



Risk evaluation of pesticide use to protected European reptile species



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ARTICLE INFO

Article history:

Received 27 January 2015

Received in revised form 30 June 2015

Accepted 2 August 2015

Available online xxxx

Keywords:

Fungicide

Habitats Directive

Herbicide

Insecticide

IUCN Red List of Threatened Species

Management plan

Special Areas of Conservation

ABSTRACT

Environmental contamination is supposed to be a reason for population declines in reptiles. Especially intensification and expansion of agriculture are leading to increased pesticide exposure risks for wildlife. In the European Union, Special Areas of Conservation (SACs) have been established for the conservation of taxa listed in Annex II of the Habitats Directive. In the SACs, agricultural land use is legal. Therefore, we conducted a risk evaluation of pesticide exposure for Annex II reptiles by calculating proportions of land use with regular pesticide applications within SACs. Using three evaluation factors (occurrence probability, physiology, life-history aspects), a species-specific risk index was created. Nearly half of the species at above-average risk by pesticide use are globally threatened with extinction (IUCN Red List of Threatened Species). About 30% of their SACs are agriculturally used and one priority subspecies of the Habitats Directive is at highest risk (*Vipera ursinii rakosiensis*). Also, all evaluated fresh-water and land-dwelling turtle species are at high risk. National variation in agricultural land use in the SACs was observed. Species at above-average risk are mainly distributed in the Mediterranean and Pannonian/Continental biogeographical regions of Europe. Conservation status according to the IUCN Red List of Threatened Species as well as national differences among the member states argue for the inclusion of pesticide risk assessments in site-specific management plans for SACs to avoid regional loss of reptilian biodiversity.

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1. Introduction

Biodiversity decline is a serious and widely recognized problem among all taxa and ecosystems over the entire globe. In reptiles, worldwide population declines have been noted (Gibbons et al., 2000). A first analysis of their global conservation status revealed that nearly one in five reptilian species is threatened with extinction, while for others one in five information is lacking (Böhm et al., 2013). The causes for declines are assorted. For ‘industrialized’ countries, habitat loss and degradation are most extensively contributing to population declines (Todd et al., 2010). In these countries, primary and secondary reptile habitats have been transformed into areas of intensive agricultural land use. As a spin-off, species additionally become more and more exposed to agrochemicals, especially pesticides (Weir et al., 2010).

Today, massive land use change can be observed in Europe, for instance, related to the growing impact from energy crops (Fargione et al., 2010). Additionally, there is a trend to grow energy crops on previously uncultivated land including former mining areas (Dauber et al., 2012). Such areas are known to serve as crucial secondary habitats for reptiles (Günther, 1996; Böhme et al., 1999). In the future, the cultivation of genetically engineered crops – which are created to stand adverse abiotic conditions like too low soil pH – might even increase the inclusion of previously non-arable areas (Pengue, 2005). It is no

surprise that solely in Europe, 18% of all reptile species are listed as threatened with extinction (Cox and Temple, 2009; Böhm et al., 2013).

The contribution of environmental contaminants, especially pesticides, to reptile declines has yet been little addressed. Even with regard to simple acute toxic effects only marginal information is available, although showing its importance. As an example, in Hermann's tortoises (*Testudo hermanni*) from southern Greece, a significantly reduced survival and symptoms of poisoning after herbicide applications was reported (Willemssen and Hailey, 2001). Evidence of potentially strong impacts on European reptile wildlife has been linked to sublethal concentrations. Wall lizards (*Podarcis bocagei*) from Portugal, for instance, revealed an increase of hemoparasites, reduced liver size, lack of energetic reserve accumulation, oxidative stress, increased thyroid activity, disturbance of sex ratio and general loss of fitness after pesticide exposure (Amaral et al., 2012a,b,c; Bicho et al., 2013). In the Americas, white blood cell counts decreased in *Caiman latirostris* due to herbicide contamination (Latorre et al., 2013), while laboratory and field studies detected a depressed clutch viability, reduced neonatal survival, hermaphroditism, and reduced testosterone concentration, i.e. endocrine disruption, in another crocodylian, *Alligator mississippiensis* (Guillette et al., 1994; Crain et al., 1997). Pesticide uptake in reptiles is supposed to be mainly via the food chain (Weir et al., 2010). Herbivorous and omnivorous species may suffer from direct ingestion of pesticides sprayed on plant surfaces, while in carnivorous and omnivorous reptiles biomagnification may play an important role (Biddinger and Gloss, 1984). In relation to nutrition, physiology influences pesticide uptake.

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Species with small body indices show a much greater increase in dietary exposure when compared to individuals of larger species (Weir et al., 2010). Another pathway of pesticide absorption in reptiles is dermal uptake from the contaminated environment (Hopkins, 2005). Again, a small body size means a greater contact surface relative to the body mass, promoting a comparatively higher uptake of pesticides (Murphy and Murphy, 1971). Dermal uptake in squamate reptiles also depends on pholidosis (Chang et al., 2009) as well as the lipid and keratinocyte composition of the skin (Roberts and Lillywhite, 1980; Palmer, 2000; Toni et al., 2007). Lastly, life-history aspects play an important role in reptilian pesticide exposure and uptake. Species with relatively small home ranges and migration rates can be highly threatened by the regionally intensive use of pesticides, as the ability for them to leave an exposure area is low. Conversely, species with larger home ranges may be more likely to come in contact with pesticides due to wide-ranging behavior (Günther, 1996; Böhme et al., 1999; Southwood and Avens, 2010). Furthermore, populations of species with relatively few offspring and species that need longer time to reach sexual maturity (K-strategists) will suffer more intensively from effects on individuals than r-strategists (Pianka, 1970).

In general, various problems arising from land use conflicts – including mechanical and chemical intensification of agriculture – are affecting protected areas (Jetz et al., 2007). With the Habitats Directive 92-43-EEC of the European Union (EU, 1992), the European Council set up the Natura 2000 network, which is “a coherent European ecological network of special areas of conservation” (EU, 1992). The goal of the Natura 2000 network is to assure the long-term conservation of Europe's natural heritage (threatened species and habitats, which are listed in different annexes), thus fulfilling a Community obligation under the UN Convention on Biological Diversity (<http://ec.europa.eu/>). Although the Habitats Directive has been criticized, among others, for the lack of flexibility concerning fixed lists of protected species (Hochkirch et al., 2013) or insufficient consideration of optimal site designation and management (Gaston et al., 2008), this network is considered as one of the largest and most important conservation networks of the whole world (Lockwood, 2006). The Natura 2000 network is comprised of ‘Special Areas of Conservation’ (SACs) designated by member states under the Habitats Directive (and also incorporates special protection areas, which they designate under the European Birds Directive) (<http://ec.europa.eu/>).

There have been three stages in the selection of SACs. (1) The member states carried out assessments on habitat types listed in Annex I and species occurrence listed in Annex II of the Habitats Directive to choose national sites. Annex II lists species which are of community interest and whose conservation requires the designation of ‘Special Areas of Conservation’ (SACs) (EU, 1992).

With regard to reptiles, 21 species and 3 subspecies are listed in Annex II. Seven are ‘priority species’ of the Natura 2000 network; these require an enhanced protection status (Table 1). (2) On the basis of national lists, the European Commission adopted a list of sites of community importance, in agreement with the member states including interests of relevant stakeholders, land owners and users, and environmental NGOs. (3) Based in the list of sites of community importance, the member states designated the SACs. The member states must take the necessary management or restoration measures within SACs to ensure the favorable conservation status of species and habitats within the biogeographical regions of Europe including regular monitoring and management plans (<http://ec.europa.eu/>).

The Natura 2000 network shall not be a system of strict nature reserves where all human activities are excluded. Most of the land is privately owned with the emphasis that future management is sustainable, both ecologically and economically (<http://ec.europa.eu/>). Hence, agricultural land use does not stop at SAC borders and at defined conditions land use within them is possible (EU, 1992).

Due to the aforementioned conservation requirements for protecting reptile diversity and the potential threats to them from pesticide use, it is crucial to test if current land use practice with regular pesticide

Table 1

Categories under the IUCN Red List of Threatened Species, “proportional land use with regular pesticide applications” (%LPA) within “Special Areas of Conservation” (SACs), species risk indices (SRIs) and pesticide risk factors (PRFs) of Annex II reptiles. Above-average PRFs are in bold.

IUCN status ^a	%LPA ^b within SAC	SRI	PRF
Critically Endangered			
<i>Gallotia simonyi</i> *	1.34 %	11	0.01
Endangered			
<i>Podarcis lilfordi</i>	3.64 %	8	0.02
<i>Chalcides simonyi</i>	3.91 %	11	0.02
<i>Hierophis (Coluber) cypriensis</i> *	1.20 %	10	0.01
<i>Vipera ursinii rakosiensis</i> *c	45.12 %	10	0.24
Vulnerable			
<i>Testudo graeca</i>	18.00 %	17	0.16
<i>Mauremys caspica</i> ^d	30.02 %	10	0.16
<i>Mauremys leprosa</i> ^d	26.84 %	12	0.17
<i>Iberolacerta (Lacerta) monticola</i>	7.29 %	11	0.04
<i>Vipera ursinii</i>	7.59 %	10	0.04
Near Threatened			
<i>Testudo hermanni</i>	21.87 %	14	0.16
<i>Emys orbicularis</i>	23.36 %	14	0.17
<i>Iberolacerta (Lacerta) bonnali</i>	0.19 %	13	0.00
<i>Lacerta schreiberi</i>	15.50 %	13	0.11
<i>Podarcis pityusensis</i>	5.14 %	11	0.03
<i>Euleptes europaea (Phyllodactylus europaeus)</i>	9.08 %	12	0.06
<i>Elaphe quatuorlineata</i>	23.60 %	7	0.09
Least Concern			
<i>Testudo marginata</i>	16.28 %	12	0.10
<i>Gallotia galloti insulanagae</i> ^e	6.13 %	12	0.04
<i>Zamenis (Elaphe) situla</i>	27.69 %	11	0.16
<i>Natrix natrix cypriaca</i> ^f	6.84 %	5	0.02
			0.09

* = priority species.

^a = The marine turtles *Caretta caretta* and *Chelonia mydas*, which are European priority species, have not been evaluated. Also the priority species *M. schweizeri* could not be evaluated due to lack of actual land cover data from Greece.

^b = Excluding Greece due to the lack of land cover data.

^c = *Vipera ursinii rakosiensis* is still listed for the Natura 2000 site ‘AT1220000’ but already extinct in Austria why this site was excluded.

^d = *Mauremys leprosa* not assessed by the IUCN but by Cox and Temple (2009);

M. caspica as part of *M. leprosa*.

^e = no specific IUCN assessment for this subspecies, but *Gallotia galloti insulanagae* is considered Near Threatened by the national Spanish Red List.

^f = no specific assessment for this subspecies.

applications is likely to affect reptiles within their SACs. With the purpose to test this, we conduct a spatial risk evaluation at the European level. Commonly, a toxicity risk assessment is divided into four steps: (1) hazard identification, (2) exposure assessment, (3) effect assessment and (4) risk characterization (Van Leeuwen, 2007). Number one can be seen as a first screening step. What differentiates risk from hazard is the likelihood of harm due to exposure. Exposure assessment comprises the measuring of exposure concentrations (here: pesticides in general), once chemicals are produced, used and emitted. Effect assessment (also known as dose–response–assessment) is the estimation of the relationship between dose or level of exposure to a substance, and the incidence and severity of an effect (here: to reptiles). Finally, the risk characterization is the estimation if adverse effects are likely to occur in a population or environmental compartment. This integrates the first three steps (US EPA, 1986; Van Leeuwen, 2007).

Up to now, reptiles have been understudied in ecotoxicology (Köhler and Triebkorn, 2013; Weir et al., 2015), i.e. not only specific laboratory data but especially data on causative relationships between pesticide use and reptile population declines are yet lacking. Therefore, detailed risk assessments on European reptile species are not possible yet and our risk evaluation should be regarded as the first attempt to contribute to the first two steps of a risk assessment (i.e., hazard identification and exposure assessment). Only combined with new data from the laboratory (or mesocosms), our results could be used to conduct an actual

risk characterization and thus execute an actual risk assessment for the here reviewed reptile species.

In the present study, we evaluate three different risks for European reptile species: (1) potential exposure, (2) potential individual sensitivity and (3) potential vulnerability of their populations. For this purpose, we identify the “proportions of land use with regular pesticide applications” (%LPA) within SACs that were created for Annex II reptiles and combine this spatial data with evaluation factors of species' ecology, physiology and biology.

2. Methods

2.1. Land use with regular pesticide applications within the SACs (“potential exposure”)

We calculated %LPA within SACs that were created for Annex II reptile species using ArcMap 10 (Esri®) and the latest version (2006, updated 2011) of the European CORINE (Coordination of Information on the Environment) land cover data. CORINE data and those for Natura 2000 sites and species were obtained from the European Environmental Agency (<http://eea.europa.eu>). In the CORINE project, mapping of the land cover was performed on the basis of satellite remote sensing images on the scale 1:100,000. Agricultural land cover classes (under the CORINE-Label “agricultural areas”), which reflect areas where pesticides are regularly applied, were chosen, these were CORINE land cover classes 211 (“non-irrigated arable land”), 212 (“permanently irrigated land”), 213 (“rice fields”), 221 (“vineyards”), 222 (“fruit trees and berry plantations”), 223 (“olive groves”), 241 (“annual crops associated with permanent crops”), 242 (“complex cultivation patterns”), 243 (“land principally occupied by agriculture with significant areas of natural vegetation”) and 244 (“agro-forestry areas”). We are aware that cultivation and pesticide use practices differ between and in these classes (often annually), but more detailed information is not available for the entire EU. Although we realize that on intensively used hay meadows pesticides are regularly applied, we excluded land cover class 231 (“pastures”) because it is not possible to distinguish between those pastures and real pastures. Conversely, parts of the European agricultural area are organic (see [Discussion](#)). Since no actual land cover data was available for Greece, this country was excluded from the evaluation.

2.2. Species risk indices and pesticide risk factors

Not only habitat exposure but also life-history traits and physiology of the considered reptile species ([Table 1](#)) remarkably differ, we created a species risk index (SRI) for each taxon reflecting its potential general risk based on literature data and – when possible – presence/absence data (coordinates from the Global Biodiversity Information Facility, GBIF (<http://data.gbif.org>) and HerpNet (<http://www.herpnet.org>) for occurrence data and pseudo absence data; see below for details). Three evaluation factors (EFs) for exposure risk were considered to define the SRI. The SRI combined with %LPA defined the species' pesticide risk factor (PRF).

2.3. Evaluation factor for habitat exposure risk (EF 1) (“potential exposure”)

Together with the spatial data on agricultural land use, EF 1 refers to the potential “exposure risk” of a species. For EF 1, we awarded 1 Risk Point (RP) when habitat exposure risk was ‘high’ and 0 when it was ‘low’. In a first step, information was obtained from the literature (Gasc et al., 1997; Böhme et al., 1999; Cox and Temple, 2009) and from the IUCN Red List of Threatened Species (<http://www.iucnredlist.org>). The literature-based estimates of habitat exposure are given in [Appendix A](#).

For 11 species and subspecies (see [Appendix A](#)), the literature-based estimates were used for evaluating their habitat exposure risk. For the

remaining nine taxa, sufficient occurrence data were available to use logistic regression models to predict the presence/absence as a function of %LPA. When the presence of a species positively correlated with %LPA, a regular occurrence in cultivated landscapes was suggested. Hence, 1 RP was awarded. 0 RP was given if there was no significant trend, so that it can be suggested that species usually do not occur within cultivated landscapes. Occurrence data were corrected for duplicates and implausible records (e.g. records far outside of a species native range). For species with ≥ 100 records ($n = 5$), we randomly chose a subset of 100 localities, respectively. For species with less than 100 but more than 10 records ($n = 4$), we considered all records as 10 is the minimum sample size per predictor (here: %LPA) in logistic regressions ([Agresti, 2007](#)). We set a 1 km-buffer around each presence record to account for potential migration and dispersal. We are aware that distances of both home ranges and dispersal capacities can remarkably differ among species and even within populations depending on habitat types and connectivity. However, 1 km is acceptable as an average maximum range ([Günther, 1996](#); [Böhme et al., 1999](#)). Because of concern on spatial autocorrelation, presence records had to be at least 2 km apart to ensure that the 1 km circles do not overlap. Consequently, species with less than 10 suitable presence points (i.e. whose 1 km buffers do not overlap) were not considered in further analyses ($n = 11$).

In a subsequent step, for each species, absence points were created in equal numbers to the presence points, respectively. For this purpose, we used a random sample of locations from SACs within the species' distribution range (<http://www.iucnredlist.org>), but where the considered species was not listed. Also absence points had to be at least 2 km apart and 1 km buffers were set. Finally, as a predictor for the presence/absence of a species, the %LPA was calculated within all buffers. Spatial data were processed using ArcMap 10. All statistical analyses were performed with the R and the MASS package (R Developmental Core Team, Vienna).

2.4. Evaluation factor for species' physiology (EF 2) (“potential individual sensitivity”)

This EF refers to the “potential individual sensitivity” caused by pesticide use. As mentioned in the [Introduction](#), species with small body indices show a much greater increase in dietary exposure when compared to individuals of larger species ([Weir et al., 2010](#)) and greater contact surface promoting a respectively higher uptake of pesticides ([Murphy and Murphy, 1971](#)). Therefore, we took the average snout-vent-length (and carapax length for turtles respectively) as a proxy to account for species' differences concerning their different physiology (taken from the literature; [Appendix A](#)). We estimated the probability distribution of the data (i.e. a quantitative variable) using the histogram function in R. The data were classified into five classes. Hence, for EF 2 we awarded 0 to 4 RPs.

2.5. Evaluation factor for life-history (EF 3) (“potential vulnerability of populations”)

This EF refers to the “potential vulnerability of populations” caused by pesticide use. Reptile species with a K-strategy, that is (1) with relatively few offspring (clutch size, hatchlings), (2) with low reproductive (clutch) frequency per year and (3) when longer time is needed to reach sexual maturity are supposed to suffer more from effects on individuals than r-strategists. We considered these three life-history aspects for reproductive potential by classifying (1) the average clutch/offspring size, (2) clutch frequency per year and (3) time to reach sexual maturity. Again, all data were literature-based ([Appendix B](#)). Data were grouped into four to seven classes (4 classes for average clutch frequency/year (0–3 RPs), 6 classes for average clutch/offspring size/year (0–5 RPs) and 7 classes average time to reach sexual maturity (0–6 RPs)). Hence, according to our classification, a species could score a maximum of 14 RPs for EF 3.

2.6. Calculation of the pesticide risk factors (PRFs)

Employing EFs 1–3, a species could maximally receive 19 RPs (cf. Appendices A–C). In a first step, the sum of the RP defined the SRI for each taxon. Based on the SRI and the %LPA within a species' SAC, we eventually calculated the PRF using a modified formula under which a species habitat can score PRFs 0–1 (Wagner et al., 2014).

$$\text{PRF} = \text{SRI} * \% \text{LPA} / 19 * 100$$

(SRI = sum of awarded RP; 19 = maximum RP that could be awarded)

Because of concerns about the robustness of our evaluation to changes in the definition, scale or number of categories, we additionally gave equal weights for the three EF by converting the awarded RP to a relative scale of 0–10, so that a species at maximum could score here 10 points for habitat, 10 points for physiology and 10 points for life-history (see Appendices A–C). The formula was changed to

$$\text{PRF}_{\text{weighted}} = \text{SRI}_{\text{weighted}} * \% \text{LPA} / 30 * 100$$

(SRI_{weighted} = sum of weighted RP;
30 = maximum points that could be awarded after weighting)

Finally, PRF and PRF_{weighted} were compared using Wilcoxon signed rank tests with continuity correction.

2.7. National variation

To demonstrate national variation in risk by pesticide use for Annex II reptiles, we additionally calculated %LPA within national SACs for all species, which are distributed in more than one EU member state. We tested if %LPA and thereby risk significantly differs between member states. Therefore, the %LPA within the national SACs of a species were compared. For all comparisons, one-way ANOVA followed by Bonferroni-corrected post-hoc tests were conducted (some data had to be Box–Cox-transformed prior to analysis).

3. Results

3.1. %LPA within the SACs and evaluation factors

The average (current) %LPA within the SACs was 14.37% (± 2.71) and ranged from less than 1% ($0.07 \pm 0.04 \text{ km}^2$) in SACs that were created for the Pyrenean rock lizard (*Iberolacerta bonnali*) to more than 45% in SACs for the subspecies *rakosiensis* of the Meadow viper (*V. ursinii*) ($201.84 \pm 60.60 \text{ km}^2$) (Table 1; Fig. 1; Appendix C). In the SACs of ten taxa, the %LPA was above-average (about 15–45%; Table 1).

As mentioned, the data for EF 2 (species' physiology, i.e. average snout-vent-length) were grouped into five classes (0–20, >20–40, >40–60, >60–80 and 120–140 cm). Most taxa (14) were classified into the first group (0–20 cm) and, therefore, received 4 RPs for EF 2. Three taxa received 2–3 RPs, while the large European colubrid snakes *Hierophis cypriensis*, *Natrix n. cypriaca* and *Elaphe quatuorlineata* only received 1 and 0 RPs (Appendix A).

For EF 3 (life-history), species received RP for three different factors: (1) “Average clutch size/number of offspring”, (2) “Average reproductions/year” (i.e., number of clutches/offspring per year) and (3) “Average time to reach sexual maturity”. Information was literature-based (Appendix B).

- (1) “Average clutch size/number of offspring” was grouped into six classes (2–4, >4–6, >6–8, >8–10, >10–12 and 14–16). *I. bonnali* and *Euleptes europaea* have the smallest clutch sizes and, therefore, received the highest RP (5). Nine species only produce an average of 4–6 eggs and received 4 RPs, seven taxa produce 6–10 eggs/offspring and received 2–3 RPs, while *N. n. cypriaca* and *E. quatuorlineata* have the largest clutch sizes (Appendix B).

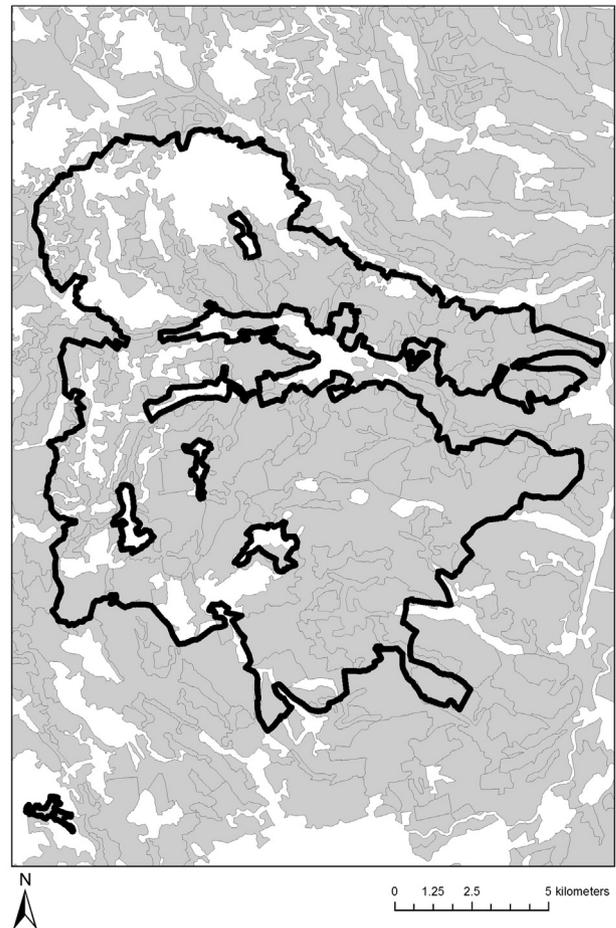


Fig. 1. Example of high agricultural land use within the Romanian Special Area of Conservation 'Dealurile Clujului de Est' (ROSCI0295), which was (among other Annex II species) created for the reptiles *Vipera ursinii rakosiensis* and *Emys orbicularis*. The borders of the protected area are black surrounded; the gray polygons indicate land cover classes where pesticides are regularly applied.

- (2) “Average reproductions/year” was grouped into four classes (1–1.5, >1.5–2, >2–2.5 and >2.5–3). Eighteen taxa received 2–3 RPs because they only reproduce on average 1–2 times per year, but *T. hermanni* and *Podarcis lilfordi* 2–3 times (Appendix B).
- (3) “Average time to reach sexual maturity” was grouped into seven classes (>8–9, >7–8, >6–7, >5–6, >4–5, >3–4 and 2–3 years). The turtles *Testudo graeca*, *T. hermanni* and *T. marginata* received 6–5 RPs because they need on average 7–8.5 years to reach sexual maturity. *E. orbicularis*, *Mauremys leprosa* and the two snakes *H. cypriensis* and *E. quatuorlineata* have an intermediate time span, while the remaining 13 taxa quickly reach sexual maturity (Appendix B).

3.2. Species risk indices and pesticide risk factors

The awarded RP amounted to the SRI, which were in average 11.1 ± 0.6 (Table 1). The turtles *T. graeca*, *T. hermanni* and *E. orbicularis* revealed the highest, *P. lilfordi* and the two snakes *E. quatuorlineata* and *N. n. cypriaca* the lowest SRI (Table 1).

Using the described formulae, the SRI or SRI_{weighted} and the current %LPA defined the final “pesticide risk factor” (PRF) or PRF_{weighted} of a species. PRF and PRF_{weighted} did not change or only little (from 0 to ± 0.07 ; Table 2). Species at above-average risk stayed the same (with one exception: *Lacerta schreiberi*; cf. Tables 1 and 2 and see

Discussion), all changes were not significant ($V = 99.5$, $p = 0.85$) and therefore we regarded the PRF (based on the sum of $RP = SRI$) as robust.

Ten species – including all six Annex II turtles – are at above-average risk by pesticide use within their SACs. In six cases, the high PRF resulted due to both high proportions of agricultural land use within the SACs and high sensitivity based on physiological and life-history aspects (i.e. high SRI). Conversely, the above-average PRF of four species (*Mauremys caspica*, *Zamenis situla*, *Vipera u. rakosiensis* and *E. quatuorlineata*) were mainly based on high proportions of agricultural land use (Table 1; Appendices A–C). Most species at above-average risk occur in the southern and south-eastern parts of Europe (Fig. 2), which represent Mediterranean and Pannonian/Continental biogeographical regions, respectively, which are also known for their high reptile species richness (Gasc et al., 1997).

3.3. Priority species and global conservation status

With regard to the seven European priority species, the two marine turtle species have not been evaluated. Moreover, *Macrovipera schweizeri* could not be evaluated due to lack of actual land cover data from Greece. From the remaining four priority species, *Gallotia simonyi*, *H. cypriensis* and *N. natrix cypriaca* are at low risk (PRFs 0.01–0.02; Table 1) within their SACs, whereas over 45% of the SACs that were created for *V. ursinii rakosiensis* are currently agriculturally used and, consequently, the highest PRF (0.32; Table 1) was assigned to this taxon.

Regarding the threat of species within their entire range and on the basis of the IUCN Red List of Threatened Species, out of the ten reptile species with above-average PRF, four are listed as Vulnerable or even Endangered, but only four as Near Threatened and two as Least Concern (Table 1).

3.4. Variations at the national scale

Seven out of 12 species, which occur in more than one EU member states, have significant differences of %LPA within their national SAC (Fig. 3; Appendices D–E). Especially in Bulgarian and Portuguese SACs that were created for Annex II reptiles, high proportions of LPA could be identified. This also accounts for Italian SACs, but only for certain



Fig. 2. In the Natura 2000 network of protected areas, most Special Areas of Conservation (all polygons represent SACs) that were created for Annex II reptile species at above-average risk by pesticide use (gray polygons) are situated in the southern parts (Mediterranean region) or the south-eastern parts (Pannonian and Continental region) of Europe compared to others (black surrounded polygons). Note that the Azores, the Canary Islands and Cyprus are excluded for better graphic representation.

species. Finally, Romanian SACs have usually low LPA, but the SACs that were created for *V. ursinii* are nearly half-covered by %LPA (Fig. 3).

Table 2

Species risk indices ($SRI_{weighted}$) after converting the Risk Points (RPs) of each evaluation factor (EF) to a relative scale of 0–10 to test for robustness of the PRF. Pesticide risk factors (PRFs) and $PRF_{weighted}$ did not significantly differ ($p > 0.05$). Above-average $PRF_{weighted}$ are in bold.

Species	$SRI_{weighted}$	$PRF_{weighted}$
<i>Gallotia simonyi</i> ^a	15.00	0.01
<i>Podarcis lilfordi</i>	12.86	0.02
<i>Chalcides simonyi</i>	15.00	0.02
<i>Hierophis (Coluber) cypriensis</i> ^a	8.93	0.00
<i>Vipera ursinii rakosiensis</i> ^a	21.79	0.33
<i>Testudo graeca</i>	28.57	0.17
<i>Mauremys caspica</i>	23.57	0.24
<i>Mauremys leprosa</i>	25.00	0.22
<i>Iberolacerta (Lacerta) monticola</i>	15.00	0.04
<i>Vipera ursinii</i>	21.79	0.06
<i>Testudo hermanni</i>	26.43	0.19
<i>Emys orbicularis</i>	26.43	0.21
<i>Iberolacerta (Lacerta) bonnali</i>	16.43	0.00
<i>Lacerta schreiberi</i>	15.71	0.08
<i>Podarcis pityusensis</i>	25.00	0.04
<i>Euleptes europaea (Phyllodactylus europaeus)</i>	15.71	0.05
<i>Elaphe quatuorlineata</i>	14.29	0.11
<i>Testudo marginata</i>	23.21	0.13
<i>Gallotia galloti insulanagae</i>	15.71	0.03
<i>Zamenis (Elaphe) situla</i>	20.71	0.19
<i>Natrix natrix cypriaca</i> ^a	5.36	0.01
		0.10

^a = priority species.

4. Discussion

According to our study, all six turtle species that are listed in Annex II of the Habitats Directive are at high risk by the use of pesticides. This is based on all three evaluated risks (exposure, sensitivity, vulnerability) and these turtles additionally show high proportions of agricultural land use within their SAC (about 16–30%; Table 1). Particularly, turtles need relatively long time to their first reproduction and, in consequence, adverse (long-term) effects of pesticide use on individuals might result in stronger effects at the population level compared to species reaching sexual maturity faster (Pianka, 1970). Considering our spatial risk evaluation as part of a first step hazard identification together with conducted laboratory/mesocosm studies, *T. hermanni* is one of the few European reptiles for which toxicological tests have revealed sensitivity to herbicide use (Willemssen and Hailey, 2001). Four out of ten species at above-average risk are listed as at least Vulnerable by the IUCN Red List of Threatened Species. However, most Annex II reptiles are endangered within their entire ranges or are listed as Near Threatened and only four of the evaluated 21 Annex II reptiles are listed as Least Concern by the IUCN (Cox and Temple, 2009). This might be the reason for the high proportion of endangered species at above-average risk. Contrariwise, Wagner et al. (2014), in their amphibian study, found that most Annex II taxa at above-average risk were listed Least Concern. But in the case of Annex II amphibians there is an inverse relationship between risk and conservation status: nearly half of them are listed as Least Concern by the IUCN (<http://www.iucnredlist.org>).

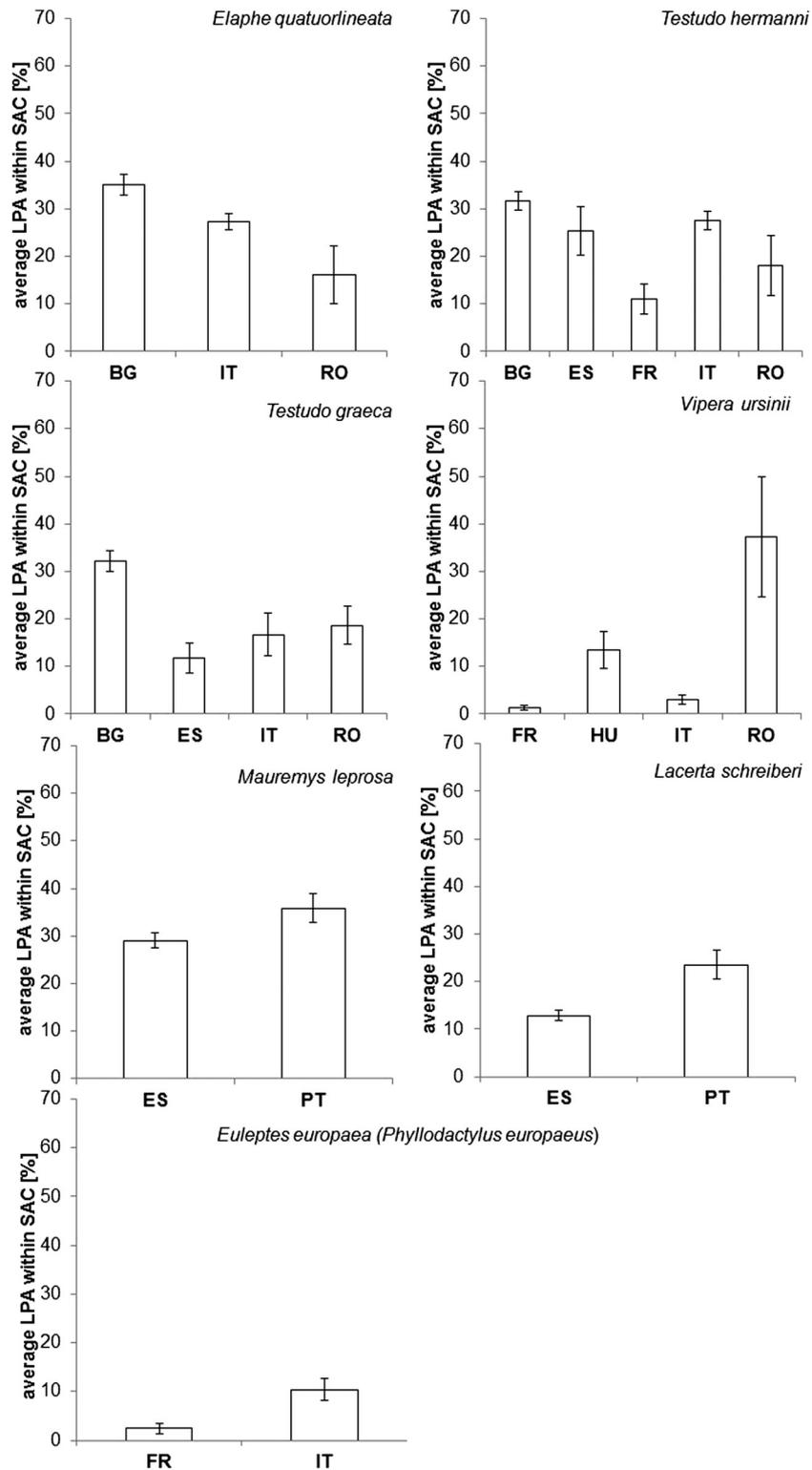


Fig. 3. National variations of “proportional land use with regular Pesticide Applications” (%LPA) (\pm SE) within Special Areas of Conservation (SACs) that were created for *Elaphe quatuorlineata*, *Testudo hermanni*, *T. graeca*, *Vipera ursinii*, *Mauremys leprosa*, *Lacerta schreiberi* and *Euleptes europaea*. Abbreviations: BG = Bulgaria; ES = Spain; FR = France; HU = Hungary; IT = Italy; PT = Portugal; RO = Romania.

The significant differences between proportions of LPA within national SAC strongly argue for species- and site-specific evaluations to avoid regional loss of reptilian biodiversity. Site-specific detailed evaluations of pesticide contamination should start in the EU member states, which reveal the highest %LPA in the SAC of their Annex II reptiles. Mainly, these are member states from the southern (Mediterranean) or south-eastern (Pannonian/Continental) regions of Europe (Fig. 2),

generally known for their higher reptile species richness compared to the rest of Europe (Gasc et al., 1997). Evaluations should include detailed information on species occurrence, population fluctuations, cultivation and pesticide application practices to possibly link reptile population declines with increasing pesticide use or use of specific formulations. With such data, the final steps of a risk assessment could be conducted (US EPA, 1986; Van Leeuwen, 2007).

4.1 . Robustness and limitations of our first attempt of a risk evaluation

Concerning the robustness of our first attempt of a risk evaluation for European reptiles to changes in the definition, scale or number of categories, we regarded the PRF (based on the simple sum of RP) as valid because no or no statistically significant differences between PRF and PRF_{weighted} were observed. However, this considered robustness may change if a species score relatively high by having the size and reproductive mode considered 'risky' even though its habitat is mostly remote from agriculture and agrochemical exposures. With regard to the Annex II reptile species, this is only the case for *L. schreiberi* (cf. Appendices A–C) and all remaining species at high risk usually occur in agriculturally used areas. However, when considering a wider range of species, this problem has to be taken into account. Conversely, pesticide drift into mountainous habitats far away from the application area has been observed in several studies (Sparling et al., 2001; Davidson et al., 2002; Davidson, 2004; Fellers et al., 2004; Davidson and Knapp, 2007).

Finally, to conduct a complete risk assessment at the European level, data on (i) detailed pesticide use, (ii) habitat contamination and effects on reptiles at the (iii) individual and especially (iv) at the population level are necessary. Such data could be obtained when specific monitoring programs will be part of the management plans of SAC. Such monitoring action should at least be considered for SAC, which are under high land use pressure and were created for Annex II reptiles that are threatened within their entire territories.

Acknowledgments

NW and SL thank the 'Graduiertenkolleg 1319 – Verbesserung von Normsetzung und Normanwendung im integrierten Umweltschutz durch rechts- und naturwissenschaftliche Kooperation' of the German Research Foundation (Graduiertenkolleg 1319) DFG for support.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <http://dx.doi.org/10.1016/j.biocon.2015.08.002>.

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