–3 m	n.a.	n.a.
5 m	5.63	1.689
10 m	5.63	1.689

9614 (a): The only available values are for the 8-m distance downwind from the middle of the tree trunk, which are assumed to
 9615 represent a 5-m distance from the edge of the orchard; the same value is used for 5 and 10 m.
 9616 n.a., not available.

9617

9618 **Table 65: Table 4:** Dermal exposures for residents (mean data on potential dermal exposures)
 9619 (adapted and amended from EFSA PPR Panel, 2010)

9620

Method of application (distance from sprayer)		
Dermal (mL spray dilution/person)		
	Adults	Children
Arable/ground boom sprayer (from BREAM)		
2 m	0.22	0.18
5 m	0.12	0.12
10 m	0.11	0.10
Orchard/broadcast air assisted applications (Lloyd <i>et al.</i> 1987) (a)		
2–3 m	n.a.	n.a.
5 m	3.68	1.11
10 m	3.68	1.11

9621 (a): The only available values are for the 8-m distance downwind from the middle of the tree trunk, which are assumed to
 9622 represent a 5-m distance from the edge of the orchard; the same value is used for 5 and 10 m.
 9623 n.a., not available.

9624

9625 Surface deposits:

9626 Dermal exposure from surface deposits based on spray drift should be based on the following
 9627 equation (EFSA PPR Panel, 2010):

9628 $SERD = (AR \times D \times TTR \times TC \times H \times DA)/BW$ (equation 4)

9629 where:

9630 • SERD = systemic exposure of residents via the dermal route (mg/kg bw/day)

9631 • AR = application rate (mg/cm²) (consider MAF, if necessary)

9632 • D = drift (%) (if multiple applications have to be taken into account, a lower percentile could be
 9633 considered for risk refinement)

9634 • TTR = turf transferable residues (%) (for products applied in liquid sprays, 5 %, and for products
 9635 applied as granules, 1 % [these values come from data obtained using the Modified Californian Roller
 9636 Method (Fuller *et al.*, 2001; Rosenheck *et al.*, 2001) and represent the upper end of the range from a
 9637 number of studies with different compounds])

9638

- 9639 • TC = transfer coefficient (cm²/h) (default values of 7300 cm²/h for adults and 2600 cm²/h for
 9640 children are recommended, TC values take into account minimal protection from clothes)
- 9641 • H = exposure duration (hours) (a default value of two hours is recommended by US EPA, 2001)
- 9642 • DA = dermal absorption (%)
- 9643 • BW = body weight (kg).

9644

9645 Values for drift percentage should be taken from table 66, as appropriate.

9646

9647

9648 **Table 66:** Ground sediments based on drift as a percentage of the application rate

Distance	Field crops (a)		Fruit crops, early stages (b)		Fruit crops, late stages (b)		Grapes (b)		Hops (b)	
	Mean	P75	Median	P77	Median	P77	Median	P77	Median	P77
2–3 m	4.1	5.6	18.96	23.96	6.96	11.01	5.25	6.90	9.95	15.93
5 m	1.8	2.3	11.69	15.79	3.73	6.04	2.32	3.07	5.91	8.57
10 m	1.0	1.3	6.07	8.96	1.6	2.67	0.77	1.02	2.91	3.70

9649

(a): From BREAM.

9650

(b): From Ganzelmeier/Rautmann (the 75th percentile is not published).

9651

P75, 75th percentile; P77, 77th percentile.

9652

9653 Based on the limited availability of data, for products applied as granules, drift from applications of
 9654 granules should be assumed to be 3 % for broadcast (in the EFSA calculator, 3% is considered as drift
 9655 on surfaces independently of the distance) and manual applications. Further refinements could be
 9656 considered based on new data. Dust drift for in-furrow applications are considered to be negligible.

9657

9658 Entry into treated crops:

9659 Entry into treated crops is based on exposure from activities such as walking in treated fields for
 9660 adults.

9661 The method used should be the same as for workers, with the same DFR and a TC based on data for
 9662 inspection activities (75th percentile: 7500 cm²/h, mean: 5980 cm²/h), and with a 15-minute

9663 exposure. TC values are only available for adults. A factor of 0.3 has been applied to the adult TC for
9664 children re-entering treated crops.

9665 For entry onto treated lawns (two hours of inhalation), exposures should be calculated in the same
9666 way as surface deposits (see below as for bystander), but using a deposition percentage of 100 %.

9667

9668 Dermal exposure of Bystander

9669 Bystanders may be exposed briefly to plant protection products via spray drift. It is assumed that it
9670 would not take more than 5 minutes for the tractor to pass a bystander during which the bystander
9671 could be exposed directly.

9672 For the estimation of bystander exposure, the same approach as for residents should be followed,
9673 except for dermal and inhalation exposure to spray drift which should be taken as 95th percentile
9674 values derived from the respective datasets. However the estimation of exposure through the four
9675 pathways should be estimated separately since they are not expected to take place at the same time.

9676

9677 Spray drift:

9678 The exposures from spray drift should be calculated using the following equation:

9679 Dermal exposure × dermal absorption percentage + inhalation exposure,

9680 where the dermal absorption percentage is that for the in-use dilution taken from the toxicological
9681 evaluation and dermal and inhalation exposures are those shown in Table 67: .

9682

9683

9684 **Table 67:** Dermal exposures for bystanders (95th percentile) (adapted and amended from EFSA
 9685 PPR Panel, 2010)

9686

Method of application (distance from sprayer)		
Dermal (mL spray dilution/person)		
	Adults	Children
Arable/ground boom sprayer (from BREAM calculator)		
2 m	1.21	0.74
5 m	0.57	0.48
10 m	0.48	0.39
Orchard/broadcast air assisted applications (Lloyd <i>et al.</i> 1987) (a)		
2–3 m	n.a.	n.a.
5 m	12.9	3.87
10 m	12.9	3.87

9687 (a): The only available values are for the 8-m distance downwind from the middle of the tree trunk, which are assumed to
 9688 represent a 5-m distance from the edge of the orchard; the same value is used for 5 and 10 m.
 9689 n.a., not available.

9690

9691 Surface deposits:

9692 Dermal exposures from surface deposits based on spray drift should be based on the following
 9693 equation (EFSA PPR Panel, 2010):

9694 $SEBD = (AR \times D \times TTR \times TC \times H \times DA)/BW$ (equation 5)

9695 where:

- 9696 – SERD = systemic exposure of bystander via the dermal route (mg/kg bw/day)
- 9697 – AR = application rate (mg/cm²) (consider MAF, if necessary)
- 9698 – D = drift (%) (if multiple applications have to be taken into account, a lower percentile could
 9699 be considered for risk refinement)
- 9700 – TTR = turf transferable residues (%) (for products applied in liquid sprays, 5 % is used, and,
 9701 for products applied as granules, 1 % is used. These values come from data obtained using

- 9702 the Modified Californian Roller Method (Fuller et al., 2001; Rosenheck et al., 2001), and
 9703 represent the upper end of the range from a number of studies with different compounds
- 9704 – TC = transfer coefficient (cm²/h) (default values of 14 500 cm²/h for adults and 5 200 cm²/h
 9705 for children are recommended; TC values take into account minimal protection from clothes)
- 9706 – H = exposure duration (hours) (a default value of two hours to cover resident exposure)
- 9707 – DA = dermal absorption (%)
- 9708 – BW = body weight (kg).

9709

9710 Values for drift percentage should be taken from Table 68: , as appropriate.

9711

9712 **Table 68:** Ground sediments as a percentage of the application rate, calculated on the basis of the
 9713 95th/90th percentile values

Distance	Field crops (a)	Fruit crops, early stages (b)	Fruit crops, late stages (b)	Grapes (b)	Hops (b)
	95th perc entile	90th perc entile	90th perc entile	90th perc entile	90th perc entile
2–3 m	8.5	29.20	15.73	8.02	19.33
5 m	3.5	19.89	8.41	3.62	11.57
10 m	1.9	11.81	3.60	1.23	5.77

9714 (a): From BREAM, arable - ground boom.

9715 (b): From Ganzelmeier/Rautmann.

9716

9717 Drift from agricultural applications of granules (general granule application, e.g. slug pellets) is
 9718 assumed to be 3 % for broadcast and manual applications (“worst case”). Dust drift for in-furrow
 9719 applications is considered to be negligible.

9720

9721 Entry into treated crops:

9722 For entry into crops, the same approach as for resident should be followed.

9723 For entry onto treated lawns, exposures should be calculated in the same way as for surface deposits
 9724 (see above), but using a deposit (% of application rate) of 100 %.

9725

9726 Conclusion on the use of human dermal exposure models:

9727 In conclusion, the equation 1, used for the 1st tier potential dermal exposure estimation for the
9728 worker could be applied for the PDE estimates of amphibians and reptiles. More specifically, the DFR
9729 values $3 \mu\text{g active substance/cm}^2$ of foliage/kg a.s. applied/ha could be used as a 1st tier assessment.
9730 Furthermore, the TC could be estimated on the basis of the fraction of the total body area of the
9731 organism(s) and its activity (contact duration with new surfaces per hour) assuming that it is in
9732 continuous contact with the treated crop for a number of hours (T). The time will depend on the
9733 behavior of the animal and it will be estimated from the time spend in the treated crop or in the
9734 contaminated field. Furthermore, for multiple applications the MAF (examples in Table 61:) could be
9735 considered. If this approach will be applied the following parameters need to be identified for the
9736 most relevant life stage of the organism, in order to carry out the respective risk assessment:

9737 - Toxicological endpoint (TEP) and the respective threshold (NOAEL and Acceptable level of
9738 dermal exposure).

9739 - The assessment factor for the conversion of the NOAEL to the toxicological threshold,

9740 - If the TEP will be derived from a study carried out via the dermal route of exposure no
9741 dermal absorption factor is needed. In this case the acceptable dermal exposure (Regulatory
9742 threshold for acceptable exposure = $\text{NOAEL}_{\text{dermal}} / \text{assessment factor}$) can be directly compared to
9743 DE. However, if it is derived from oral exposure (Regulatory threshold for acceptable
9744 exposure = $\text{NOAEL}_{\text{oral}} / \text{assessment factor}$), information on both oral and dermal absorption is
9745 necessary [oral absorption for correction of the oral dose in order to get the systemic threshold and
9746 the dermal absorption for the estimation of the systemic dermal exposure ($\text{SDE} = \text{DExDA}$) from the
9747 dermal exposure (DE) (equation 3)].

9748 - Body surface area in contact with the foliage,

9749 - Behavior of the animal and time spend in the treated field.

9750 NOTE: Please consider that I have described here the approach that is followed in the mammalian
9751 toxicology exposure estimation and how it can be applied for Amphibians and Reptiles.

9752

9753 In respect to information that could be retrieved from the resident and bystander dermal exposure
9754 during application as a result of spray drift, the data presented on Table 64: , Table 65: and Table 67:
9755 are from direct measurements of simulated human exposure with different application techniques
9756 and cannot provide any information to be directly used for the estimation of amphibians and reptiles
9757 exposure.

9758 For the A&R dermal exposure due to contact with surface deposits, the principle of the approach
9759 followed for resident and bystander exposure could be applicable as well (equation 4 and 5). For the
9760 A&R exposure estimation the 90th , 95th or 100th percentile of ground sediments as a percentage of
9761 the application rate (Table 7) should be used. In this case, it will be necessary also to identify the
9762 points mentioned above (TEP, DA, TC etc).

9763

9764 **Dermal exposure models used in bird risk assessment**9765 **4) Dermal exposure models from US-EPA for birds**

9766 From Technical Description and User's Guidance Document for the Terrestrial Investigation Model
 9767 (TIM) (US-EPA 2015). Page 50 – 54 of 77

9768

9769 The dermal exposure estimate consists of two parts:

9770 1. exposure to direct intercept (= overspray of the bird – half of the body surface of the bird)
 9771 and

9772 2. contact to plant surfaces (dislodgeable pesticide residues on foliage)

9773

9774 Equation 6.1. $D_{\text{dermal}}(t) = (D_{\text{intercept}}(t) + D_{\text{contact}}(t)) * F_{\text{red}} * F_{\text{field}}$

9775

9776 F_{field} is a probabilistic element and calculated by Monte Carlo Simulations.

9777

9778 **Table 69:** Parameters used for equations to estimate pesticide exposure concentrations through
 9779 dermal exposure. From US EPA TIM model user's guide
 9780 ([https://www.epa.gov/sites/production/files/2015-](https://www.epa.gov/sites/production/files/2015-06/documents/timv3_0_tech_manual.pdf)
 9781 [06/documents/timv3_0_tech_manual.pdf](https://www.epa.gov/sites/production/files/2015-06/documents/timv3_0_tech_manual.pdf))

Symbol	Parameter Description	Variable Type*	Units
A_{rate}	Application rate from label	Constant	lb a.i./A
BW	Body weight	Random	g/bird
$C_{\text{plant}}(t)$	Concentration of the pesticide in crop foliage at time t	Random	mg/kg
DAF	Dermal absorption fraction	Constant	none
$D_{\text{contact}}(t)$	Incidental Dermal Contact Dose	Random	$\mu\text{g pesticide/g-bw}$
$D_{\text{dermal}}(t)$	Dose through dermal exposure for a pesticide at time t	Random	$\mu\text{g pesticide/g-bw}$
$D_{\text{intercept}}(t)$	Intercepted Dermal Dose	Random	$\mu\text{g pesticide/g-bw}$
DPR	Dislodgeable pesticide residues	Constant	mg/m^2
F_{dfr}	Dislodgeable foliar residue adjustment factor	Constant	kg/m^2
F_{field}	Fraction of on field exposure	Random	none
F_{red}	Dermal route equivalency factor	Constant	none
$R_{\text{foliar contact}}$	Rate of foliar contact (6.01)	Constant	$\text{cm}^2\text{foliage/cm}^2\text{body surface (per hour)}$
SA_{total}	Total surface area of bird	Random	cm^2
TPR	Total pesticide residues	Constant	mg/kg

* "Constant" indicates that the parameter is set to one value. "Random" indicates that the parameter's value varies based on a distribution of possible values.

9782

9783

9784 **Exposure from Interception (overspray):**

$$\text{Equation 6.2. } D_{\text{intercept}(t)} = \frac{(A_{\text{rate}} * 11.2) * (SA_{\text{total}} * 0.5) * DAF}{BW}$$

$$\text{Equation 6.3. } SA_{\text{total}} = 10 * BW^{0.667}$$

9785

9786

9787 The application rate is multiplied by 11.2 to convert the application rate from lb a.s./A to metric units
9788 to generate a concentration value expressed in µg a.s./g BW.

9789

9790 **Dermal exposure from contact to plant surfaces:**

$$\text{Equation 6.4. } D_{\text{contact}(t)} = \frac{C_{\text{plant}(t)} * F_{\text{dfr}} * R_{\text{foliar contact}} * (SA_{\text{total}} * 0.079) * 0.1}{BW}$$

9791

$$\text{Equation 6.5. } F_{\text{dfr}} = \frac{DPR}{TPR}$$

9792

$$\text{Equation 6.6. } F_{\text{red}} = \frac{LD_{50(\text{avian oral})}}{LD_{50(\text{avian dermal})}}$$

9793

9794

9795 The dislodgeable foliar residue adjustment factor (F_{dfr}) is needed because the total residues are
9796 expressed in terms of mass of pesticide per unit fresh mass of vegetation (mg a.s./mg foliage), while
9797 dislodgeable pesticide residues are expressed in terms of mass of pesticide per surface area of the
9798 vegetation. It is the quotient of dislodgeable residues and total residues measured immediately after
9799 pesticide application. If the residue data are not available then a default F_{dfr} value of 0.62 (based on
9800 mean residue value from foliage of 28 mg a.s./m²/45 mg a.s./kg) is applied.

9801 The EPA value of 2.8 µg a.s./cm² of foliage per kg a.s. applied/ha is 25% (0.25) of the application rate
9802 as dislodgeable residues. The applied rate in lb/acre was converted to kg/ha with a factor of 1.12 (0.25
9803 lb/ha * 1.12 = 0.28 kg/ha = 2.8 µg a.s./cm²). The 25% of dislodgeable foliar residues of the application
9804 rate is based on the arithmetic mean of 60 measurements of 4 pesticides in 14 different crops (see
9805 section D 6.2 of Appendix D in the USEPA 2012 SOP for residential pesticide exposure assessment,
9806 p.491-497). A very high variability was observed. The standard deviation of the arithmetic mean of

9807 0.25 is 0.23. Due to the low number of pesticides measured and the high variability observed in these
9808 measurements it is recommended to use the dislodgeable foliar residues of 3 µg a.s./cm² of foliage per
9809 kg a.s. applied/ha which is used in the human risk assessment which is approximately the 90th
9810 percentile of the underlying dataset (see section 11.1.1 dermal exposure models used in human risk
9811 assessment).

9812 Amphibian and reptile specific formulas for the animal's surface area are needed. The dermal
9813 adsorption fraction (DAF) would need to be adjusted for amphibians and reptiles. The default value is
9814 1 and could be used as a conservative starting point.

9815 As birds will mainly be exposed via their legs the total surface area of a bird is multiplied by 0.079.
9816 For amphibians and reptiles the full surface area could come in contact with foliar residues. It may
9817 be possible to refine this assumption if data from contact surface of the animal with different crop
9818 types become available e.g. the sides of the animal are in contact with cereals and the ventral side is
9819 in contact with crops where animals can climb (e.g. orchards).

9820 No data are available for foliar contact rate ($R_{\text{foliar contact}}$) of birds legs. As a surrogate values from the
9821 estimates for farm workers hands were used (11.9 – 5,050 cm²/h). A default factor of 6.01 cm²
9822 foliage/ cm² body surface is used for birds. This factor would need to be adjusted for amphibians and
9823 reptiles. Such a factor could be derived from information on the speed of movement and surface
9824 area of the animal in contact with foliage during movement.

9825 The dermal route equivalency factor (Fred) is applied to estimated dermal exposures in order to
9826 derive an estimate of the equivalent oral dose. This is needed for calculating the total overall dose
9827 (from oral and dermal uptake) and to compare to a toxicity endpoint based on oral exposure (e.g.
9828 oral acute LD50). In situations where avian dermal and oral LD50 data are available for a pesticide,
9829 Fred is calculated by dividing the oral LD50 by the dermal LD50. Since EPA does not have a data
9830 requirement for avian acute toxicity testing via the dermal route, it is expected that a chemical-
9831 specific dermal LD50 will rarely be available. In cases where a chemical-specific dermal LD50 value is
9832 not available, it can be generated automatically by TIM using Equation 6.7 (Appendix G, reproduced
9833 from USEPA, 2004). This equation is based on available avian dermal and oral toxicity data. Although
9834 the data set is limited to 25 chemicals (primarily organophosphate insecticides), it has the advantage
9835 of being based on avian toxicity data for both routes of exposure.

9836 It is not expected that oral toxicity and dermal toxicity data are available for amphibians and reptiles.
9837 This constitutes a problem for adding up the exposures and comparing them to one endpoint (either
9838 dermal or oral LD50). Whether the dermal route equivalency factor for mammals or birds could be
9839 extrapolated to amphibians and reptiles is highly uncertain. Because of the specific functions of
9840 amphibian skin for gas exchange and water regulation it is expected that amphibians will be more
9841 sensitive to dermal exposure than birds or mammals.

9842 Overall it is concluded that the dermal exposure model from the US-EPA for birds could provide a
9843 basis for suggesting an exposure model for amphibians and reptiles. However, it would be necessary
9844 to use amphibian and reptile specific factors such as dermal absorption fraction (DAF), the surface
9845 area of the animal, foliar contact rate.

9846

9847 **Inhalation exposure**9848 **Inhalation exposure models used in human risk assessment**9849 **5) Inhalation exposure of worker**

9850 Worker inhalation exposure may be to vapour and/or airborne aerosols (including dust). Exposure *via*
 9851 the inhalation route is considered to have limited contribution to the total exposure in comparison to
 9852 the dermal route. Currently, the exposure of worker *via* the inhalation route is considered for tasks
 9853 carried out indoors. The estimated exposure is depended on the Task Specific Factor which can be
 9854 used in the first tier of exposure and risk assessment and the only available set of exposure data is
 9855 for harvesting and re-entry in ornamental greenhouses. Worker exposure estimates for the
 9856 inhalation route after outdoor applications are only necessary in exceptional cases (e.g. for volatile
 9857 substances). In this case *an ad hoc* approach is necessary.

9858

9859 **6) Inhalation exposure of Resident**

9860 Resident inhalation exposure may take place as a result of exposure to drift or to vapour.

9861 For the estimation of exposure via drift the mean and the 75th percentile of exposure to spray
 9862 solution for both adults and children following arable/ground boom applications as well as orchard
 9863 broadcast applications are given on Table 70: and Table 71: .

9864

9865 **Table 70:** Inhalation exposures for residents (75th percentile from data on potential dermal and
 9866 inhalational exposures) (adapted and amended from EFSA PPR Panel, 2010)

Method of application (distance from sprayer)	These values are the 75th percentiles for residents (assuming average breathing rates for inhalation exposures)	
Inhalation (mL spray dilution/person)		
	Adults	Children
Arable/ground boom sprayer(from BREAM)		
2 m	0.00010	0.00022
5 m	0.00009	0.00017
10 m	0.00009	0.00013
Orchard/broadcast air assisted applications (Lloyd <i>et al.</i> 1987) (a)		
2–3 m	n.a.	n.a.
5 m	0.0021	0.00164

10 m	0.0021	0.00164
------	--------	---------

9867 (a): The only available values are for the 8-m distance downwind from the middle of the tree trunk, which are assumed to
 9868 represent a 5-m distance from the edge of the orchard; the same value is used for 5 and 10 m.
 9869 n.a., not available.

9870

9871 **Table 71:** Inhalation exposures for residents (mean data on potential dermal and inhalational
 9872 exposures) (adapted and amended from EFSA PPR Panel, 2010)

Method of application (distance from sprayer)	These values are the mean values (assuming average breathing rates for inhalation exposures)	
Inhalation (mL spray dilution/person)		
	Adults	Children
Arable/ground boom sprayer		
2 m	0.00009	0.00017
5 m	0.00008	0.00014
10 m	0.00007	0.00011
Orchard/broadcast air assisted applications (a)		
2–3 m	n.a.	n.a.
5 m	0.00170	0.00130
10 m	0.00170	0.00130

9873 (a): The only available values are for the 8-m distance downwind from the middle of the tree trunk, which are assumed to
 9874 represent a 5-m distance from the edge of the orchard; the same value is used for 5 and 10 m.
 9875 n.a., not available.

9876

9877 Exposures to vapour should be estimated using the method that has been developed in the UK (CRD,
 9878 2008) and Germany (Martin et al., 2008), based on the highest time-weighted average exposure for a
 9879 24-hour period, according to the volatility of the active substance:

9880 $SERI = (VC \times IR \times IA)/BW$, where:

9881 • SERI = systemic exposure of residents via the inhalation route (mg/kg bw per day)

9882 • VC = vapour concentration (mg/m³)

9883 • IR = inhalation rate (m³/day)

9884 • IA = inhalation absorption (%)

9885 • BW = body weight (kg).

9886

9887 For moderately volatile compounds (vapour pressure ≥ 0.005 Pa and < 0.01 Pa), exposures should be
 9888 calculated assuming a default concentration in the air of $15 \mu\text{g}/\text{m}^3$ and daily average breathing rates
 9889 as reported in Table 64: , resulting in:

9890 • an adult value of $15 \mu\text{g}/\text{m}^3 \times 0.23 \text{ m}^3/\text{day}/\text{kg} \times 60 \text{ kg} = 3.45 \mu\text{g}/\text{day}/\text{kg} \times 60 \text{ kg} = 207 \mu\text{g}/\text{day}$

9891 For compounds with low volatility (vapour pressure < 0.005 Pa), exposures should be calculated
 9892 assuming a default concentration in the air of $1 \mu\text{g}/\text{m}^3$ and daily average breathing rates as reported
 9893 in Table 65: , resulting in:

9894 • an adult value of $1 \mu\text{g}/\text{m}^3 \times 0.23 \text{ m}^3/\text{day}/\text{kg} \times 60 \text{ kg} = 0.23 \mu\text{g}/\text{day}/\text{kg} \times 60 \text{ kg} = 13.8 \mu\text{g}/\text{day}$.

9895 Any future possibility of modifying the vapour pressure value and the concentration in the air will
 9896 allow a refinement of the exposure calculations.

9897

9898 7) Inhalation exposure of Bystander

9899 Bystander inhalation exposure may happen via exposure to spray drift or to vapour. The 95th
 9900 percentile of bystander inhalation exposure via drift assuming high inhalation breathing rate both for
 9901 arable/ground boom sprayers and orchard broadcast air assisted applications for adults and children
 9902 are given on Table 72: . The exposure to vapours should be calculated in the same way as for
 9903 residents.

9904

9905 **Table 72:** Inhalation exposures for bystanders (95th percentile) (adapted and amended from EFSA
 9906 PPR Panel, 2010)

Method of application (distance from sprayer)	95th percentiles for bystanders (assuming high breathing rates for inhalation exposures)	
Inhalation (mL spray dilution/person)		
	Adults	Children
Arable/ground boom sprayer(from BREAM calculator)		
2 m	0.00050	0.00112
5 m	0.00048	0.00083
10 m	0.00051	0.00076
Orchard/broadcast air assisted applications ((Lloyd <i>et al.</i> 1987) (a)		
2–3 m	n.a.	n.a.

5 m	0.0044	0.0035
10 m	0.0044	0.0035

9907 (a): The only available values are for the 8-m distance downwind from the middle of the tree trunk, which are assumed to
 9908 represent a 5-m distance from the edge of the orchard; the same value is used for 5 and 10 m.
 9909 n.a., not available.

9910

9911 **Conclusion on the use of residents and bystander inhalation exposure assessment:**

9912 Inhalation exposure of both residents and bystanders may happen as a result of exposure to airborne
 9913 spray during application or due to exposure to vapours. In respect to information that could be
 9914 retrieved from the resident and bystander inhalation exposure during application as a result of spray
 9915 liquid inhalation, the data presented on Table 70: and Table 71: are from direct measurements of
 9916 simulated human exposure with different application techniques and cannot provide any information
 9917 to be directly used for the estimation of amphibians and reptiles inhalation exposure.

9918 For the estimation of A&R exposure due to inhalation of vapours, the principle of the approach
 9919 followed for resident and bystander exposure could be applicable as well (equation 6). In this case
 9920 the vapour concentration for compounds of low volatility is assumed to be 1µg/m³ while for
 9921 moderately volatile 15 µg/m³. However, for the application of equation 6 in case of A&R the
 9922 inhalation rate of the animals is needed.

9923

9924 **Inhalation exposure in bird risk assessment**

9925 **8) Inhalation exposure model for birds from US EPA**

9926 The inhalation exposure model from the US-EPA for birds is probably a good basis for suggesting an
 9927 exposure model for amphibians and reptiles after adjusting the bird specific factors.

9928 From Technical Description and User's Guidance Document for the Terrestrial Investigation Model
 9929 (TIM)

9930 Page 44-50

9931 As in the human inhalation exposure there is a differentiation in dose received from spray droplets
 9932 and from vapour.

9933 F_{field} is the fraction of field exposure – it is calculated by Monte Carlo simulation.

9934 The oral dose equivalence factor F_{re} – is applied to estimated inhalation exposures in order to derive
 9935 an estimate of the equivalent oral dose. This is needed for calculating the total overall dose (from
 9936 oral, dermal and inhalation uptake).

$$\text{Equation 5.1. } D_{inhalation(t)} = (D_{spray(t)} + D_{vapor(t)}) * F_{re} * F_{field}$$

9937

9938

Table 5.1. Parameters Used for Equations in Section 5 to Estimate Pesticide Exposure through Inhalation

Symbol	Parameter Description	Variable Type*	Units
A_{rate}	Application rate from label	Constant	lb a.i./A
B_{vol}	The volume-based biotransfer factor; function of Henry's law constant and Log Kow	Constant	$\mu\text{g/L}$ fresh weight leaf/ $\mu\text{g/L}$ air
BW	Body weight	Random	g/bird
$C_{air(drops)(t)}$	Pesticide concentration in a volume of air for the time step immediately following the pesticide application	Constant	$\mu\text{g/mL}$
$C_{air(t)(vol)}$	Concentration of the pesticide in air at time t (resulting from volatilization); function of $M_{pesticide}$, m_{plant} , and B_{vol}	Random	$\mu\text{g/mL}$
CH	Height of crop	Constant	m
D	Fraction of hour where pesticide is applied	Constant	none
$D_{inhalation(t)}$	Dose through inhalation for a pesticide at time t	Random	$\mu\text{g pesticide/g-bw}$
$D_{spray(t)}$	Droplet Inhalation Dose	Random	$\mu\text{g pesticide/g-bw}$
$D_{vapor(t)}$	Volatilization inhalation dose; function of pesticide concentration in air, volume of inhaled air, and body weight of the bird	Random	$\mu\text{g pesticide/g-bw}$
F_{AM}	The ratio of avian to mammalian pulmonary membrane diffusion rates from USEPA 2004	Constant	none
F_{field}	Fraction of on field exposure	Random	none
F_{re}	The avian route equivalency factor	Constant	none
$F_{respired}$	Volumetric fraction of droplet spectrum not exceeding the upper size limit of respired particles for birds	Constant	none
H	Henry's law constant	Constant	$\text{atm}\cdot\text{m}^3/\text{mol}$
IS	Inhalation scale factor	Random	none
Kow	Octanol-water partition coefficient	Constant	none
LD_{50}	Lethal dose sufficient to kill 50% of exposed individuals	Constant	$\text{mg/kg} = \mu\text{g/g}$
$M_{pesticide}$	The pesticide concentration on the treated field at time t (accounting for dissipation); function of application rate	Random	mg
m_{plant}	The mass of plant (crop) per hectare based on user input	Constant	kg
R	Universal gas constant (8.205 e^{-5})	Constant	$\text{atm}\cdot\text{m}^3/\text{mol}\cdot\text{K}$
RH	Height of spray release	Constant	m
R_{rate}	Respiration rate	Random	mL/h
T	Air temperature	Constant	K
V_{air}	The volume of air in 1 ha to a height equal to the height of the crop canopy	Constant	L
$V_{inhalation}$	Volume of air respired	Random	mL
ρ_{plant}	The density of the crop tissue assumed as fresh leaf (0.77)	Constant	kg/L

* "Constant" indicates that the parameter is set to one value. "Random" indicates that the parameter's value varies based on a distribution of possible values.

9939

9940

9941 *Inhalation of droplets:*

$$\text{Equation 5.2. } D_{\text{spray}(t)} = \frac{C_{\text{air}(t)(\text{drops})} * V_{\text{inhalation}} * F_{\text{respired}}}{BW}$$

9942

9943 Fraction of applied pesticide spray (F_{respired}) – only droplets with a size of <100 μm are considered to
 9944 be inhaled. The default value of the fraction of spray inhaled is 0.28.

9945

9946 **Pesticide concentration in a volume of air ($C_{\text{air}(t)(\text{drops})}$):**

$$\text{Equation 5.3. } C_{\text{air}(t)(\text{drops})} = \frac{D * A_{\text{rate}} * 0.112}{RH}$$

9947

9948 The height of spray release (RH) is a constant value of either 1m or 3.3 m

9949 $D=0.025$ based on 90s duration of direct spray inhalation for ground spray applications

9950 $D=0.0083$ based on 30s duration of direct spray applications

9951 The factor of 0.112 is used to convert lb a.i./A to metric units and to give a concentration expressed
 9952 in $\mu\text{g a.i./mL}$.

9953

9954 **Calculation of Inhaled Air Volume ($V_{\text{inhalation}}$):**

$$\text{Equation 5.4. } V_{\text{inhalation}} = 3 * R_{\text{rate}} * S_1$$

9955

9956 The inhalation rate is varied randomly from a beta distribution of values from 0.9 to 1.1 (mean = 1)
 9957 (factor S_1) to allow variation depending on the different activity levels between different hours (this
 9958 is because all exposure routes are considered in a probabilistic approach).

9959 A factor of 3 is applied to account for greater volumes inhaled in the field than in the laboratory.

9960

9961 Allometric equation to calculate the respiration rate:

$$\text{Equation 5.5. } R_{\text{rate}} = 60 * \left(284 * \left(\frac{BW}{1000} \right)^{0.77} \right)$$

9962

9963

9964

9965 Inhalation of vapour phase:

9966 Two compartments are considered: crop leaf and air between crop and soil. Dissipation between the
 9967 total pesticide mass applied to a 1-ha treated field ($M_{\text{pesticide}}$; Equation 5.8) combined with
 9968 dissipation between the time of application and time t are used to estimate the total mass of
 9969 pesticide available for partitioning between crop leaf and canopy air. The density of the crop tissue
 9970 (ρ_{plant}) assumed to be fresh leaf is 0.77 kg/L, based on the Hazardous Waste Identification Rule
 9971 (HWIR) Farm Food chain Model (USEPA, 1999). The air compartment volume (V_{air}) is represented by
 9972 a 1-ha area, with a height set at the top of the canopy at time of application (Equation 5.9). The
 9973 available pesticide residue is then partitioned between the two compartments (air and leaf mass)
 9974 through the application of the volume-based biotransfer factor (B_{vol}) developed for the HWIR model
 9975 (Equation 5.10). It is assumed that the air temperature (T) is a constant value of 298.1 K (equivalent
 9976 to 25°C, 77°F). A temperature of 25°C was chosen because Henry's law constant and octanol-
 9977 water partition coefficient (K_{ow}) values for pesticides are frequently available at this temperature;
 9978 however, the relevance to the actual environment at the time of pesticide application is an
 9979 uncertainty. The total available residues establish an upper limit of available pesticide concentration
 9980 in the air as a result of volatilization from (treated) leaf surfaces.

$$\text{Equation 5.6. } D_{\text{vapor}}(t) = \frac{C_{\text{air}(t)(\text{vol})} * V_{\text{inhalation}}}{BW}$$

9981

$$\text{Equation 5.7. } C_{\text{air}(t)(\text{vol})} = \frac{M_{\text{pesticide}}}{V_{\text{air}} + \left(\frac{m_{\text{plant}} * B_{\text{vol}}}{\rho_{\text{plant}}} \right)} * e^{-rt}$$

$$\text{Equation 5.8. } M_{\text{pesticide}} = A_{\text{rate}} * 1.12 * 10^6$$

$$\text{Equation 5.9. } V_{\text{air}} = CH * 10^7$$

$$\text{Equation 5.10. } \text{Log } B_{\text{vol}} = 1.065 * \text{Log } K_{\text{ow}} - \text{Log} \left(\frac{H}{RT} \right) - 1.654$$

9982

9983

9984 It is concluded that the exposure estimates for birds could in principle be used for amphibians and
 9985 reptiles after adjusting it with amphibian and reptile specific factors for inhaled air volumes.

9986 Ventilation rates and oxygen consumption of different reptile groups and birds and mammals were
 9987 compared in a review article by Bennett 1973. Higher ventilation rates of homeotherms are
 9988 principally the result of a greater ventilation frequency in mammals and a greater tidal volume in
 9989 birds. The inhaled volume of air per minute is about 3.6 times and 4.9 times greater in birds and
 9990 mammals compared to reptiles.

9991 It is expected that the contribution of inhalation exposure to the total exposure is much less than
 9992 oral and dermal exposure and therefore it is considered not necessary to assess inhalation exposure
 9993 by default. However an inhalation exposure assessment may be needed if a substance is volatile and
 9994 very toxic to reptiles. Inhaled volumes in amphibians are likely to be even less than for reptiles as
 9995 their skin has an important function for gas exchange. Therefore it is not considered necessary to
 9996 conduct an inhalation exposure assessment for amphibians.

9997

9998

9999 **Oral exposure**

10000

10001 **Herptox model of US-EPA**10002 **Oral exposure in T-Rex / T-Herps models from USEPA.**

10003 The oral exposure assessment procedure used by the USEPA is based on very similar principles as the
 10004 SANCO EFSA Guidance documents for birds and mammals (EFSA, 2009). The overall process is based
 10005 on the estimate of residues on dietary items after application of a given plant protection product.
 10006 Several models are available for foliar and granular application, but also for seed treatment. The
 10007 Kenaga transfer coefficients, modified by Fletcher, are used to determine residual concentrations of
 10008 PPPs on dietary items. Half lives of ASs (first order kinetics), application rate, number of
 10009 application(s), interval between applications are taken into account to simulate exposure estimates
 10010 for 1 year. Only Upper bound Kenaga results are used for risk evaluation, as a protective measure.
 10011 The T-Rex model has been developed to fit various birds and mammal species (see Table 73:). It is
 10012 assumed that the mass fraction of water will be 80% for herbivores and insectivores, but only 10%
 10013 for granivores. A major difference with the SANCO model lies in the species-specific scenarios used.
 10014 In the USEPA models, the species are theoretical species with either herbivorous, insectivorous or
 10015 granivorous regimen, used as worst case scenarios in a first tier approach.

10016

10017 **Table 73:** Food intake estimates for bird and mammalian species in the T-Rex model (USEPA)

Species	Organism / Body weight (g)	Food Intake (g.day ⁻¹)		Percent body weight consumed (g.day ⁻¹)	
		Herbivores/ Insectivores	Granivores	Herbivores/ Insectivores	Granivores
Small mammal	15	14.3	3.2	95	21
Medium Mammal	35	23	5.1	66	15

Large Mammal	1000	150	34	15	3
Small Bird	20	23	5	114	25
Medium Bird	100	65	14	65	14
Large Bird	1000	291	65	29	6.5

10018

10019

DRAFT

10020

10021 The European process uses the concept of focal species. These species are real species potentially
 10022 exposed in their habitat to the PPP applied and biological data from scientific literature about their
 10023 diet and habitat preferences. According to the type of crop, the feeding regimen is adjusted and food
 10024 intake rate adjusted accordingly. The model also takes into account other variables like the
 10025 interception rate for foliar application on all crop types. The scenarios are more detailed to fit all
 10026 potential used and crop types.

10027

10028 **Table 74:** Examples of food intake rates based on SANCO document (EFSA Journal, 2009; 7(12):
 10029 1438

Species	Organism / Body weight (g)	Food Intake (g.day ⁻¹)		Percent body weight consumed (g.day ⁻¹)	
		Herbivores/ Insectivores	Granivores	Herbivores/ Insectivores	Granivores
Small herbivorous mammal (<i>Apodemus sylvaticus</i>)	21.7	-	3.7	-	17
Small insectivorous Mammal (<i>Sorex araneus</i>)	9.7	5.3	-	55	-
Large herbivorous Mammal (<i>Oryctolagus cuniculus</i>)	1543	771	-	50	-
Small Granivorous Bird (<i>Carduelis cannabina</i>)	15.3	-	4.3	-	28
Medium Insectivorous Bird (<i>Glareola pratinctola</i>)	75	23.2	-	31	-
Large Herbivorous Bird	3108	1709	-	55	-

(Anser anser)					
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10030

10031 T-Herps is only used if standard risk evaluation with the T-REX models for amphibians and reptiles
 10032 exceeds the Level of Concern for acute (0.1) or chronic exposures

10033 The model has been adapted from the T-Rex model developed by the USEPA. Currently, it has only
 10034 been approved to assess exposure of terrestrial life stages of insectivorous herptiles (i.e. no
 10035 herbivorous species have been considered). The model is based on the assumption that herptiles, as
 10036 poikilotherm species, have a lower metabolic rate, a lower caloric intake requirement and, as a
 10037 consequence, a lower Food Intake Rate (FIR). Evidence of this difference is provided by the estimated
 10038 caloric requirements for free living iguanid lizards as compared with passerine birds:

10039

10040 Iguanids :

10041

10042 $FMR = 0.0535 (BW)^{0.799}$

10043

10044 Passerine birds :

10045 $FMR = 2.123(BW)^{0.749}$

10046

10047 FMR: free-living metabolic rate (kcal/day)

10048 BW: body weight (g)

10049

10050 These equations indicate that the metabolic rate of birds can be 40 times higher than reptiles of
 10051 similar body weight. This difference tends to decrease when body weight increases. As a
 10052 consequence, using an avian food intake allometric equation instead of specific herptiles models
 10053 would result in an over-estimation for reptiles and terrestrial-phase amphibians.

10054 T-Herps has been developed for the California Red Legged Frog (CRLF). The following specific points
 10055 have been included to adjust the basic model to Herptiles

10056

10057 Food intake rate

10058 The following equation was developed for an insectivorous iguanid (Nagy, 1987 cited in T-Herps
 10059 document).

10060 $FI = 0.013(BW)^{0.773}$

- 10061 FI = Food Intake (g/day)
- 10062 *It is assumed that terrestrial-phase amphibians and reptiles have similar caloric requirements. At*
10063 *least one study was conducted (and cited in the T-Herps literature) to compare food intake values of*
10064 *juvenile bull frogs (*Rana catesbeiana*) (Modzelewskii and Culley, 1974, cited in T-Herps) with*
10065 *estimates obtained with this equation. The results indicate that juvenile bullfrog had daily FI ranging*
10066 *from 3 to ca 7% of their BW. Estimates ranged from 3% to 5% BW, which is considered close enough*
10067 *to fit the purpose.*
- 10068
- 10069
- 10070 Including small mammals and amphibians as potential dietary items.
- 10071 T-Herps (as well as T-Rex, the standard model for bird and mammal exposure estimates) evaluates
10072 exposure from consumption of grass, plants, insects, seeds and fruits. T-Herps includes different prey
10073 items, as it is recognized that some herpetofauna consume small mammals and amphibians. Heptile
10074 prey items are assumed to eat insects. These insects carry residues based on the Kenaga values. The
10075 prey size can be altered in the spreadsheet, to adjust for a specific prey.
- 10076 For mammalian preys, two estimates of exposure are calculated by assuming that the prey item
10077 consumes either short grass or large insects.
- 10078 Estimated daily exposure of small mammals is determined as in the general T-REX model. (It is
10079 assumed that the same could be done with the European Guidelines).
- 10080 The amount of pesticide (mg) consumed is determined by multiplying the weight of the prey item by
10081 the dose in the prey item (mg.kg⁻¹).
- 10082 The resulting exposure estimate is determined as the pesticide mass consumed (mg/bw of assessed
10083 species).
- 10084 The mass of the prey item can be altered in the program. Default values are set at 35g for mammals.
- 10085
- 10086 Water content of food items
- 10087 Wet weight of food intake is used in the FIR equation. Water content of various potential food items
10088 is used in the models developed by the USEPA. The highest mean water content of the taxonomic
10089 group of prey item is used in dose calculation. For instance, default values of 69% for insects and 85%
10090 for amphibians are used.
- 10091 Body weight of Herptiles
- 10092 The spreadsheet is designed to include small, medium and large animals. The default values
10093 correspond to CRLF data. Critical review of values is recommended in order to adapt prey items to
10094 the assessed species.
- 10095 Limitations and uncertainties

- 10096
- 10097 T-Herps has been developed only for terrestrial herptiles exposure resulting from the consumption of
10098 terrestrial organism. No evaluation for aquatic organisms is included. If there is no evidence of
10099 bioaccumulation in aquatic organisms, this should not be a concern. If there is evidence of
10100 bioaccumulation in aquatic organisms, this should be included in the risk characterization phase.
- 10101 Metabolism, biotransformation or elimination from the prey item is not considered. T-Herps assumes
10102 that the prey animal is consuming its daily intake of contaminated food before being consumed by
10103 the species of interest.
- 10104 Bioaccumulation of contaminants in prey items is not included. As a consequence, exposure estimate
10105 is likely to be underestimated.
- 10106 Residues present on the prey item (as a result of direct spray) is not being considered
- 10107 A default median value of 35g is assumed for small mammal preys. Larger prey animals will result in
10108 lower dietary-based RQs. It is not known if it is better to use dietary-based or dose-based RQs. If
10109 these RQs do not exceed the level of concern, it is suggested to use smaller prey items in the model.
- 10110 T-Herps does not include temperature influence on the food intake allometric equation, although
10111 there is evidence indicating that temperature will influence the FIR.
- 10112 The allometric equation used to estimate FIR provides a constant daily value. T-Herps, however,
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