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# Risk assessment considerations for plant protection products and terrestrial life-stages of amphibians

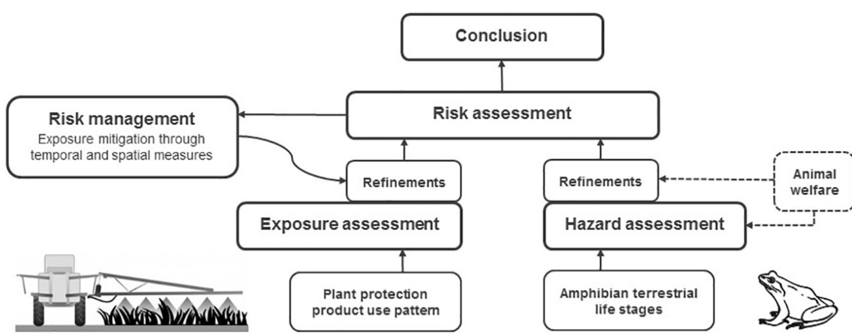
Lennart Weltje [a,*], Andreas Ufer [a], Mick Hamer [b], Peter Sowig [c], Sandra Demmig [d], Friedrich Dechet [e]

[a] BASF SE, Crop Protection – Ecotoxicology, Speyerer Strasse 2, D-67117 Limburgerhof, Germany
[b] Syngenta, Jealott's Hill International Research Centre, Bracknell, Berkshire RG42 6EY, United Kingdom
[c] Bayer CropScience, Industriepark Höchst H871, D-65926 Frankfurt am Main, Germany
[d] Syngenta Agro GmbH, Am Technologiepark 1-5, D-63477 Maintal, Germany
[e] Industrieverband Agrar e.V., Mainzer Landstrasse 55, D-60329 Frankfurt am Main, Germany

## HIGHLIGHTS

- Exposure and toxicity data for pesticides and terrestrial amphibians were considered in a risk assessment context
- Dermal exposure of terrestrial amphibians appears more important than dietary exposure
- Therefore, a screening risk assessment approach is presented for dermal toxicity to terrestrial amphibians
- The exposure-driven approach uses existing vertebrate data and would significantly reduce amphibian (vertebrate) testing
- The approach can be used to identify pesticide applications of low concern and those that need further consideration

## GRAPHICAL ABSTRACT

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## ABSTRACT

Some amphibians occur in agricultural landscapes during certain periods of their life cycle and consequently might be exposed to plant protection products (PPPs). While the sensitivity of aquatic life-stages is considered to be covered by the standard assessment for aquatic organisms (especially fish), the situation is less clear for terrestrial amphibian life-stages. In this paper, considerations are presented on how a risk assessment for PPPs and terrestrial life-stages of amphibians could be conducted. It discusses available information concerning the toxicity of PPPs to terrestrial amphibians, and their potential exposure to PPPs in consideration of aspects of amphibian biology. The emphasis is on avoiding additional vertebrate testing as much as possible by using exposure-driven approaches and by making use of existing vertebrate toxicity data, where appropriate. Options for toxicity testing and risk assessment are presented in a flowchart as a tiered approach, progressing from a non-testing approach, to simple worst-case laboratory testing, to extended laboratory testing, to semi-field enclosure tests and ultimately to full-scale field testing and monitoring. Suggestions are made for triggers to progress to higher tiers. Also, mitigation options to reduce the potential for exposure of terrestrial life-stages of amphibians to PPPs, if a risk were identified, are discussed. Finally, remaining uncertainties and research needs are considered by proposing a way forward (road map) for generating additional information to inform terrestrial amphibian risk assessment.



* Corresponding author.
*E-mail address:* Lennart.weltje@basf.com (L. Weltje).

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## 1. Introduction

Several factors threatening amphibians have been implicated in the global decline of this taxonomic group. These are: habitat destruction and modification (including road kill); infectious diseases (*e.g.*, chytridiomycosis and ranavirus infections); introduced/exotic/invasive species that compete with, prey on, and parasitize native amphibians; commercial use; contaminants and climate change (including UV-B radiation) (Cooke, 1972; Collins, 2010; IUCN, 2016). Of those factors, habitat loss and degradation is the most important one. Agricultural activities are considered likely to have an impact on amphibian populations, primarily *via* habitat modifications, but also directly *via* mechanical operations that may cause physical damage and the use of fertilizer during migration periods (Liczner, 1999; Schneeweiss and Schneeweiss, 1999; Berger et al., 2011; Berger et al., 2012). However, it is unclear if plant protection products (PPPs) contribute directly to local population declines or even to what extent amphibians are actually exposed to PPPs. Field monitoring studies have yielded contrasting results regarding the potential influence of PPPs. For example, Davidson (2004) described a statistical association between historical PPP use (mainly organophosphate and carbamate insecticides) and downwind amphibian population declines in California. In contrast, Greenberg et al. (2016) found no effect of the herbicide triclopyr on the amphibian community (consisting of 13 species) in an Appalachian hardwood forest monitored over 5 years, while Guerra and Aráoz (2015) in their 3-year monitoring study, found a higher number of amphibian individuals and species (in total 12) in sugarcane (where 11 PPPs were applied) and lemon plantations (in which 10 PPPs were applied) in Argentina, compared to secondary forests (9 species). Regardless, some level of exposure cannot be excluded and worst-case full overspray laboratory testing indicates a potential for acute toxicity to juvenile terrestrial life-stages of some PPPs (Relyea, 2005; Belden et al., 2010; Brühl et al., 2013). On the other hand, exposure of terrestrial life-stages of amphibians under more realistic conditions showed no effects of an overspray application at the registered use rate (*e.g.*, for a lambda-cyhalothrin formulation in an extended laboratory trial (Berger et al., 2015), for glyphosate (Edge et al., 2013) and pyraclostrobin (Cusaac et al., 2015) formulations in semi-field trials). Also, juvenile American toads (*Bufo americanus*) exposed dermally to filter paper moistened with a worst-case carbaryl concentration (Webber et al., 2010), as well as adult tiger salamanders (*Ambystoma tigrinum*) exposed to soil sprayed with malathion at twice the maximum use rate showed no signs of toxicity (Henson-Ramsey et al., 2008). Furthermore, national incident reporting schemes, such as that in the UK (http://www.hse.gov.uk/pesticides/topics/reducing-environmental-impact/wildlife/wiis-quarterly-reports.htm) contain no evidence for amphibian mortalities after PPP applications.

Terrestrial life-stages of some amphibian species may occur in agricultural landscapes; either because their migration routes to or from their breeding sites lead them through agricultural fields (FERA 2011; Berger et al., 2013; Lenhardt et al., 2015) or because they use arable fields as a habitat (*e.g.*, Bosman and van den Munckhof, 2006; Guerra and Aráoz, 2015). Consequently, these amphibians are expected to be exposed to agricultural practice. This includes mechanical operations such as tillage, mowing and harvesting, but also alternative weed management practices like grooming or flaming, which may physically impact animals occurring in the field. Liczner (1999) showed that physical damage was positively correlated with body length and negatively with mowing height and driving speed. In addition, there is the potential for chemical exposure to fertilizers and PPPs.

The European Regulation 1107/2009 concerning the placing of PPPs on the market (EC, 2009), requires the risks for aquatic and terrestrial life-stages of amphibians to be assessed based on available information. Similar wording can be found in the European Food and Safety Authority (EFSA) Guidance Document for risk assessment for aquatic organisms in edge-of-the-field waters (EFSA, 2013). However, there is no specific guidance available on how to perform a risk assessment for amphibians, but recently EFSA published a scientific opinion on this topic (EFSA, 2018). Also, there are no specific testing requirements for aquatic or terrestrial life-stages of amphibians, since the general consideration was that the risk to this group would be covered by aquatic, avian and mammalian data. This is, in part, related to the need to minimize vertebrate testing, which is also laid down in regulation 1107/2009 (EC, 2009). Accordingly, there are no standardized testing methods available for terrestrial amphibians (Johnson et al., 2017). The only available internationally validated tests are the amphibian metamorphosis assay (AMA; OECD TG 231, 2009) and the larval amphibian growth and development assay (LAGDA; OECD TG 241, 2015a) which use the exclusively aquatic clawed frog *Xenopus* sp. These *Xenopus* assays are specifically designed and conducted to investigate effects of waterborne chemicals on thyroid-governed developmental processes, rather than to generate toxicity data for use in risk assessment. Amphibian species with terrestrial life-stages are largely unavailable commercially and permissions would be needed for field collection and testing of native species. Besides the protected status of amphibian species, collecting specimens from the wild is problematic for a number of reasons, including: limited spatial and particularly temporal availability; the unknown (contamination) history of field-collected animals, and potential diseases and parasite infections (Blaustein et al., 2012) which may influence test outcomes (for an example on wood frog (*Lithobates sylvaticus*) tadpoles, see Pochini and Hoverman, 2017). Furthermore, in many species, a fully reliable species determination can only be done by taxonomic experts (see Plötner, 2010).

Amphibians possess unique biological features, some of which require special consideration in the risk assessment (EFSA, 2018). For most species, these features include the metamorphosis from aquatic larva to terrestrial juvenile, hibernation and terrestrial migration. Further, amphibians are poikilotherms (*i.e.* adopt the external temperature) and in the terrestrial life-stage exhibit both cutaneous gas and water exchange as well as respiration through lungs. With very few exceptions, terrestrial life-stages are exclusively carnivorous (Johnson et al., 2017), and may fast for prolonged periods of time. All of the above factors may influence an amphibian's exposure to PPPs. A major consideration and difference to other terrestrial vertebrates is the role of dermal exposure and the relative importance of this route; *e.g.*, birds and mammals have feathers and fur, respectively, while amphibians have a semi-permeable skin (Smith et al., 2007; Katagi and Ose, 2014).

Despite the abovementioned differences to other vertebrates, some conclusions on the potential risk to amphibians can be drawn by using available regulatory vertebrate toxicity data, that are generated for active substances and formulated products (see Weltje et al., 2013; Crane et al., 2016). The standard regulatory vertebrate data package consists of aquatic data, *i.e.* fish acute and chronic tests, and terrestrial data, *i.e.* avian and mammalian acute and chronic tests. In addition, there may be useful studies in the human health data package (*e.g.*, *in vitro* dermal absorption and dermal toxicity studies in mammals). Further, Berger et al. (2015) showed a correlation between the results from acute dermal terrestrial amphibian toxicity tests and rat acute inhalation toxicity studies.

This paper describes an approach to risk assessment for terrestrial life-stages of amphibians, which starts with an extended entry screen that is based on available vertebrate toxicity data and a critical examination of the potential for exposure. Thereafter, it considers options for toxicity testing of amphibians and performing a risk assessment in a tiered way. Finally, knowledge gaps are identified that require further research and, for these activities, a road map is proposed. In this context, a road map is understood to be a way forward in prioritising, developing and implementing the tools and