Biological Conservation 191 (2015) 667–673

Contents lists available at ScienceDirect

Biological Conservation

journal homepage: www.elsevier.com/locate/bioc

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.

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 (Willemsen 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 crocodilian, *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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http://dx.doi.org/10.1016/j.biocon.2015.08.002

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 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).

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			
Gallotia simonyi[*]	1.34 %	11	0.01
Endangered			
Podarcis lilfordi	3.64 %	8	0.02
Chalcides simonyi	3.91%	11	0.02
Hierophis (*Coluber*) *cypriensis*[*]	1.20%	10	0.01
Vipera ursinii rakosiensis[*c]	45.12 %	10	**0.24**
Vulnerable			
Testudo graeca	18.00 %	17	**0.16**
Mauremys caspica[d]	30.02 %	10	**0.16**
Mauremys leprosa[d]	26.84%	12	**0.17**
Iberolacerta (*Lacerta*) *monticola*	7.29 %	11	0.04
Vipera ursinii	7.59 %	10	0.04
Near Threatened			
Testudo hermanni	21.87 %	14	**0.16**
Emys orbicularis	23.36 %	14	**0.17**
Iberolacerta (*Lacerta*) *bonnali*	0.19 %	13	0.00
Lacerta schreiberi	15.50 %	13	**0.11**
Podarcis pityusensis	5.14%	11	0.03
Euleptes europaea (*Phyllodactylus europaeus*)	9.08 %	12	0.06
Elaphe quatuorlineata	23.60%	7	**0.09**
Least Concern			
Testudo marginata	16.28 %	12	**0.10**
Gallotia galloti insulanagae[e]	6.13 %	12	0.04
Zamenis (*Elaphe*) *situla*	27.69 %	11	**0.16**
Natrix natrix cypriaca[*f]	6.84%	5	0.02
			Ø 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 been 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 gallotia insulanagae* is considered Near Threatened by the national Spanish Red List.

[f] = no specific assessment for this subspecies.

Up to now, reptiles have been understudied in ecotoxicology (Köhler and Triebskorn, 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