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Review

AMPHIBIANS AT RISK? SUSCEPTIBILITY OF TERRESTRIAL AMPHIBIAN LIFE STAGES TO PESTICIDES

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Abstract—Current pesticide risk assessment does not specifically consider amphibians. Amphibians in the aquatic environment (aquatic life stages or postmetamorphic aquatic amphibians) and terrestrial living juvenile or adult amphibians are assumed to be covered by the risk assessment for aquatic invertebrates and fish, or mammals and birds, respectively. This procedure has been evaluated as being sufficiently protective regarding the acute risk posed by a number of pesticides to aquatic amphibian life stages (eggs, larvae). However, it is unknown whether the exposure and sensitivity of terrestrial living amphibians are comparable to mammalian and avian exposure and sensitivity. We reviewed the literature on dermal pesticide absorption and toxicity studies for terrestrial life stages of amphibians, focusing on the dermal exposure pathway, that is, through treated soil or direct overspray. In vitro studies demonstrated that cutaneous absorption of chemicals is significant and that chemical percutaneous passage, P (cm/h), is higher in amphibians than in mammals. In vivo, the rapid and substantial uptake of the herbicide atrazine from treated soil by toads (*Bufo americanus*) has been described. Severe toxic effects on various amphibian species have been reported for field-relevant application rates of different pesticides. In general, exposure and toxicity studies for terrestrial amphibian life stages are scarce, and the reported data indicate the need for further research, especially in light of the global amphibian decline. Environ. Toxicol. Chem. 2011;30:2465–2472. © 2011 SETAC

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INTRODUCTION

The International Union for Conservation of Nature Categories of Vulnerable, Endangered, or Critically Endangered include 32.5% of the total number of amphibian species but only 12 and 23% of birds and mammals, respectively [1]. According to Quaranta et al. [1], amphibians are more sensitive to environmental changes and contamination than birds or mammals primarily for two reasons. First, most species spend the first part of their life in aquatic environments and the second part in terrestrial environments: they may face alteration and contamination of both [2–4]. Second, amphibian skin is highly permeable and is physiologically involved in gas, water, and electrolyte exchange with the environment [1]; therefore, it is highly susceptible to physicochemical stressors such as ultraviolet B radiation, pathogens, or xenobiotics.

In a current evaluation of the global amphibian decline, pollution is seen as the most important threat to amphibian populations after habitat loss [3]. De Lange et al. [5] modeled population vulnerability to contaminants (DDT and chlorpyrifos) of 144 species belonging to seven taxonomic vertebrate groups and showed that reptiles and amphibians contained the most vulnerable species. Assessment of vulnerability was based on ecological traits such as life history, feeding biology, internal contaminant distribution, toxicokinetics, toxicological sensitivity, and behavioral characteristics. Davidson and Knapp [6] assessed factors driving the occurrence and decline of amphibian populations in proximity to agricultural landscapes. A correlation between intense pesticide use and amphibian population decline was revealed, and the degree of protection from windborne pesticide exposure was a significant predictor of southern mountain yellow-legged frog *(Rana muscosa)* occurrence in the United States [6]. However, Bradford et al. [7] did not find any association between any pesticide-related metric and *Rana muscosa* population declines but found a relationship with the apparent pattern of spread of chytridiomycosis, an infectious fungal disease of amphibians caused by *Batrachochytrium dendrobatidis*.

A great variety of pesticides and fertilizers is increasingly applied, often in combination, representing a significant suite of pollutants. Thus, defining and attributing cause and effects in field studies investigating amphibian decline is difficult, because the agricultural landscape is in continuous flux: crop rotation, land use changes, differences in chemical use, and varying formulations and application rates [8]. Furthermore, pesticides also interact with other stressors [8–10]. For example, pesticide exposure can be an important cofactor suppressing the amphibian immune system and facilitating the outbreaks of infectious diseases such as chytridiomycosis, resulting in reduced adult fitness or mortality [8]. Infectious diseases have been associated with the decline of frog populations on six continents [8].

The scope of this review is to evaluate whether terrestrial amphibian life stages (juveniles and adults) are at risk if exposed to a pesticide, disregarding costressors. The focus was on data related to dermal exposure via pesticide-treated soil or direct overspray because this is seen as the main pathway in the terrestrial habitat [11]. We examined studies relating effects in amphibian species to defined rates or doses of pesticides applied. Monitoring data describing body burdens of frogs collected from polluted sites were not taken into account, because these data can neither be related to specific

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pesticide treatments (doses, concentrations, or application rates) nor can they be linked to a single exposure pathway (aquatic or terrestrial, dermal or dietary). Furthermore, the question was whether terrestrial amphibian life stages potentially exposed to pesticides are at higher risk than mammals or birds. Currently, no specific risk assessment for amphibians in the terrestrial habitat is performed in the authorization process of pesticides, assuming that the results of risk assessment procedures based on toxicity data for bird and mammal species will also be sufficiently protective for amphibians. No data sets for direct risk comparison between birds or mammals and amphibians for specific substances (pesticides) were available for evaluation. However, higher risk for amphibians might possibly exist if there is enhanced chemical uptake or higher sensitivity to pesticides. Moreover, the uptake route across the skin caused by overspray, which is possibly relevant for amphibians, is not taken into account in the risk assessment for birds and mammals, in which only contamination by oral uptake is considered. Studies describing significant adverse effects on amphibians of authorized field application rates of pesticides currently used would indicate that amphibian populations in the field might be at risk. Available data on aquatic amphibian exposure and toxicity were recently reviewed in particular for an assessment of sublethal effects by Mann et al. [8]. The present review elucidates the status of the experimental research on terrestrial amphibian life stages.

The literature search was performed within the literature database ISI Web of Knowledge (2011, Thomson Reuters). Searching for 'amphibian*' within the subject area 'toxicology' led to 1,104 hits. The number of hits decreased to 126 when refining the search for 'adult' or 'juvenile*,' confirming that the main part of the published ecotoxicological research concerns eggs, embryos, or tadpoles. After a further search refinement for 'pesticide*,' 23 publications remained. Excluding all studies in which postmetamorphic amphibians were exposed in an aquatic environment, fewer than 10 papers remained. Refining the search in this way in other databases such as the SETAC journals database, RATL: A Database of Reptile and Amphibian Toxicology Literature (RATL; 2011, Canadian Wildlife Service, Hull, Québec, Canada), Google Scholar (2001), or vifabio (2011, Goethe University, Frankfurt [Main], Germany), a free biologists' database, the number of relevant literature sources decreased in a similar way. Thus, as a first result, the literature search revealed that, compared with studies simulating pesticide exposure and effects in the aquatic habitat, the number of studies simulating and examining exposure and effects in the terrestrial amphibian habitat is low.

EXPOSURE: AMPHIBIAN SKIN PROPERTIES AND CHEMICAL ABSORPTION

In terrestrial habitats, amphibians are likely to be exposed to chemicals when they move across agricultural fields to reach suitable habitats for foraging and reproduction [12–14]. Chemicals may reach amphibians by direct application, drift, runoff, or residues on soil and plant material [2]. Amphibian skin is highly permeable, functioning as a respiratory organ and regulating water uptake in both terrestrial and aquatic morphs [15,16]. Amphibians in terrestrial habitats obtain water mainly by dermal absorption. Toads take up water predominantly through a highly vascularized pelvic patch of skin [12]. Through this patch, along with water, metals [17] and pesticides [18] can also be absorbed. The musculature underlying the pelvic patch may be used to regulate contact of the skin with the soil surface and help facilitate water movement through lymph channels into venous circulation [19]. Thus, amphibians moving across agricultural fields may be at risk of chemical exposure when they come in contact with soil or plants. Agricultural chemicals present on the vegetation or in soils can leach or diffuse into small pockets of water and subsequently affect amphibians [12].

Smith et al. [11] suggested that amphibians may have the ability to taste with their skin and to examine the suitability of water prior to absorbing it, a process potentially beneficial to amphibians in contact with contaminated substrates. Takahashi [20] reported that gray treefrogs (*Hyla versicolor, Hyla chrysoscelis*) avoided oviposition in pools contaminated with Roundup® (formulated glyphosate). However, Hatch et al. [21] demonstrated that juvenile western toads (*Bufo boreas*) and cascade frogs (*Rana cascadae*) avoided urea-soaked paper towels but not urea in soil, even though exposure to urea-treated soil resulted in significant mortality of both species [11]. Furthermore, juvenile American toads (*Bufo americanus*) also did not avoid soils contaminated with atrazine [12]. Therefore, it is deduced that amphibians are not able to avoid contaminated terrestrial substrates.

Willens et al. examined the percutaneous absorption of the insecticide malathion by anuran skin in vitro [22,23]. In the diffusion cell model, the total absorption (percentage of rate administered, 26 $\mu g/cm^2$) of ventral and dorsal skin of the American bullfrog *(Rana catesbeiana)* was 81 and 69% and for the cane toad (*Bufo marinus*) 83 and 77% [22]. In contrast, with the harvested perfused anuran pelvic limb (HPAPL) model, 46% absorption of the total malathion dose administered was measured in frog skin (pelvic limb) [23]. The HPAPL model maintains the anatomic and physiologic integrity of the skin of the pelvic limb, so it permits a more accurate physiological representation of in vivo cutaneous pharmacokinetics than diffusion cell models. Under this assumption, a 46% uptake of the total rate administered might be taken as a more realistic figure [23]. However, the HPAPL model examines the chemical uptake through limb skin, whereas, under field conditions, substances are also likely to be absorbed ventrally or dorsally.

Furthermore, neither model completely replicates natural exposure conditions. For example, in the case described above, ethanol was used as the application vehicle together with malathion, whereas in field applications a variety of vehicle solvent combinations may be used, influencing the absorption kinetics. Therefore, the risk arising from true environmental exposure may be higher than in the reported experiments because malathion is more likely to partition to the skin from an aqueous or oily formulation [23].

Quaranta et al. [1] also compared in vitro the percutaneous passage of two test substances (mannitol and antipyrine) and three commonly used herbicides (atrazine, paraquat, and glyphosate) through the ventral skin of adult green frog *(Rana esculenta)* and in pig ear skin (mammals). The percutaneous passage P (cm/h) of all tested substances was greater through frog than pig skin and on a logarithmic scale correlated linearly with the K_{OW} of the test substance. Relations between percutaneous passage of frog and pig (P_{frog}/P_{pig}) were 302 for atrazine, 120 for antipyrine, 66 for mannitol, 29 for paraquat, and 26 for glyphosate. Furthermore, chemical diffusion occurred one or two orders of magnitude more quickly in frog than in pig, depending on the chemical's hydrophobicity [1]. This was linked to the inversely proportional relationship between thickness and permeability of epithelia, with a stratum corneum of pig epithelium roughly 10 times thicker than the tested frog epithelium [1]. However, since for atrazine the relation between

frog and pig percutaneous passage differed by more than one order of magnitude (302), the authors assumed that possibly the structure of the *stratum corneum* as well as the composition and geometry of barrier lipids might also contribute to a greater permeability of frog skin [1]. Another parameter influencing the magnitude of xenobiotic body burdens is the skin (surface)-to-body-ratio, which is maximized in amphibians to allow percutaneous gas, water, and ion exchange with the environment and is minimized in mammals to avoid temperature loss [1].

Mendez et al. [12] assessed the uptake of atrazine by American toads (*Bufo americanus*) in vivo, exposing them to atrazine-treated soil. This mimics a realistic field exposure, because atrazine is a pre-emergent herbicide detectable in agricultural soils, and toads hydrate on moist surfaces, such as soil, rather than in open water [24]. In the present study, adult dehydrated toads were exposed to soil spiked with 20 ml radiolabeled atrazine–treated water (460 μg/L). As the animal rehydrated, radiolabeled atrazine was taken up rapidly across the pelvic patch and accumulated mainly in the gall bladder and intestine. Mendez et al. [12] concluded that exposure of adult life stages of amphibians through direct uptake of atrazine from soils and runoff water is relevant and should be considered in risk assessment procedures. However, cutaneous absorption relative to the total rate administered was not reported, and no toxic effects resulting from the chemical uptake were described in the article.

Henson-Ramsey et al. [25] assessed the toxicokinetics of malathion in tiger salamanders (*Ambystoma tigrinum*) exposed to contaminated soil surfaces. Malathion was applied to soil at a field-relevant rate (50 μg/cm^2) and a higher rate (100 μg/cm^2). Tiger salamander burdens ranged from 0.35 to 1.46 μg/g, with a median level of 0.86 μg/g, and malathion and its metabolite malaoxon were detected in all sampled tissues: epaxial muscle, liver, other viscera, and avisceral carcass (muscle, skin, and bone). No evidence of bioaccumulation was observed. Additional feeding of malathion-treated earthworms did not increase malathion body burdens. Malathion uptake resulted in inhibition of brain cholinesterase activity in the salamander species (see Table 1).

Shah et al. [26] compared the dermal penetration of the insecticides carbaryl, parathion, DDT, dieldrin, and permethrin into insects (American roaches [*Periplaneta americana*] and tobacco hornworm larvae [*Manduca sexta*]) and vertebrates (grass frog [*Rana pipiens*], Japanese quail [*Coturnix japonica*], and ICR laboratory mice [*Mus musculus*]). In general, insecticides were absorbed more quickly by vertebrate species than by insects. Carbaryl was absorbed with a half-time penetration rate of 6 min by grass frogs compared with 4,600 min for American roaches. All other insecticides were absorbed more quickly by mice or birds than by amphibians. At the end of the observation period (48 h), recovery rates of the total doses administered were highest in insects for three of five insecticides assessed. Insecticides were applied dorsally to frogs in a 1-cm^2 area using a Hamilton syringe, resulting in grass frog body burdens (in relation to total dose administered) of 85% for parathion, 96% for carbaryl, 41% for DDT, 23% for dieldrin, and 56% for permethrin. Distribution of the insecticides in blood and liver of the grass frog was low, namely, 6 to 10% in blood and 2 to 4% in liver. Thus, the insecticides were distributed mainly in the remaining carcass (25–80% of the recovered doses). Excretion of the radiolabeled molecules by frogs was low. For Shah et al. [26] differences in the species sensitivity to the tested insecticides originate from processes such as pesticide transport to site of action, metabolism, storage, and excretion rather than from substance skin penetration, because differences in total penetration between taxa were less than two- to threefold, whereas medium lethal doses differed up to 200,000-fold (DDT).

Smith et al. [11] state that the exposure of amphibian species through transport of contaminants across the skin may be the most significant route of exposure, in contrast to bird and mammal species, for which dermal exposure is considered a moderate contributor to overall exposure. For terrestrial vertebrates other than amphibians, ingestion is considered to be the predominant exposure route, because in many mammalian and avian species fur and feathers might serve as a protective barrier against chemical exposure [11]. However, Vyas et al. [27] emphasized the relevance and importance of the dermal exposure route for birds and the lack of consideration thereof.

McComb et al. [28] performed a computer simulation to predict worst-case, normal, and minimum exposure rates of mammals, birds, and amphibians to glyphosate in forests. For this purpose, the authors defined taxa-specific dermal penetration rates as a possible explanatory variable in exposure scenarios, with penetration rates of 7.5% for birds, 10% for mammals, and 50% for amphibians. The values for daily food consumption rates were inversely allocated to the vertebrate groups, with 25 to 50% of body weight for birds and mammals and a daily consumption of 10% of their body weights by amphibians. These and further assumptions lead in general to a higher modeled exposure for birds and mammals than for amphibians. However, pesticide exposure in agricultural fields with less plant interception than in forests and higher application rates likely to reach soil or amphibians may lead to a different ranking in exposure risks.

The results of these five studies on amphibian pesticide absorption confirm that the transport of contaminants across the skin is very likely a significant route of exposure for amphibians, as also stated in the review by Smith et al. [11]. Because the environmental risk assessment for terrestrial vertebrates does not take dermal exposure into account at present, these results point at an essential characteristic of the exposure scenarios that must be considered for amphibians in the future, in contrast to birds and mammals. To date, no standard exposure scenarios and risk assessment procedure has been defined for terrestrial amphibians [29].

TOXICITY OF PESTICIDES TO AMPHIBIAN TERRESTRIAL LIFE STAGES

Toxicity data for dermal exposure

Nine studies have reported toxicological data for juvenile or adult amphibians exposed dermally to pesticides (see Table 1). Among these, six studies assessed lethal effects [10,30–34], and three studies assessed behavior [35] or brain cholinesterase inhibition [25,36] as sublethal endpoints in the tested amphibians. The latter studies demonstrated dose-dependent brain cholinesterase-inhibiting effects in amphibians caused by dermal malathion exposure. Malathion also caused significant lethal effects in toads, as reported by Taylor et al. [10]. Boyd et al. [30] showed for northern cricket frogs (*Acris crepitans*) that recently metamorphosed individuals are especially susceptible to pesticides (DDT), probably because of a larger surface-to-volume ratio compared with adults. Furthermore, they showed that, independent of the life stage, northern cricket frogs collected from amphibian populations with no prior contact with the pesticide were more sensitive to DDT, suffering higher mortality, than individuals collected from amphibian populations frequently exposed to DDT previously.

Table 1. Overview of studies examining pesticide effects after dermal exposure (treated substrate or direct overspray)[a]

Species	Life stage	Pesticide: Substance	Pesticide: Class	Exposure	Endpoint	Toxicity data	Literature source
Northern leopard frog (*Rana pipiens*) Wood frog (*Rana sylvatica*) American toad (*Bufo americanus*)	Adult	Aminocarb (Matacil®)	Insecticide	Dermal (aerial forest spray)	Behavior	175 g a.i./ha: no significant change in amphibian activity	Bracher and Bider [35]
Northern slimy salamander (*Plethodon glutinosus*)	Adult	Malathion	Insecticide	Dermal (filter paper)	Behavior, ChE inhibition	3 × 5.6 kg/ha: 34% ChE inhibition; no behavioral effects	Baker [36]
Eastern red-backed salamander (*Plethodon cinereus*)				Dermal (filter paper)	Behavior, ChE inhibition	2 × 2.2–9.0 kg/ha: no significant inhibition of ChE activity; no behavioral effects	
Northern slimy salamander (*Plethodon glutinosus*)				Dermal (sprayed forest ground)	Abundance, ChE inhibition	10 × 5.6 kg/ha: no effect on abundance or ChE inhibition	
Tiger salamander (*Ambystoma tigrinum*)	Adult	Malathion	Insecticide	Dermal (soil)	ChE inhibition	50 μg/cm^2: 50–65% ChE inhibition; 100 μg/cm^2: 90% ChE inhibition; no symptoms (lethargy, tremors, decreased responsiveness, anorexia)	Henson-Ramsey et al. [25]
				Dermal + dietary		Additional dietary exposure did not increase ChE inhibition	
Northern cricket frog (*Acris crepitans*)	Juvenile + adult	DDT	Insecticide	Dermal (filter paper)	Mortality	0.31 mg/cm^2: 0–80% mortality 0.47 mg/cm^2: 5–50% mortality 0.63 mg/cm^2: 15–70% mortality 0.79 mg/cm^2: 15–50% mortality	Boyd et al. [30]
American toad (*Bufo americanus*)	Juvenile	Carbaryl (Sevin®)	Insecticide	24 h dermal (paper towel)	Mortality, feeding behavior, weight	2 mg/L: no mortality, no effect on feeding behavior and growth	Webber et al. [31]
Woodhouse's toad (*Bufo woodhousii*)	Adult	Malathion	Insecticide	Dermal (ventral)	Weight	0.0011 mg/g: 0% mortality 0.011 mg/g: 40% mortality	Taylor et al. [10]
		Malathion + bacteria				0.0011 mg/g: 80% mortality 0.011 mg/g: 100% mortality	
Great Plains toad (*Bufo cognatus*)	Juvenile	Pyraclostrobin (Headline®)	Fungicide	Dermal (overspray)	Mortality	880 ml/ha: >50% mortality (after 72 h)	Belden et al. [32]
		Propiconazole + trifloxystrobin (Stratego®)				880 ml/ha: 7% mortality (after 72 h)	
		Propiconazole + azoxystrobin (Quilt®)				102–10,200 ml/ha: 4–22% mortality (after 72 h)	
Emerald glass frog (*Centrolene prosoblepon*)	Juvenile	Glyphosate (Glyphos®) + Cosmo Flux®	Herbicide + adjuvant	Dermal (overspray)	Mortality	LC1: 1.97 kg a.e./ha; LC50: 4.5 kg a.e./ha	Bernal et al. [34]
Banded robber frog (*Pristimantis taeniatus*)	Adult					LC1: 1.93 kg a.e./ha; LC50: 5.6 kg a.e./ha	
Common lesser toad (*Rhinella granulosa*)	Juvenile					LC50: 6.5 kg a.e./ha	
Daudin's tree frog (*Scinax ruber*)	Juvenile					LC1: 0.32 kg a.e./ha; LC50: 7.3 kg a.e./ha	

(Continued)

Table 1. (*Continued*)

Species	Life stage	Pesticide		Exposure	Endpoint	Toxicity data	Literature source
		Substance	Class				
South-american crested toad (*Rhinella typhonius*)	Juvenile					LC1: 1.56 kg a.e./ha; LC50: 14.8 kg a.e./ha	
Tungara frog (*Engystomops pustulosus*)	Juvenile	Glyphosate (Glyphos®) + Cosmo Flux®	Herbicide + adjuvant	Dermal (overspray)	Mortality	LC1: 7.02 kg a.e./ha; LC50: 19.6 kg a.e./ha	
Cane toad (*Rhinella marina*)	Juvenile					LC1: 5.08 kg a.e./ha; LC50: 22.8 kg a.e./ha	
Yellow-striped poison frog (*Dendrobates truncatus*)	Adult					LC1: >7.38 kg a.e./ha; LC50: >7.38 kg a.e./ha	
Wood frog (*Rana sylvatica*)						1.6 ml a.i./m^2: 68% mortality (after 24 h)	
Woodhouse's toad (*Bufo woodhousii*)	Juvenile	Glyphosate (Roundup®)	Herbicide	Dermal (overspray)	Mortality	1.6 ml a.i./m^2: 86% mortality (after 24 h)	Relyea [33]
Gray tree frog (*Hyla versicolor*)						1.6 ml a.i./m^2: 82% mortality (after 24 h)	

[a] a.i. = active ingredient; ChE = brain cholinesterase; a.e. = acid equivalent; LC1 = concentration that will kill 1% of the sample population; LC50 = concentration that will kill 50% of the sample population.

Terrestrial exposure of American toad (*Bufo americanus*) metamorphs for 24 h on paper towels soaked in a 2 mg/L carbaryl (formulation Sevin®) solution did not have any negative impact on feeding behavior, growth, or survival [31]. However, although the concentration was realistic for the aquatic environment, the exposure was not verified to be adequate for the terrestrial environment, and the study design might underestimate terrestrial exposure and effects. The commonly used fungicide formulations Headline®, Stratego®, and Quilt® resulted in significant mortality of juvenile Great Plains toads (*Bufo cognatus*) when applied at rates relevant for the terrestrial environment [32]. The fungicide formulation Headline (active ingredient pyraclostrobin) caused the most severe effects, with >50% mortality in juvenile toads at the corn label application rate. The glyphosate formulation Roundup caused significant mortality (68–86%) when Relyea [33] exposed juvenile gray tree frogs (*Hyla versicolor*), wood frogs (*Rana sylvatica*), and Fowler's toad (*Bufo woodhousii fowleri*) directly at an application rate of 1.6 ml active ingredient (a.i.)/m^2 in a worst-case scenario, assuming no interception by vegetation during application. Bernal et al. [34] performed, in their opinion, a more realistic exposure scenario by employing field-relevant glyphosate rates (1.85–29.5 kg a.e. [acid equivalents] of glyphosate/ha; field rate 3.69 kg a.e. of glyphosate/ha) and providing soil and leaf litter in the experimental units. The resulting lower lethal effects compared with the results reported by Relyea (for median lethal dose [LD50] values see Table 1) could be related to the altered exposure scenario but also to the use of a different glyphosate formulation (Glyphos®) combined with a specific adjuvant (Cosmo flux®) used for coca control in Colombia. Bracher and Bider [35] performed a field experiment spraying the insecticide Matacil® (aminocarb) over forest areas by aircraft at the maximum allowed field rate (175 g a.i./ha, Agriculture Canada) and assessing the activity of the forest animal community. Activity was assessed by evaluating footprints on sand transects and determining the total number of transect crossings per day for each species. The amphibian activity at the treated site did not differ statistically from the activity at the control site.

Toxicity data for oral or subcutaneous exposure

In seven evaluated studies, adult or juvenile amphibians were exposed to pesticides subcutaneously by injection or orally through food items (dietary; see Table 2). In six of the seven studies, sublethal effects were assessed; in three of them, lethal effects were also assessed.

Dieldrin, DDT, and malathion (insecticides) exposure caused immunosuppressive effects in the tested amphibians [37,38]. The DDT-metabolite *p,p*-DDE (l,l-dichloro-2,2-bis[P-chlorophenyl]ethylene) decreased CYP26 gene and protein expression, thereby possibly affecting health and reproductive ability [39]. Exposure to pentachlorophenol (fungicide) did not cause mortality but significantly reduced food consumption [40]. McComb et al. [28] determined LD50 values for amphibians exposed to glyphosate (herbicide) and did not detect any effects on liver or kidney tissues. Harri [41] determined the median lethal dose of DDT in European grass frogs *(Rana temporaria)*. Further details on the experimental design, amphibian species exposed, and test results are reported in Table 2.

McComb et al. [28] compared mammalian and amphibian toxicity data with toxicity data for Swiss–Webster laboratory mice *(Mus musculus)* to evaluate the degree to which dose responses of model organisms (laboratory rodents) could be

Table 2. Overview of studies examining pesticide effects after oral or subcutaneous exposure (injection)[a]

Species	Life stage	Pesticide: Substance	Pesticide: Class	Exposure	Endpoint	Toxicity data	Literature source
Northern leopard frog (*Rana pipiens*)	Adult	DDT Dieldrin	Insecticide	Dietary	Immunosuppression	75 ng/g: significant reduction of antibody level 2.1 ng/g: significant reduction of antibody level	Albert et al. [37]
Northern leopard frog (*Rana pipiens*)	Adult	DDT Malathion Dieldrin	Insecticide	Injection	Immunosuppression	923 ng/g: immunosuppressive effects 990 ng/g: immunosuppressive effects 50 ng/g: immunosuppressive effects	Gilbertson et al. [38]
European common frog (*Rana temporaria*)	Adult	*p,p*-DDE	Insecticide metabolite	Injection	CYP26 gene and protein expression	Sublethal doses of 0.01–10 mg/kg: dose-specific significant decrease, implications in health and reproductive ability	Leiva-Presa et al. [39]
African clawed frog (*Xenopus laevis*)	Subadult	Pentachlorophenol	Fungicide/ bactericide	Dietary	Mortality/feeding behavior	64.8–2604 μg/g meal worm (27 days): 0% mortality NOAEL for reduced food consumption: 638 μg/g	Schuytema et al. [40]
Rough-skinned newt (*Taricha granulosa*)	Adult	Glyphosate	Herbicide	Injection	Mortality/behavior	LD50: 1,250 mg/kg, NML: 500 mg/kg; no effects on behavior	McComb et al. [28]
Tailed frog (*Ascaphus truei*)				Injection	Mortality/histopathological changes	LD50: >2000 mg/kg, NML: >2,000 mg/kg; no effects on liver/kidney tissues	
Western red-backed salamander (*Plethodon vehiculum*)				Injection	Mortality/histopathological changes	LD50: 1,170 mg/kg, NML: <1,200; no effects on liver/kidney tissues	
Ensatina salamander (*Ensatina eschschlotzii*)				Injection	Mortality	LD50: 1,070 mg/kg, NML: <900 mg/kg	
Pacific giant salamander (*Dicamptodon ensatus*)				Injection	Mortality/histopathological changes	LD50: <2,000 mg/kg, NML: <2,000 mg/kg; no effects on liver/kidney tissues	
Rough-skinned newt (*Taricha granulosa*)				Oral	Mortality	LD50: >2,600 mg/kg, NML: >2,600 mg/kg	
European common frog (*Rana temporaria*)	Adult	DDT	Insecticide	Oral (gelatin capsule)	Mortality	20-days LD50: 7.6 mg/kg	Harri [41]

[a] DDE = *p,p*-DDE = (l,l-dichloro-2,2-bis[P-chlorophenyl]ethylene); NOAEL = no-observed-adverse effect level; LD50 = dose that will kill 50% of the sample population; NML = no-mortality level.

used to predict dose–response relationships of other wildlife species. Therefore, the technical glyphosate isopropylamine salt (herbicide) was injected intraperitoneally. The LD50 values of glyphosate ranged from 800 to 1,340 mg/kg for mammals and from 1,170 to >2,000 mg/kg for amphibians and were in the center of the mammalian range for laboratory mice. Therefore, McComb et al. concluded that white laboratory mice are adequate to model the sensitivities of seven wildlife animal species of nine species included in the experiment. According to McComb et al. [28], injection is not representative of normal field exposure, but this administration method was used because it ensures precise dosage. Thus, it is questionable whether the toxicity data related to the injected doses is representative for field situations, because differences in processes such as absorption, distribution in the body, and transport to organs between amphibian and mammal species may result in other toxicity responses compared with a direct injection of the pesticides.

CONCLUSIONS

The reported results of the evaluated studies indicate that the transport of pesticides across the skin is likely to be a significant route of exposure for amphibians, as was also stated by Smith et al. [11], and that pesticides can diffuse one or two orders of magnitude more quickly into amphibians than into mammals [1]. We also found exposure and toxicity studies for terrestrial amphibian life stages to be scarce. Only 13 studies linking field-relevant dermal or dietary exposure to terrestrial toxicity data could be evaluated. However, the few existing toxicity data suggest that amphibians can be sublethally or even lethally affected by field-relevant terrestrial pesticide application rates. The paucity of published data on terrestrial amphibian life stages is remarkable, especially with the variety of pesticide formulations in use for crop protection; the countless possible combinations thereof; the numerous costressors such as ultraviolet B radiation, pathogens, and parasites; and the differences in amphibian species sensitivity, indicating the need for further research. However, it should be kept in mind that examining single pesticides at high concentrations and without addressing the effects of costressors may lead to an underestimation of the role of pesticides in affecting amphibian populations [42]. Nevertheless, such data would allow for an approximation of the risk to amphibians posed by pesticide use.

For the aquatic environment, Aldrich [29] found the acute aquatic risk for amphibian eggs and larvae to be adequately represented by aquatic invertebrate and fish data. For the terrestrial life stages of amphibians, the verification of a sufficient protection from unacceptable risks by using the vertebrate data from bird and mammal studies in the risk assessment of pesticides is imperative.

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