



Impact of agricultural pesticides on amphibian (frog & toad) populations

Deepak Tiwari

Department of Zoology, University of Allahabad, Uttar Pradesh, India

Abstract

Amphibians (frogs/toads) are one of the most endangered vertebrates in the world and contamination with agricultural pesticides has been broadly cited as a significant factor in the global demise of amphibians. The paper summarizes experimental, field and meta-analytic findings on the effects of agrochemicals, e.g. herbicides, insecticides, and fungicides, on amphibian survival, development, growth, immune functioning, and reproductive health at various life stages. The most important mechanisms of action are direct acute toxicity, sublethal physiological and behavioral effects including endocrine imbalance, immunosuppression and perturbed growth and metamorphosis and indirect effects which are mediated by habitat degradation and food-web changes. The meta-analyses and numerous field studies all show that pesticides exposure has negative impacts on amphibian survival, body status, and population sustainability. Specific laboratory investigations go on to point at certain issues that can be raised regarding commonly used chemicals like atrazine, neonicotinoids, and some pyrethroid insecticides. The paper ends by recommending management and conservation actions which focus on reducing off-target exposure to pesticides by building buffer zones and more effective pesticide practices, implementing an integrated pest management (IPM) approach, undertaking regular monitoring of the population of amphibians in agricultural landscapes, and prioritizing research into pesticide combinations and actual exposure situations.

Keywords: Amphibians, development, growth, integrated pest management

Introduction

Amphibians play a central role in freshwater and terrestrial environments and are generally considered major bioindicators of environmental wellbeing. Regulates the population of insects, such as crop pests, disease vectors, supports nutrient cycling by having a dual aquatic-terrestrial life cycle, and is a major food source to reptiles, birds, mammals and fish (Blaustein and Wake, 1990; Wells, 2019) [2, 18]. This sensitivity to adverse environmental influences increases their ecological significance as a result of which the population dynamics in amphibians are an early indicator of ecosystem destruction. Amphibians have some biological characteristics that increase their susceptibility to chemical pollutants. Their skin is very permeable and facilitates the exchange of gases and water and also enables the absorption of pollutants in the soil, plants and water at high rates (Duellman and Trueb, 1994) [7]. Moreover, their biphasic cycle of life, which is usually made up of aquatic eggs and larvae and then terrestrial or semi-terrestrial juveniles and adults, places them in a position where they are exposed to pesticides in various habitats and in different stages of their development (Mann *et al.*, 2009) [10]. Farmlands usually overlap with the breeding wetlands of amphibians, and seasonal migrations often force their movement across the pesticide-treated fields, which means that both acute and chronic exposure is more likely to occur (Brühl *et al.*, 2013) [5].

Amphibian populations have never been as reduced on a global scale since the late twentieth century and over 40 percent of the world amphibians now face the threat of extinction (Stuart *et al.*, 2004; List, 2004) [9, 17]. These losses are commonly known as multifactorial, and they come as a result of habitat destruction and fragmentation, new infectious disease outbreaks like chytridiomycosis brought about by *Batrachochytrium dendrobatidis*, climate change, invasive species, and environmental pollution (Collins and

Storfer, 2003; Hayes *et al.*, 2010) [6]. Agricultural pesticides have become one of such drivers and a significant and widespread stressor on its own, as well as in combination with other threats.

Farm pesticides such as herbicides, insecticides, and fungicides are supposed to interfere with the workings of nature and have become widespread using both spatial and temporal scales worldwide. These chemicals get into the non-target habitat through runoff, spray drift, atmospheric deposition and leaching in to the ponds, streams, and the terrestrial environment around them which amphibians use (Relyea, 2005) [12]. Various laboratory and field experiments have shown that pesticides may have both direct lethal and various sublethal effects, such as retarded growth and development, impaired behavior, endocrine disruption, and immunosuppression (Boone and Bridges, 2003; Rohr and McCoy, 2010) [3]. The concerns of such sublethal effects are even more terrifying since these effects might not permanently cause death, but instead they can diminish fitness, make organisms more vulnerable to predators and pathogens and eventually, the effects can be felt at the population scale.

Notably, there is no single exposure to pesticides. It has been indicated that agrochemicals may act synergistically with pathogens and environmental stresses to increase the effects of diseases and suppress immune defenses of the amphibians (Rohr *et al.*, 2009) [15]. As an example, parasite infections and fungal diseases are known to be more vulnerable to pesticide-induced immunosuppression, which already causes huge amounts of amphibian declines globally (Hayes *et al.*, 2006) [8]. Moreover, pesticide mixtures, or combinations, are more realistic representatives of exposure conditions in the real world, but their interaction effects have not yet been fully studied. According to amphibians' ecological values combined with poor tolerance to chemical stressors reported, and the ongoing increase of the

agricultural industry on a global scale, the effects of pesticides used in agriculture on the populations of the frogs and toads are a scientific and conservation challenge. There is a need to synthesize the experimental, field, and meta-analytic evidence in a comprehensive way to explain the mechanisms of toxicity, determine risks on populations, and provide information on management strategies. This kind of information is essential in coming up with mitigation strategies that will be able to strike a balance between agricultural production and conservation of biodiversity and sustainability of the ecosystem in the long term.

Exposure Pathways and Ecological Context

Agricultural pesticides have a unique exposure to amphibians, which is associated with life-history factors, physiological factors, and geographic location near agroecosystems. Exposure is possible by various direct and indirect routes acting both in aquatic and terrestrial environments and differing with the ecology of different species, landscape architecture, and agricultural activities. Direct overspray as the pesticides are applied on agricultural fields is one of the primary routes of exposure. Numerous species of frogs and toads make seasonal terrestrial migrations among non-breeding areas and the aquatic breeding places, crossing frequently croplands and field margins where pesticides are being actively used. When taking part in such movements, people can be sprayed directly by spray droplets or dust contaminated with pesticides, which causes acute dermal exposure (Brühl *et al.*, 2013) [5]. In amphibians, protection of agrochemicals is very dangerous, unlike most vertebrates, which have protective scales or fur.

The second prominent route is through the dermal and respiratory intake of contaminated soil, vegetation and surface water. The amphibian skin is very permeable and it is very important in osmoregulation and respiration. This physiological process helps dissolved chemicals such as pesticides to be absorbed quickly in case they are deposited on the soils, plants or litter (Duellman and Trueb, 1994) [7]. Amphibians often have close contact with wet substances resulting in large internal dosages even at low environmental concentrations. One of the most important

and least vital ways of pesticide intake in the earthly phase of life is thus cutaneous exposure (Quaranta *et al.*, 2009) [11]. The third is dietary exposure, where amphibians eat invertebrate food that can either bioaccumulate or harbour residues of the pesticides. Insects and other arthropods in agricultural landscapes regularly have quantifiable insecticides and herbicides, which are passed on to amphibians during trophic interactions. In aquatic environments, amphibian larvae can also consume polluted algae, detritus or suspended solids and further augment internal exposures. This type of dietary absorption may result in chronic and sublethal exposure to it, which impairs growth, behavior, and the immune system. The fourth route is the fourth significant pathway of exposure, which is the embryonic and larval exposure to water. Pesticides are conveyed to ponds, ditches, wetlands, and streams where amphibians breed and develop larvae (Relyea, 2005) [12]. Since tadpoles and eggs spend a long time in the water, they are highly susceptible to dissolved pesticides and their metabolites. The pulse exposures after rain events may overlap with critical developmental periods, which leads to death or permanent developmental impacts (Mann *et al.*, 2009) [10]. Particularly vulnerable creatures are innovative amphibians that have a life-cycle of migration between terrestrial and aquatic breeding locations. An example is when adults come across pesticides when they are migrating on the land, and eggs and larvae come across the polluted water bodies. Such cumulative risk and potentially less population resilience is a result of this cumulative exposure which is repeated and stage-specific. Exposure intensity and duration are highly affected by the ecological context. Environmental availability depends on pesticide persistence, chemical formulation (e.g. wettable powders, granules, emulsifiable concentrates), application frequency and timing (Boone and Bridges, 2003) [3]. The movement of pesticides into amphibian habitats is prolonged by highly persistent compounds or formulations that increase penetration, whereas landscape factors like soil type, slope, vegetation cover, and hydrological connectivity define the movement of pesticides into aquatic environments. Downslope wetlands of agricultural fields or without vegetated buffer zones are some of the most susceptible to contamination.

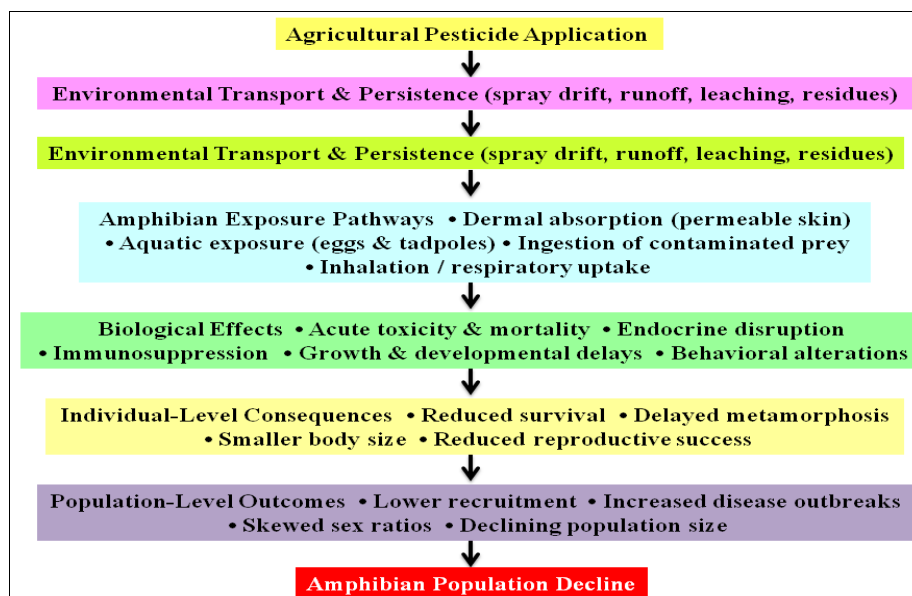


Fig 1: Conceptual flowchart showing direct and indirect effects of agricultural pesticides on frogs and toads

Field surveys and residue analysis can be used to present solid evidence that exposure to pesticides is not a mere theoretical consideration that does not happen in real-world situations. Research has detected a large variety of herbicides, insecticides, and fungicides in surface waters, sediments, and amphibian tissues found in farmlands (Brühl *et al.*, 2013) ^[5]. Notably, such residues usually contain parent compounds as well as biologically active metabolites. Most amphibians do not experience a single chemical, but rather, a complex combination of multiple pesticides is found in tissue analyses, and there are concerns on whether there is an additive or synergistic effect that is not appropriately captured using a single-compound toxicity test (Sparling *et al.*, 2010) ^[16]. In general, physiological sensitivity, multi-habitat lifecycle and proximity to agricultural settings expose amphibians to high risks of being exposed to pesticides. Knowledge of these exposure pathways in their ecological setting is critical in the effective measurement of risks, interpretation of toxicological data, and development of effective mitigation measures to decrease the effects of pesticides on frog and toad populations.

Types of Effects on Amphibians

The effects of agricultural pesticides on amphibians occur on an individual, population and ecosystem levels, with both lethal and sublethal effects occurring as a spectrum. Due to multiple exposures of frogs and toads at different stages of their life cycle and from different habitats, such effects tend to compound and interact over time resulting in the long-term population decline even in the absence of noticeable mass mortality events.

Acute Toxicity and Mortality

There are also pesticides that are simply fatal to the amphibians at the levels of occurrence in farmlands. Several groups of agrochemicals have been reported to cause acute toxicity, especially organophosphates (e.g., chlorpyrifos, Malathion), carbamates (e.g., carbaryl), older-generation insecticides, which disrupt neural transmission, or metabolic activities (Sparling *et al.*, 2010) ^[16]. Laboratory toxicity tests show that lethal concentration (LC 50) is highly species-dependent, development, temperature and formulation dependent (Boone and Bridges, 2003) ^[3]. These stages are more vulnerable relative to adults since they undergo rapid cell division, lack detoxification mechanisms, and are exposed to toxic substances over a long period in water environments (Mann *et al.*, 2009) ^[10]. Nevertheless, terrestrial adolescents and adults are also susceptible, especially when there is a migration period and the juveniles and adults can directly receive an overspray or direct contact with freshly treated soils (Bruehl *et al.*, 2013). The amphibian mortality has been reported in the field and has been associated with pesticide applications or even post-rainfall runoff suggesting that laboratory-observed acute toxicity can be put into practice with resulting losses in the actual population (Relyea, 2005) ^[12].

Sublethal Physiological Effects

Although acute mortality has ecological importance, the sublethal effects are becoming known as important causes of amphibian population decline as much as acute mortality. They do not necessarily lead to immediate death but might lead to impaired fitness, lower reproductive success and exposure to other stressors.

Endocrine Disruption and Reproductive Impairment

One of the most thoroughly researched sublethal pesticide effects on amphibians is the endocrine disruption. Certain experimental and field studies have reported that the use of herbicides including atrazine disrupts the steroid hormone balance, resulting in the alteration of the testosterone and estrogen levels, decreased sperm production, hermaphroditism and feminization of male frogs (Rohr and McCoy, 2009) ^[15]. These disturbances have the potential to interfere with mating, fertility, and offspring viability and end up distorting sex ratios and decreasing growth rates in population. Even though the extent of the effects of atrazine at environmentally significant concentrations is still a point of contention, an extensive literature has been recorded on consistent biomarker-level and endocrine responses to low-dose and chronic exposures in amphibian reproductive systems (Hayes *et al.*, 2006) ^[8]. Other pesticides, those with hormone-mimicking or hormone-disrupting properties, are raising similar concerns.

Immunosuppression and Increased Disease Susceptibility

Exposure to pesticides may impair the ability of amphibians to resist pathogens and parasites because of weakened immunity. Surveys have indicated that some insecticides and herbicides suppress the functions of leukocytes, antibodies and antimicrobial defenses on the skin. This immunosuppression is especially frightening in the light of the worldwide effect of infectious illnesses like chytridiomycosis and ranaviral diseases. Experimental studies indicate that amphibians exposed to pesticides are more prone to parasitic infections and fungal pathogens and additionally chemically-stressed amphibians have been shown to counteract disease in a synergistic relationship and contribute to the rapid depletion of the population (Rohr *et al.*, 2009) ^[15]. Therefore, pesticides can not only lead to direct damages but also increase the impact of other significant contributors of the amphibian loss.

Growth and Developmental Delays

Growth patterns, developmental rates, and metamorphic time can all be dramatically changed by exposure to pesticides during the larval stage of development. Such agrochemicals have been found to cause reduced growth, slowed and increased metamorphosis, and lower body size at metamorphosis that has been strongly associated with post-metamorphic survival and reproductive success in tadpoles (Boone and James, 2003) ^[3]. In a meta-analysis of data in many studies, similar adverse effects of pesticides on amphibians were found in survival and growth, which showed that these effects could be generalized to species and chemical classes (Baker *et al.*, 2013) ^[1]. Reduced size at metamorphosis may raise the risk of predation, low competitive ability, and future fecundity and thereby convert sublethal developmental impacts into effects at the population level.

Behavioral Changes

Sublethal exposure to pesticides may also interfere with the amphibian behavior, including foraging efficiency, predator shunning, learning, and reproductive signals. Larvae have been reported to exhibit altered swimming performance and low escape responses, which causes the larvae to be more vulnerable to predation (Relyea & Mills, 2001) ^[13].

Depending on the exposure in adults, calling behaviour, mate attraction and territoriality can be influenced, thus resulting in low reproductive success even when the individuals survive the exposure (Bridges, 1997). Behavioral impairments are especially challenging to identify in fieldwork and may have significant ecological consequences since they do not create obvious pathological evidence that might cause direct effects on survival and reproduction.

Indirect Ecological Effects

In addition to direct physiological effects, there are also indirect ecological effects of pesticides that may have significant effects on amphibians. Agrochemicals have a tendency of dwindling the number and variety of aquatic and terrestrial invertebrates, forming the major food source of most amphibians (Relyea, 2005) [12]. Lack of food and nutrition may be negative growth, lengthening the lifespan of the larva, and death due to starvation or predation.

Pesticides can also change predator prey interactions by killing off predators or competitors selectively resulting in trophic imbalances. Along with that, herbicides and fungicides may negatively impact the quality of habitats by removing aquatic plants and altering the periphyton and microbial communities and by modifying nutrient cycling in wetlands (Mann *et al.*, 2009) [10]. These habitat-mediated effects have the potential to last long after pesticide use, and can currently act on more scale than direct toxicity.

Synthesis

All these factors, including acute toxicity, sublethal physiological interference, behavioral maladaptation, and indirect ecological impact, create a complicated net of stressors on amphibians in agricultural landscapes. Although the level of pesticides may not be high enough to result in instant death, their summative and interactive impacts may undermine the population resiliency and play a significant role in causing the long-term changes of frogs and toads.

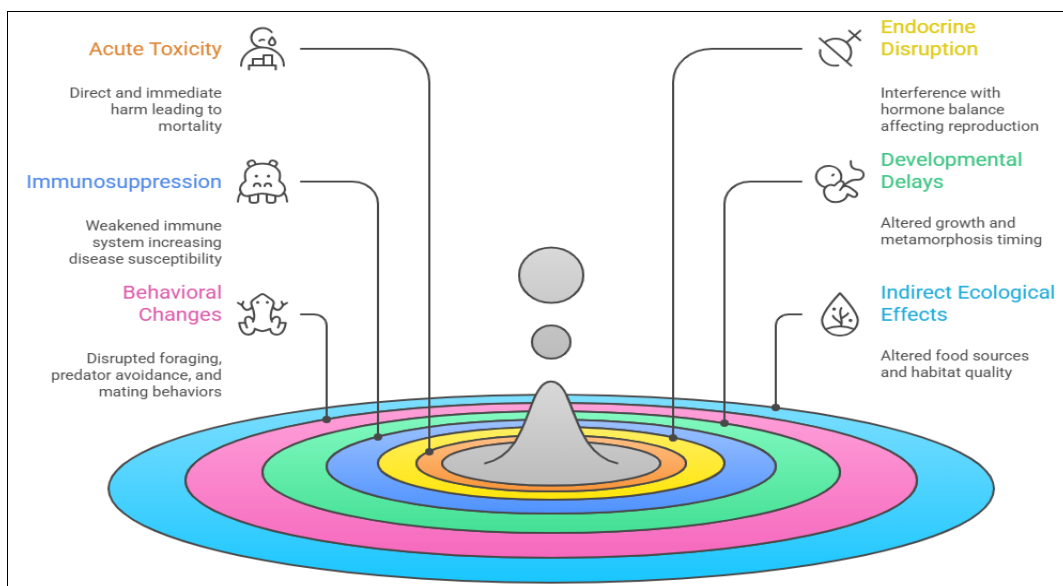


Fig 2: Type of effect in Amphibians

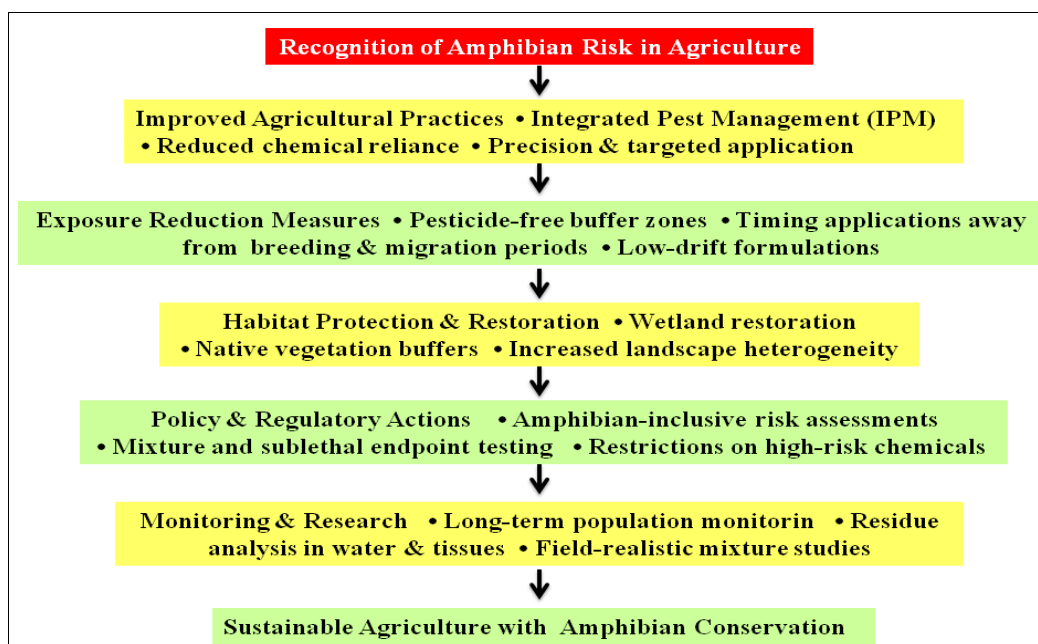


Fig 3: Integrated framework for mitigating pesticide impacts on amphibian populations in agricultural landscapes

Evidence Synthesis

Meta-analyses and Broad Syntheses

An increasing number of meta-analytic and review-based studies demonstrate that, the relative to agricultural pesticides, amphibians are exposed to the consistent negative impacts of agricultural pesticides in strong and quantitative measures. It is one of the most impactful meta-analysts that combined data of dozens of lab and field experiments and evaluated lethal and sub-lethal effects of pesticides and fertilizers on amphibians. The analysis has documented a huge negative effect size on the survival (about -0.90) and a quantifiable decrease in growth (about -0.07), which showed that agrochemicals have a significant impact on amphibian viability (by species, life stages, and chemical classes) (Baker *et al.*, 2013) ^[1]. Notably, these effects were not specific to a given taxonomic group and type of pesticide, pointing at an overall proneness of amphibians to the impact of agriculture chemicals. General regional and global overarching syntheses also highlight disproportionately high risks among amphibians that live in agricultural landscapes. The species which move through cultivated land to breeding ponds are especially vulnerable because they experience pesticides both on land and in water (Brühl *et al.*, 2013) ^[5]. Reviews point out that terrestrial exposure pathways, including direct overspray and contact with contaminated soils, have traditionally been underrepresented in the risk assessment process where aquatic toxicity has been the major component of toxicity (Mann *et al.*, 2009) ^[10]. The surveys of the fields in Europe and North America regularly report the occurrence of herbicides, insecticides, and fungicides in wetland water, sediments, and tissues of amphibians within agricultural catchments confirming that exposure is extensive and chronic and not unique. Taken together, all these syntheses suggest that pesticide exposure can be a widespread stressor that can affect the amphibian population dynamics on landscape scales.

Chemical-Specific Findings

Atrazine is one of the herbicides that has received a remarkable level of scientific scrutiny because of its popularity and possible endocrine disruptive impact. Several experimental studies document atrazine-induced changes in sex hormone regulation, gonadal development and reproductive morphology on amphibians including low androgen levels and feminization or demasculinization of male amphibians (Hayes *et al.*, 2006) ^[8]. Although the extent of population-wide effects at environmentally significant concentrations is controversial, weight-of-evidence analyses have consistently determined biomarker-level endocrine and immunological reactions following atrazine exposure (Rohr and McCoy, 2010) ^[15]. Latest reviews still rank atrazine as a chemical of concern to the amphibian endocrine and immune system, especially when it is used chronically and with other stress agents. The neonicotinoid insecticides and amphibian's literature is relatively new, yet the literature is growing at an accelerated rate. Initially created as systemic insecticides to attack crop pests, neonicotinoids are currently known to be environmentally contaminant and biologically active outside of their target. Laboratory and mesocosm experiments prove that the exposure to neonicotinoids may disrupt larval development, slow growth, change the swimming behavior,

and have carryover effects that extend into juvenile life on land (Robinson *et al.*, 2019) ^[14]. Even though the acute toxicity to amphibians is usually less than the insects, sub-lethal effects are worrying in regard to long-term population implications. There are indications of direct physiological effects as well as indirect effects that are mediated by decreased prey abundance but there are significant gaps in our understanding of real-life concentrations, formulation-specific toxicity, and cross-contamination with other agrochemicals. Control studies in laboratories have also revealed that other classes of pesticides such as pyrethroids, organophosphates and some fungicides have lethal and sublethal effects on amphibians. These substances have the ability to interfere with neural transmission, metabolisms as well as immune functions resulting to death or reduced functioning (Sparling *et al.*, 2010) ^[16]. Nevertheless, their environmental applicability differs across the board based on environmental endurance, modes of application, and dynamics within the landscape transport. Consequently, it is found that field exposure probability and, therefore, population-level risk vary significantly across compounds, and even across agricultural settings (Hayes *et al.*, 2006) ^[8].

Mechanisms linking pesticides to population declines

The effects of agricultural pesticides on the declines in the amphibian populations involve a series of mechanisms that are interdependent and have various effects at both stages of life and at ecological levels. These processes tend to work together making their effects more destructive and lessening the population resistance in agricultural landscapes. A decreased survival and recruitment is one of the main mechanisms. Exposure to pesticides at the embryonic and larval stages often causes death and interferes with normal development. The agrochemicals used tend to cause delayed metamorphosis or metamorphose at a reduced body size in the larvae, which are highly correlated with poor juvenile survival, reduced dispersal capability, and low reproductive capacity in adulthood (Boone and Bridges, 2003; Baker *et al.*, 2013) ^[1, 3]. Since the recruitment of juveniles into the breeding population is a key factor in the persistence of the population, a slight decrease in the growth of larvae or their survival can have long-lasting consequences of population losses.

Reproductive impairment: Another important route by which pesticides because demographic change is reproductive impairment. Numerous pesticides disrupt the female hormonal regulation causing hormonal imbalance, maldevelopment of gonadal development, decreased gametes production and deformed mating behavior (Hayes *et al.*, 2006; Rohr and McCoy, 2010) ^[8]. These disturbances cause decreasing fecundity and mating success, sex ratio distortions, and eventually reduce the growth rates of populations. The chronic, low dose long term exposure is especially worrying because the effects of these on reproductive processes might take some time to build up without causing filibustre mortality.

Disease amplification: Ecology The pesticide effects on the amphibian populations are further exacerbated by the amplification of disease. According to a great number of studies, the exposure to pesticides may inhibit the immune system, making all the defenses, both innate and adaptive,

weaker (Rohr *et al.*, 2009) ^[15]. Amphibians with compromised immunity are more vulnerable to pathogens like chytrid fungi (*Batrachochytrium dendrobatidis*) and ranaviruses which are some of the largest causes of amphibian worldwide losses. Under these circumstances, pesticides are the indirect causes of population collapse because they raise the occurrence and severity of diseases, and turn previously sublethal stress into massive losses of demographics. Besides direct effects on the individuals, pesticides are also able to decrease the amphibian populations due to habitat-mediated decreases in carrying capacity. Repeat pollution of breeding ponds and adjoining land environments impairs the water quality, decreases aquatic vegetation and changes microbial and invertebrate populations required to support larval growth (Mann *et al.*, 2009) ^[10]. The destruction or modification of the appropriate breeding habitat actually reduces the number of individuals that a landscape is capable of supporting. Such habitats can have a long-term effect of acting as a demographic sink, in which local reproduction is insufficient to replace mortalities, causing recolonization to be slowed by the refugia around it, and prompting the initiation of regional losses (Brühl *et al.*, 2013) ^[5]. Combined, diminished recruitment, reproductive failure, augmented disease vulnerability and depreciated habitat generate strengthening feedbacks damaging amphibian population health. The combination of these mechanisms in areas where farming is absorbed may result in long-term population suppression or local extirpation even in cases where pesticide levels do not often result in mass mortality events.

Management and Mitigation Strategies

Landscape-Level Measures

The landscape-scale interventions that minimize the exposure and improve the quality of the habitat could help mitigate the effect of pesticides on amphibians. One of the most effective ways of reducing spray drift and run off into amphibian habitats is to create pesticide free buffer zones around breeding ponds, wetlands and terrestrial migration corridors. Reclamation and rehabilitation of wetlands with native vegetation can also reduce pesticides loads, by means of sedimentation, uptake by plants, and degradation by

microbes and at the same time provide high-quality habitats of breeding and refuge (Mann *et al.*, 2009) ^[10]. By enhancing the heterogeneity of landscapes, including keeping margins of fields uncultivated, and linking blocks of habitat, possible shelters can be created which minimize cumulative exposure and aid recolonization.

Agricultural Practice Changes

Integrated pest management (IPM) plans at farm level can significantly decrease the use of chemical pesticides and still maintain good crop production. The methods of IPM such as crop rotation, biological control and precision application reduce the total chemical application and reduce off-target exposure (Boone and Bridges, 2003) ^[3]. It is also possible to minimize risk by timing the pesticide applications to avoid time windows of maximal amphibian activity, including breeding migrations and periods of larval development. Where control of chemicals is required, using less persistent and less toxicity formulations and application techniques to reduce drift and runoff can have a profound impact of reducing effects of non-target amphibians.

Regulatory and Research Priorities

The existing system of risk assessment of pesticides does not provide sufficient considerations of biology and real-life exposure conditions of amphibians. Environmental risk assessment should be done by the regulatory agencies such that it involves the use of amphibian outcome endpoints, various stages of life, and realistic exposure of the mixture instead of using single-compound, single-species toxicity tests (Sparling *et al.*, 2010) ^[16]. This also requires the implementation of long-term monitoring programs, which will monitor the outcomes of amphibian population and pesticide residues in water, sediment, and tissues in the agricultural landscapes (Smalling *et al.*, 2013). Future studies need to focus on sublethal outcomes, which are immune dysfunction, endocrine interference, behavior, and carryover of aquatic larvae to terrestrial juveniles. It is vital to know how these indirect impacts can be converted into effects at the population level to formulate science-based policymaking that can reconcile between agricultural productivity and amphibian protection.

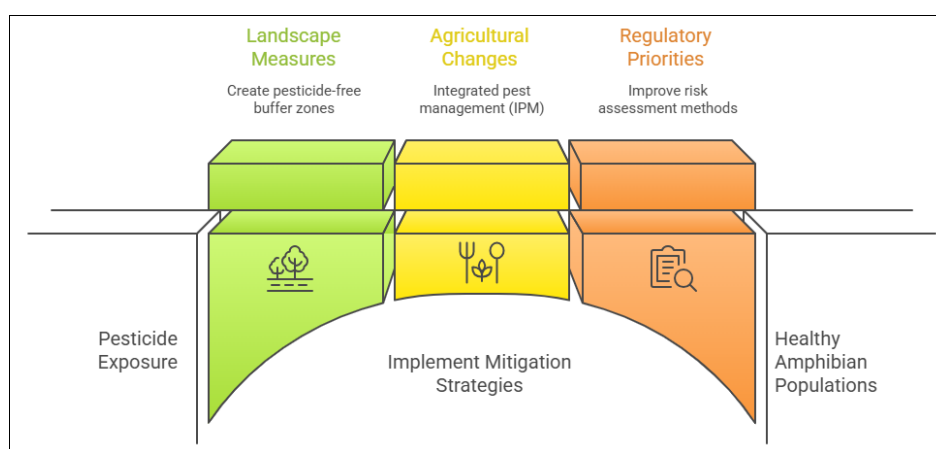


Fig 4: Protecting Amphibians from Pesticides

Limitation and effects of Pesticides on Frogs and Toads

Although significant progress has been made in the field of ecotoxicology, there are still a number of research gaps that hamper a clear understanding of the impacts of pesticides on

amphibian species especially frogs and toads in various ecological perspectives. It is necessary to address these gaps in order to translate the results of the experiment into effective conservation and regulatory measures. The main

weakness is that there are few long-term, population-scale studies that directly relate measured environmental levels of pesticides with demographic change like survival, recruitment, and population growth rates. The majority of the current studies are based on short term laboratory or mesocosm studies that address individual level outcomes, usually under controlled scenarios that do not acknowledge the temporal dynamics and complexity of actual agricultural landscapes (Sparling *et al.*, 2010) ^[16]. It is still challenging to measure the level of persistence of the population in more than one generation without long-term field data that can combine exposure, life-history characteristics, and population dynamics. The other notable gap is that of interactive effects of multiple stressors. The amphibians in the farm areas are not often subjected to isolated single chemicals, but complex combinations of herbicides, insecticides, fungicides and their metabolites are frequently found. These chemical stressors tend to be accompanied by non-chemical stresses like emerging infectious diseases, climate induced drought, habitat fragmentation and nutritional stress. The interaction between pesticides and pathogens can potentially be synergistic (such as disease effects), which can severely exceed the effects of individual stressors (Rohr *et al.*, 2009) ^[15]. Nevertheless, the experimental designs that explicitly test such interactions are still relatively strong and field-realistic, and therefore we can predict the outcome of processes in the real world to a limited extent. The generality of existing knowledge is further limited by the presence of geographic and taxonomic bias. The relatively small proportion of temperate species is the reason why the majority of studies have been carried out in North America and Europe. On the contrary, the amphibian diversity of the world is concentrated in tropical areas, especially South and Southeast Asia, Africa and Latin America, and is the focus of fast agricultural growth and intensification of pesticides (Stuart *et al.*, 2004) ^[17]. This shortage of data in these areas presents tropical amphibians and various agro-ecological systems as underrepresented in risk assessment and conservation planning. Lastly, absence of standardization of protocols of measuring sub lethal endpoints and pesticide residue load in amphibian tissue complicates the cross-study comparability and synthesis. Techniques of immune system, endocrine interference, behavior and tissue residues have been highly diverse in the context of different studies and thus it is hard to compare the results to come up with meta-analyses or to come up with regulatory levels pertinent to amphibians (Mann *et al.*, 2009) ^[10]. Creating convergent sampling, analysis and reporting systems would significantly contribute to the strength and similarity of the future studies. To conclude, geographically inclusive, long-term and integrative research methods will be necessary to advance amphibian conservation in the agricultural landscapes. By focusing on population-level outcomes, multi-stressor interactions, standardized methodologies, and tropical systems will be essential in bridging gaps in existing knowledge and guide the science-based pesticide management policies.

Conclusion

The body of experimental, field-based and synthetic evidence that is available clearly shows that agricultural pesticides have widespread and far-reaching negative impacts on amphibians including frogs and toads. Exposure has been reported to lower survival, retard growth and

development, impair immune functions and alter endocrine and reproductive functions across a broad selection of chemical classes. These impacts can be seen at different phases of life, such as aquatic larvae to terrestrial juveniles and adults and are more pronounced in those species that breed in agricultural wetlands or migrate between treated fields. Though the intensity and effects of effects of pesticides depend on both the type of pesticides, formulations, concentration, timing of exposure and the sensitivity of the species, the broad trend that is coming out of meta-analyses and field studies on the residue levels and species sensitivity has shown that the exposure to pesticides is not a one off or hypothetical hazard however, all the ecological evidence points to the fact that pesticides are a wide spread and ecologically significant stress factor. Notably, the facts indicate that pesticides may have far-reaching effects besides being lethal. Sublethal effects of delayed metamorphosis, smaller body size at maturity, weaker immune protection, and reproductive physiology are possible and can be reflected in less recruitment and low long-term population viability. These sublethal effects when combined with other stresses prevalent to agricultural landscapes such as habitat loss, hydrologic change, climate variability, and infectious disease, can drive populations above recovery thresholds. Through this, pesticides can be both major stressors and intensifiers of other agents of amphibian decline, leading to the development of demographic sinks and local population expanses. Conservation and management wise, the results highlight the importance of intersectoral solutions. It will take agricultural practices, including application of integrated pest management practices, proper timing and location of the use of chemicals, preserving pesticide-free buffer zones near breeding areas and migration pathways, to reduce the risk of pesticides to the amphibians. Policy-level environmental risk assessment should go beyond simplistic, single-chemical, single-phase of toxicity studies to include amphibian-relevant endpoints, realistic exposure situations and chemical mixtures. No less important is the broadening of long-term studies and fieldwork that connects exposure measured to demographic consequences especially in unexplored areas and biodiversity-rich tropical landscapes. To sum up, the conservation of amphibians in agricultural areas is part of the ecological requirement and a pilot project to balance food production and protection of the biodiversity.

References

1. Baker NJ, Bancroft BA, Garcia TS. A meta-analysis of the effects of pesticides and fertilizers on survival and growth of amphibians. *Science of the total environment*, 2013;449:150–156.
2. Blaustein AR. Declining amphibian populations: a global phenomenon?. *Trends in ecology and evolution*, 1990;5:203–204.
3. Boone MD, Bridges CM. Effects of pesticides on amphibian populations. *Amphibian conservation*, 2003, 152–167.
4. Bridges CM. Tadpole swimming performance and activity affected by acute exposure to sublethal levels of carbaryl. *Environmental Toxicology and Chemistry*, 1997;16(9):1935–1939.
5. Brühl CA, Schmidt T, Pieper S, Alscher A. Terrestrial pesticide exposure of amphibians: an underestimated

- cause of global decline?. Scientific reports,2013;3(1):1135.
6. Collins JP, Storfer A. Global amphibian declines: sorting the hypotheses. Diversity and distributions,2003;9(2):89–98.
 7. Duellman WE, Trueb L. Biology of amphibians. JHU press, 1994.
 8. Hayes TB, Case P, Chui S, Chung D, Haeffele C, Haston K, Tsui M. Pesticide mixtures, endocrine disruption, and amphibian declines: are we underestimating the impact?. Environmental health perspectives,2006;114(1):40.
 9. List IR. The IUCN red list of threatened species. Disponí vel em:< http://www.iucnredlist.org/info/categorias_criativa2001.html>. Acesso em, 2004, 12.
 10. Mann RM, Hyne RV, Choung CB, Wilson SP. Amphibians and agricultural chemicals: review of the risks in a complex environment. Environmental pollution,2009;157(11):2903–2927.
 11. Quaranta A, Bellantuono V, Cassano G, Lippe C. Why amphibians are more sensitive than mammals to xenobiotics. PLoS One,2009;4(11):7699.
 12. Relyea RA. The lethal impact of Roundup on aquatic and terrestrial amphibians. Ecological applications,2005;15(4):1118–1124.
 13. Relyea RA, Mills N. Predator-induced stress makes the pesticide carbaryl more deadly to gray treefrog tadpoles (*Hyla versicolor*). Proceedings of the National Academy of Sciences,2001;98(5):2491–2496.
 14. Robinson SA, Richardson SD, Dalton RL, Maisonneuve F, Bartlett AJ, De Solla SR, Waltho N. Assessment of sublethal effects of neonicotinoid insecticides on the life-history traits of 2 frog species. Environmental Toxicology and Chemistry,2019;38(9):1967–1977.
 15. Rohr JR, McCoy KA. A qualitative meta-analysis reveals consistent effects of atrazine on freshwater fish and amphibians. Environmental health perspectives,2009;118(1):20.
 16. Sparling DW, Linder G, Bishop CA, Krest S, Eds. Ecotoxicology of amphibians and reptiles. CRC Press, 2010.
 17. Stuart SN, Chanson JS, Cox NA, Young BE, Rodrigues AS, Fischman DL, Waller RW. Status and trends of amphibian declines and extinctions worldwide. Science,2004;306(5702):1783–1786.
 18. Wells KD. The ecology and behavior of amphibians. University of Chicago press, 2019.