

EFFECTS OF PESTICIDES ON AMPHIBIANS AND TENTATIVE SOLUTIONS: REVIEW



 Daniel Brice
Nkontcheu Kenko^{1,2}

¹Zoology Laboratory, Department of Animal Biology and Conservation,
Faculty of Science, University of Buea, Cameroon.

²Biology and Applied Ecology Research Unit, Dschang School of Science and
Technology, University of Dschang, Cameroon.

^{1,2}Email: kenko.daniel@ubuea.cm



ABSTRACT

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Amphibians are among the main affected non-target groups, victim of pesticides. This review puts into evidence worldwide studies on lethal and sub-lethal effects of pollutants on amphibians and proposes solutions to handle the issue. Literature review was carried out from articles and books, on 123 studies. The number of pesticide formulations used worldwide is very high. Pesticide effect on the environment and the biota in general have been assessed in many studies using tools such as biomonitoring, bioassays in laboratory and semi-field conditions and Ecological Risk Assessment (EcoRA) with models. The ecological effects of pollutants are varied and often inter-related. Effects in the organism or at the ecological level are usually considered an early warning indicator of potential human health impact. Effects can either be lethal or sub-lethal comprising cancer, tumours, lesions, reduction of reproductive potential, immune suppression, disruption of endocrine system, cellular and Deoxyribonucleic acid (DNA) damage, and teratogenic effects. Amphibian conservation can be done via the protection of genetic resources, cleaning habitat, sustainable harvesting, captive breeding, cloning and reintroduction programmes. Proper use of pesticides and reinforcement of regulation remains a necessity to protect environmental health.

Contribution/ Originality: This review is a critical evaluation of available data on pesticide effects on amphibians. We would like to clarify the state of knowledge in pesticide ecotoxicology in amphibians in order to identify perspectives. After the identification of various threats, proposals have been made for amphibian conservation programmes.

1. INTRODUCTION

Poverty, starvation, famine and hunger related diseases are among the main challenges faced by populations worldwide. In order to overcome these challenges, the development of the agricultural stands as a solution especially in less developed countries in which economic factors push more and more people into agriculture [1]. Unfortunately, crop production is seriously hindered by pest and diseases [2, 3], therefore farmers must make use of pesticides to fight against harmful organism and increase the yield [4]. Pesticide use in agriculture is intended to combat pests in order to improve the field; unfortunately, extensive use of pesticide in developing countries is often accompanied with improper use [5]. Large quantities of pesticides are released into the environment on a routine basis with severe consequences on organisms. The amount of pesticides coming in direct contact with or consumed by target pests is an extremely small percentage of the amount applied, less than 0.3% in most studies [6]. The

larger proportion of pesticides (99.7%) applied gets into the aquatic system through leaching, surface run-off, spray drift, soil erosion and volatilisation. The big problem with pesticides in general is non-target effects [7]. Pesticides act on living organisms with lethal effects (death) that are very easy to appreciate or sub-lethal effect such as reduction of growth, reduced reproductive capacities, teratogenic effects, that are not easy measure *in situ* [8]. Pesticides have been reported to cause genotoxicity, teratogenicity and cell damage in animal models and humans [9].

The biggest contributors of amphibians' population declines include habitat loss (destruction and fragmentation), pollution (pesticides, fertilizers and industrial wastes), exotic species, predators, competition for resources such as food and breeding sites, climate change and diseases [10]. Wetlands are very suitable habitats for amphibians, unfortunately, agriculture is the main challenge to wetland conservation [11]. Native amphibians in agricultural landscapes exhibit endocrine disruption effects due to agricultural inputs [12]. Amphibian populations are declining worldwide [13] and declines are particularly severe in the New World tropics where amphibian diversity is among the highest on the planet [14]. Over the past 25 years, reduction in amphibian populations have been occurring more dramatically [15]. The permeable skin of amphibians makes them susceptible to desiccation [16] hence, amphibians are threatened more than other vertebrate classes [15] because they like shallow, standing and temporary ponds around agricultural fields for reproduction; this increases their exposure to contaminants [17]. Amphibians are facing a major extinction crisis that requires quick measures at all levels: research, law and policy [18]. Pesticides such as Dichlorodiphenyltrichloroethane (DDT) (180g/ha), endosulfan (800 to 300g/ha) and dieldrin (14g/ha) has been reported as serious danger to amphibians in sub-Saharan Africa [19]. Amphibians as part of the aquatic community compete with groups such as snails, chironomid larvae and dipteran larvae for periphyton [20]. Among pollutants, pesticides are the main causes of amphibian population declines [21] even though relating wildlife declines to pesticides is not obvious because of many co-variables [22]. Surfactants are commonly incorporated into pesticides formulation and may also be risky to the biota [23]. Precipitation, elevation, surrounding urban and agricultural land use, and spatial location are covariates of pesticide toxicity [21] hence the necessity to assess multiple stressors [24].

Amphibians play a tremendous role in the regulation of insect pest populations and food webs [25]. Living both on land and in water, and having a naked skin used for breathing, amphibians are highly exposed to contaminants, making them good bio-indicators of environmental changes. Amphibians play a pivotal role in ecosystem as secondary consumers in many food chains. Tadpoles have significant impact in nutritional cycling. They are herbivorous to omnivorous and are the prey items for both invertebrates and vertebrates. Adult amphibians are the best biological pest controllers. Amphibians are regarded as good ecological indicators. Due to high degree of sensitivity, either during tadpole stage or as adults, they respond to very slight change in the environment more than mammals. Pollutant effects on amphibians have been assessed at the laboratory, mesocosm and landscape levels [22] but few pesticides have been tested on amphibians [26]. Even though, exposure to environmental pollutants such as agricultural pesticides has been identified as one of the ultimate causes of decline, ecotoxicological studies are extremely rare especially in tropical regions [27]. This review puts into evidence worldwide studies on lethal and sub-lethal effects of pesticides on amphibians and proposes potential solutions. This will bring more information on various effects on pesticides for better recommendations to pesticides users and for decision makers, the outcome being the protection of human and environmental health.

2. MAIN GROUPS OF CHEMICAL POLLUTANTS

A non-exhaustive list of water chemical pollutants includes heavy metals, pesticides, hydrocarbons, organohalogen compounds (Polychlorinated Biphenyls-PCBs, Polybrominated biphenyls-PBBs, Polychlorinated dibenzodioxins-PCDDs and Polychlorinated dibenzofurans-PCDFs), detergents and surfactants, chlorophenols, organometallic compounds, gases and radioactive isotopes [28].

2.1. Case of Agrochemicals

Pesticides are substances or mixtures of substances destined to repel, destroy or fight against pests of plants or vectors of animal and human diseases [29]. Based on their chemical composition, there are organic (contain carbon in their chemical structure) and inorganic (do not contain carbon and are usually derived from mineral ores) pesticides. According to their target, pesticides can be divided into insecticides (kill insects), fungicides (kill fungi), herbicides (destroy weeds), nematicides (nematodes), molluscicides (molluscs), avicides (birds) and son on. Based on their toxicity, pesticides can be classified as shown in Table 1.

Table 1. Classification of pesticides according to their toxicity [30].

WHO Class	Toxicity Level	Oral LD ₅₀ for the Rat (mg/kg of body weight)	Example
Class Ia	Extremely Hazardous	<5	Dieldrin
Class Ib	Highly Hazardous	5 -50	Eldrin
Class II	Moderately Hazardous	50 -2000	DDT
Class III	Slightly Hazardous	2000 -5000	Malathion
Class IV	Virtually Non-Toxic	>5000	Carbetamide

Note:

- DDT: Dichlorodiphenyltrichloroethane.
- WHO: World Health Organization.
- LD50: Dose that can kill 50% of the population

Pesticides use in agriculture is a common rule worldwide: there are many documented surveys on pesticide use in agriculture [4, 31-48].

The number of pesticide formulations used worldwide in very high. For instance, 107 pesticides commercial names corresponding to 54 active ingredients, were reported to be used by farmers of the Fako Division, South-West Cameroon in a single crop season by small-scale farmers and the CDC (Cameroon Development Corporation) [37] thirty-one pesticide commercial names corresponding to 18 active ingredients were reported to be used by market gardeners in Fotouni (West Cameroon) with Chlorothalonil occupying the top position [36]; thirty-two pesticide commercial names (17 active ingredients) were recorded in Balessing, a locality of the West Region of Cameroon [45]. Some of these pesticides especially fungicides have a heavy metal component. These agrochemicals have many negative effects on the environment and the biota.

2.2. Agrochemical Effects on Environment and the Biota

Pesticide effect on the environment and the biota in general have been assessed in many studies using tools such as biomonitoring, bioassays in laboratory and semi-field conditions and Ecological Risk Assessment (EcoRA) with models. Pesticides such cypermethrin, lambda-cyhalothrin, cadusafos and malathion have been reported to be very risky to water, *Daphnia* and fish in the Benoe stream, South-West Cameroon [49] furthermore, chlorpyrifos-ethyl, chlorothalonil and cypermethrin were reported to be risky to streams [50] whereas, the mixture imidacloprid+lambda-cyhalothrin is very toxic to amphibians [51]. Pesticide residues above have been detected in water bodies in Yaoundé, Centre Region of Cameroon [48]. How do these agrochemicals act on amphibians?

3. POLLUTANTS EFFECTS ON AMPHIBIANS

The ecological effects of pollutants are varied and often inter-related. Effects in the organism or at the ecological level are usually considered an early warning indicator of potential human health impact. Effects can either be lethal [52] and easy to appreciate (death), or sub-lethal, not easy to assess in routine experiments: cancer, tumours, lesions; reproduction failure, immune suppression, disruption of endocrine system [53] cellular and DNA damage, and teratogenic effects [54] and intergenerational effects. These associated stresses need not be large to have a synergistic effect with organic minor pollutants. Worldwide studies have revealed that, pollutants have lethal and sub-lethal effects on amphibians. Mortality is generally expressed as a dose-response curve

graphically represented as the relationship between the quantity of the contaminant and the magnitude of the induced impact [55].

3.1. Lethal Effects

3.1.1. Fungicides

Fungicides generate lethality in amphibians but the LC₅₀ is higher. Bioassays with fungicides have been mainly done with copper sulphate, Chlorothalonil, Pyraclostrobin, Captan and Spiroxamine.

Copper sulphate is the chemical compound with the chemical formula : CuSO₄, 5H₂O; it is a protectant foliar fungicide often used in ponds to control algae [56]. This chemical had a 120h LC₅₀ of 0.058 mg/L (Table 2) with the larvae of the green toad *Bufo viridis* [15].

Table 2. Toxicity value for fungicides summarized from studies done on tadpoles.

Species	Duration	Pollutant	LC ₅₀	Reference
<i>Bufo viridis</i>	120h	Copper Sulphate	0.058mg/L	[15]
<i>Rana utricularia</i>	24h	Mancozeb	800ppb	[57]
<i>R. utricularia</i>	24h	Fosetyl-Al	45.280ppb	[57]
<i>R. utricularia</i>	24h	Chlorothalonil	28ppb	[57]
<i>Agalychnis callidryas</i>	96h	Chlorothalonil	26.6 µg/L	[58]
<i>Isthmohyla pseudopuma</i>	96h	Chlorothalonil	25.5 µg/L	[58]
<i>Smilisca baudinii</i>	96h	Chlorothalonil	32.3 µg/L	[58]

Note:

- Al: Aluminium.
- LC₅₀: median lethal concentration; is the amount of a substance required to kills 50% of a test organism.
- ppb: parts per billion (1 ppb = 1 ug/L).

Chlorothalonil, used to control a wide range of fungal diseases on a broad range of crops, is moderately toxic to birds, honeybees and earthworms but considered to be more toxic to aquatic organisms [56]. Chlorothalonil had an LC₅₀ value of 59.36 µg/L following 8-days acute toxicity assays carried out on individually reared *Agalychnis callidryas* tadpoles [59]. Chlorothalonil is a fungicide used on maize, potatoes and fruits; it has been detected in superficial water, rain and air, so human beings and other living organisms can be heavily exposed if they use water from these sources. This fungicide is very toxic to tadpoles and those that survive have very weak immune system [59]. Chlorothalonil has also been reported to be very toxic to amphibians (*Agalychnis callidryas*, *Isthmohyla pseudopuma* and *Smilisca baudinii*) with a 96h LC₅₀ ranging from 25.5 to 32.3µg/L (Table 2); this high toxicity was accompanied by a spontaneous rupture of linea alba and posterior evisceration in tadpoles that died after 24h of exposure [58].

Pyraclostrobin is a fungicide used to control major plant pathogens in cereals and other crops; Captan is a dicarboximide fungicide used on fruit and other crops; this carcinogenic compound may also cause endocrine disruption; Spiroxamine is a systemic fungicide used to control common fungal diseases on cereals and fruits [56]. These three fungicides brought about an acute mortality of 100% after 1 hour and 40% after seven days of exposure in juvenile of the European common frogs, *Rana temporaria* [60].

3.1.2. Insecticides

In general, there is a higher aquatic risk associated with insecticides as compared to other pesticide families [49]. Organochlorine insecticides are particularly toxic to amphibians; DDT (110g/Ha), dieldrin (800g/Ha) and endosulfan (>200g/Ha) are among the most toxic, causing death of adult amphibians [19]. Endosulfan, diazinon, chlorpyrifos, alpha-cypermethrin, malathion, dimethoate and fenitrothion have been used in bioassays with amphibians.

Endosulfan is an insecticide and acaricide with low aqueous solubility and is volatile; it may have a tendency to leach to groundwater while Diazinon is a general-purpose insecticide, moderately soluble and highly volatile, highly

toxic to most aquatic [56]. These two insecticides generated high adult mortality especially Diazinon which is an enzyme inhibitor. Endosulfan is a ubiquitous environmental contaminant. The chemical is semi-volatile and persistent to degradation processes in the environment. Endosulfan and Diazinon showed evidence of bio-concentration because of the significant positive correlation between accumulated residues and mortalities [61]. Diazinon (Basudin) also induced a dose-dependent mortality in tadpoles of *Ptychadena bibroni* with a 96h LC₅₀ of 0.86 µg/L [62] (Table 3).

Table 3. Toxicity value for insecticides summarized from studies done on amphibians.

Species	Duration	Pollutant	LC ₅₀	Stage	Reference
<i>Bufo regularis</i>	96h	Diazinon	0.44mg/L	Adults	[61]
<i>Bufo regularis</i>	96h	Endosulfan	0.73mg/L	Adults	[61]
<i>Xenopus laevis</i>		Methoxychlor	72nM	Adult females	[63]
<i>Hoplobatrachus chinensis</i>	24h	Chlorantraniliprole	5.37mg/L	Tadpoles	[25]
<i>Hoplobatrachus chinensis</i>	24h	Flubendiamide-abamectin	4.90mg/L	Tadpoles	[25]
<i>Hoplobatrachus chinensis</i>	72h	Chlorantraniliprole	1.74mg/L	Tadpoles	[25]
<i>Hoplobatrachus chinensis</i>	72h	Flubendiamide-abamectin	1.45mg/L	Tadpoles	[25]
<i>Amietophrynus regularis</i>	24h	Imidacloprid+lambda-cyhalothrin	3.66 mg/L	Tadpoles	[51]
<i>Bufo Melanostictus</i>	48h	Chlorpyrifos	1.47ppm	Tadpoles	[64]
<i>Bufo Melanostictus</i>	48h	Dimethoate	8.89ppm	Tadpoles	[64]
<i>Rhinella arenarum</i>	504h	Dimethoate	12.82mg/L	Larvae	[65]
<i>Rhinella arenarum</i>	504h	Dimethoate	16.38mg/L	Embryos	[65]
<i>Ptychadena bibroni</i>	96h	Diazinon	0.860µg/L	Tadpoles	[62]
<i>Bufo variabilis</i>	96h	Alpha-cypermethrin	15.62µg/L	Gosner Stage 19	[66]
<i>Rana berlandieri</i>	24h	Imidacloprid	184.5µg/L	Tadpole	[57]
<i>Rana berlandieri</i>	24h	Carbaryl	51.581µg/L	Tadpole	[57]
<i>Rana berlandieri</i>	24h	Chlorpyrifos	1.13µg/L	Tadpoles	[57]
<i>Pseudacris triseriata</i>	24h	Imidacloprid	388.5µg/L	Tadpoles	[57]
<i>Pseudacris triseriata</i>	24h	Carbaryl	58.08µg/L	Tadpoles	[57]
<i>Pseudacris triseriata</i>	24h	Chlorpyrifos	1.13µg/L	Tadpoles	[57]
<i>Bufo americanus</i>	24h	Imidacloprid	468µg/L	Tadpoles	[57]
<i>Bufo americanus</i>	24h	Carbaryl	63.17µg/L	Tadpoles	[57]
<i>Bufo americanus</i>	24h	Chlorpyrifos	1.32µg/L	Tadpoles	[57]

Chlorpyrifos is an organophosphate insecticide with a low aqueous solubility. Chlorpyrifos has been reported to be highly toxic to birds, fish, aquatic invertebrates and honey bees, and is moderately toxic to aquatic plants, algae and earthworms [56]. As shown in Table 3, chlorpyrifos significantly reduced tadpoles' survival of Asian common toads with 1.46ppm as 48h LC₅₀ [64].

Alpha-cypermethrin is a widely used pyrethroid insecticide considered to be a serious water pollutant [56]. A study on the effect of this compound on tadpoles of the variable green toad *Bufo variabilis* revealed a 96h LC₅₀ of 15.62 µg/L [66] so it is highly toxic to amphibians (Table 3).

In an outdoor mesocosm study, malathion at low concentration (10-250µg/L), has indirect effect on amphibians. Moderately toxic to mammals, Malathion is a cholinesterase inhibitor and a neurotoxin, highly toxic to honey bees and aquatic species with the exception of algae [56]. This insecticide affected zooplankton, phytoplankton, periphyton and larval amphibians; the Leopard frog (*Rana pipiens*) which has a long time to metamorphosis had serious population reduction while the effects were little on wood frogs *Rana sylvatica* [67].

Fenitrothion is an insecticide with low aqueous solubility; moderately toxic to mammals, it is considered to be an endocrine disrupter and a cholinesterase inhibitor [56]. In green frogs and bullfrogs, fenitrothion at doses between 2 and 9ppm caused 50% mortality [68] (Table 3).

Dimethoate is an organophosphate insecticide, highly soluble in water, it is highly toxic to birds and honeybees, moderately toxic to most aquatic species and earthworms [56]. Banned in Cameroon [69] this insecticide is still used by many farmers [37]. Dimethoate caused an acute mortality of 100% after 1 hour and 40% after seven days of exposure in juvenile of the European common frogs, *Rana temporaria* [60]. Dimethoate represents a risk on *Rhinella arenarum* survival (Table 3) with larvae being more vulnerable than embryos [65].

3.1.3. Herbicides

The most detrimental impact of a contaminant on an exposed organism is to cause death. Because of confounding effects and the inability to provide controlled environments in the field, toxicity of substances are most commonly assessed through laboratory trials [55]. Worldwide, bioassays have been done mainly with Atrazine, Propanil, Glyphosate, Bromoxynil-octanoate, Fenoxapto-P-ethyl and 2,4-D.

Atrazine (2-chloro-4-ethylamino-6-isopropylamino-s-triazine) is a selective, pre- and post-emergence herbicide, used on a variety of terrestrial food crops, non-food crops, forests, residential turf, gold course turf, recreational area and rangeland. In plants, it inhibits photosynthesis by blocking the protein D1 of photosystem II [70]. Atrazine generated 100% mortality in one-week old tadpoles of *Ptychadena bibroni* at 96h. This high lethality decreased in successive developmental stages of two, three- and four-weeks tadpoles at 96h and the respective mortality were 90%, 80% and 75%. The LC₅₀ values ranged between 230.058 and 431.323 µg/L of different stages of development (Table 4). The accidental or intentional release of this herbicide in water is then very detrimental to amphibians' survival [71].

Propanil is a post-emergence herbicide used for broad-leaved and annual grass weed control in rice and other crops [56] it significantly reduced tadpoles' survival of Asian common toads with a 48h LC₅₀ of 1.47ppm [64].

Glyphosate is a broad-spectrum synthetic herbicide, highly soluble in water [56] thus requires surfactants too increase activity [72]. It is more toxic to tadpoles than embryos [72]. Glyphosate (Round-up) caused a large reduction in the survival of three amphibian species, the grey tree frog, *Hyla versicolor*; American toad, *Bufo americanus*; and leopard frog, *Rana pipiens* in pond mesocosms [26]. In laboratory bioassays, glyphosate (round-up) caused substantial mortality in the three species; from 96% to 32% in juvenile wood frog, from 100% to 18% in juvenile tree frog and from 100% to 24% in juvenile toad [26]. Moreover, Glyphosate is very toxic to amphibians especially the Ultra-Max (ULT) formulation with a 2.42mg/L LC₅₀ at 48h [73].

Bromoxynil-octanoate is an herbicide used for post-emergence control of annual broad-leaved weeds and Fenoxapto-P-ethyl is a post-emergence herbicide used to control annual and perennial grasses [56]. These two compounds generated an acute mortality of 100% after 1 hour and 40% after seven days of exposure in juvenile of the European common frogs (*Rana temporaria*) [60].

The herbicide known as 2,4-D is a selective, systemic compound, highly soluble in water: this volatile herbicide has a low potential to leach to groundwater: it is non-persistent in soil but may persist in aquatic systems under certain conditions [56]. 2,4-D had a LC₅₀ of 536.2mg/L following 8-days acute toxicity assays carried out on individually reared *Agalychnis callidryas* tadpoles [59] (Table 4).

Table 4. Toxicity value for herbicides summarized from studies done on amphibians.

Species	Duration	Pollutant	LC ₅₀	Stage	Reference
<i>Ptychadena bibroni</i>	96h	Atrazine	230.058-431.321µg/L	Tadpoles	[71]
<i>Hoplobatrachus chinensis</i>	24h	Penoxsulam	4.68mg/L	Tadpoles	[25]
<i>Engystomops pustulosus</i>	96h	Glyphosate	2799µg/L	Tadpoles	[72]
<i>Hypsiboas crepitans</i>	96h	Glyphosate	2203µg/L	Embryo	[72]
<i>Hypsiboas crepitans</i>	96h	Glyphosate	1424µg/L	Tadpoles	[72]
<i>Rana marina</i>	96h	Glyphosate	2270µg/L	Embryo	[72]
<i>Rana marina</i>	96h	Glyphosate	2170µg/L	Tadpoles	[72]
<i>Rana humboldti</i>	96h	Glyphosate	3336µg/L	Embryo	[72]
<i>Rana humboldti</i>	96h	Glyphosate	2121µg/L	Tadpoles	[72]
<i>Rhinella arenarum</i>	48h	Glyphosate (ULT)	2.42 mg/L	Tadpoles	[73]
<i>Rhinella arenarum</i>	48h	Glyphosate (INF TM)	38.76 mg/L	Tadpoles	[73]
<i>Rhinella arenarum</i>	48h	Glyphosate (Glifoglex TM)	73.77 mg/L	Tadpoles	[73]
<i>Rhinella arenarum</i>	48h	Glyphosate (C-K FAV TM)	77.52 mg/L	Tadpoles	[73]
<i>Hoplobatrachus chinensis</i>	72h	Penoxsulam	1.29mg/L	Tadpoles	[25]
<i>Hoplobatrachus chinensis</i>	48h	Penoxsulam	0.021mg/L	Tadpoles	[25]
<i>R. pipiens</i>	24h	Glyphosate (Herbicide)	20.47ppb	Tadpoles	[57]
<i>R. pipiens</i>	24h	Prodiamine (Herbicide)	840.83ppb	Tadpoles	[57]
<i>R. pipiens</i>	24h	Dimethylamine salt of 2,4-D, mecoprop and dicamba (Herbicide)	432ppb	Tadpoles	[57]
<i>Bufo Melanostictus</i>	48h	Propanil	1.46ppm	Tadpoles	[64]
<i>Bufo Melanostictus</i>	48h	Glyphosate	45.94ppm	Tadpoles	[64]
<i>Engystomops pustulosus</i>	96h	Glyphosate	3904µg/L	Embryo	[72]

Note: • ULT : Ultra-Max (Commercial name).
 • INF: Infosate (Commercial name).
 • C-K FAV: C-K Yuyos FAV (Commercial name).
 • TM: trademark.

3.2. Mixture of Pesticides

Most often, many farmers mix pesticide before use, for many reasons: save time, effectiveness [37] therefore some studies have been done of the effect of pesticide mixtures on the biota. A study on the impact of agricultural inputs on amphibians in China revealed that chlorantraniliprole (insecticide) and penoxsulam (herbicide) exhibited moderate toxicity to the Chinese tiger frog while flubendiamide-abamectin was highly toxic to this amphibian. The joint toxicity of chlorantraniliprole + flubendiamide-abamectin was synergistic after 24h while other combinations were antagonistic [25]. This makes the response of the tiger frog to pollutants very complicated to understand. When combined with other stressors, pollutants most often fail to exhibit synergistic effects [74].

Organophosphates and Carbamates (cholinesterase-inhibiting pesticides) were more strongly associated with population declines of the Yosemite toad (*Bufo canorus*), the California red-legged frog (*Rana aurora draytonii*), the foothill yellow-legged frog (*R. boylei*), the Cascades frog (*R. cascadae*), and the mountain yellow-legged frog (*R. muscosa*) in California, than any other class of pesticides [21]. Carbaryl is an obsolete carbamate insecticide with a suspected endocrine disruptor function [56]. Its toxicity may be influenced in the presence of covariates. For instance, Ultraviolet B (UV-B) radiation intensity positively influenced tadpole survival to metamorphosis and the presence of UV-B radiation made carbaryl less toxic [24].

Tadpoles exhibited narcosis when exposed to surfactants such as Nonylphenol Ethoxylates (NPE) and alcohol alkoxyolate in a static-renewal acute toxicity test, and the toxicity increased under high temperature and low oxygen conditions [23]. Table 5 gives LC₅₀ values of common agricultural surfactants on amphibians.

Table 5. LC₅₀ at 48hours of surfactants on amphibians at the tadpole stage.

Amphibian Species	Compounds	LC ₅₀ (mg/L)	Reference
<i>Crinia insignifera</i>	Nonylphenol (Teric GN*)	3.8	[23]
<i>Crinia insignifera</i>	Nonylphenol (Agrab®600*)	3.5	[23]
<i>Crinia insignifera</i>	Alcohol alkoxyate (BS1000®*)	6.0	[23]
<i>Limnodynastes dorsalis</i>	Nonylphenol (Agrab®600™)	4.1	[23]
<i>Limnodynastes dorsalis</i>	Alcohol alkoxyate (BS1000®*)	14.3	[23]
<i>Xenopus laevis</i>	Nonylphenol (Teric GN*)	2.8	[23]
<i>Xenopus laevis</i>	Nonylphenol (Agrab®600*)	2.3	[23]
<i>Bufo marinus</i>	Nonylphenol (Teric GN*)	5.1	[23]
<i>Bufo marinus</i>	Nonylphenol (Agrab®600*)	5.4	[23]
<i>Litora moorei</i>	Nonylphenol (Agrab®600*)	4.6	[23]
<i>Heleioporus eyrei</i>	Nonylphenol (Agrab®600*)	12.1	[23]
<i>Heleioporus eyrei</i>	Alcohol alkoxyate (BS1000®*)	25.4	[23]

Note: *Commercial names.

3.3. Sub-Lethal Effects

Pesticides have many sublethal effect on amphibians: endocrine disruption, altered growth and behavioural changes [22]. Pesticides showed significant sub-lethal effects on activity and growth, and in all cases these effects occurred at concentrations lower than or equal to the corresponding LC₅₀ values. For instance, the nematicides terbufos and ethoprophos and the fungicide chlorothalonil have been reported to be highly toxic with evident effects below 100µg/L [59]. Measurable concentrations of endosulfan have been detected in amphibians (*Hyla regilla*) as well as 4,4'-dichlorodiphenyldichloroethylene, 4,4'-DDT, and 2,4'-DDT residues [75] evidence of their bioaccumulation potential.

3.3.1. Physiological and Biochemical Implications

Pollutants have many physiological effects on amphibians. Most often, the direction of the effect is not obviously predictable. Agricultural activities have been reported to cause a reduction in the amount of plasmatic retinol in males of *Rana catesbeiana* [76]. Pesticides have been reported to alter spermatogenesis and metamorphosis in amphibians; Moreover, pesticide extracts are able to cause delayed metamorphosis, skewed sex ratios and altered gonadal differentiation in *Xenopus laevis* and *Bufo bufo* [12]. Pesticides lower egg hatching in amphibians especially carbaryl which significantly generated low egg hatching in *Ambystoma jelfersonianum*; most eggs exposed to pesticides that even succeeded to hatch did not survive to metamorphosis; those that succeed to metamorphose took a longer time [57]. Physiological and biochemical effects of pesticides have been mainly assessed with atrazine, methoxychlor, diazinon, chlorpyrifos, glyphosate and chlorothalonil.

Atrazine appeared capable of altering spermatogenesis, but the contexts and generality of these effects could not be firmly established [77]. Atrazine has possible effects on the glycogen level in tadpoles. Glycogen level decreased with increased pesticide concentration with no significant difference [71]. One of the worst effects of this herbicide is to make the leopard frog (*Rana pipiens*) hermaphrodite, bearing both male and female sex organs [78] this is responsible of amphibian population decline all over the world since atrazine reaches water bodies very easily. Atrazine was associated with increased aromatase gene expression, either increase or decrease in time to metamorphosis and altered sex hormone concentrations [77].

Methoxychlor or 1,1-(2,2-dichloroethylidene) bis(4-methoxybenzene) is a chemical transformation product with some insecticidal action [56]. Methoxychlor inhibited oocyte maturation in *Xenopus laevis* with a median inhibition concentration of 72nM with a dose-dependent, reversible and early-acting mechanism [63].

Diazinon (Basudin) significantly decreased glycogen levels in the amphibia *P. bibroni*, a hypoglycaemia that most often preceded mortality [62]. This pesticides also affects the brain and tongue cholinesterase (ChE) in *H. regilla* [75]. In the same line, chlorpyrifos has been reported to be a potent ChE inhibitor [75].

Tadpoles exposed to four glyphosate formulations at 48h, showed decreases in the activities of B-esterases, and Glutathion S-transferase (GST) with inhibition rates ranging from 71.52% to 86.12% [73].

Chlorothalonil (fungicide) hindered the development of the amphibian *Smilisca baudinii* at 20µg/L with no effect on the total length and body weight [58]. Tadpoles of *Isthmohyla pseudopuma* exposed to 3µg/L chlorothalonil had a lower ChE activity. A significant increase in GST activity was observed in livers of *Smilisca baudinii* exposed to 10 to 20µg/L chlorothalonil with a dose-response relationship. Chlorothalonil had no significant effect on Liver Lipid Peroxidation (LPO) liver levels [58].

Dimethoate had neurotoxic outcomes on *Rhinella arenarum* as it hindered the compound butyrylcholinesterase at doses between 0.5 and 1 mg/L [65]. Because of its dangerousness, dimethoate has been prohibited in Cameroon [79].

On the long run, superoxide dismutase (SOD) and glutathione peroxidase (GPX) enzyme activities may be potential biomarkers for monitoring contaminant levels in the environment [80] while body glycogen level may also be suitable as a biomarker of environmental contamination in amphibians [62].

3.3.2. Morphological and Anatomical Changes

Malformation, length alteration and weight modification are the three main morphological effects of pesticides on amphibians [81]. Bioassays have been done with endosulfan, diazinon, malathion, propanil, chlorpyrifos, glyphosate, atrazine, malathion, alpha-cypermethrin, chlorothalonil and dimethoate.

Endosulfan and diazinon created morphological changes in *Bufo regularis* [71]. Malathion significantly shortens developing *Xenopus laevis* tadpoles at concentrations of 1.0 to 2.5 mg/L following a 72h exposure [82].

Propanil and chlorpyrifos together with dimethoate and glyphosate are responsible for retarded metamorphosis, skin ulcer, oedema and malformation in Asian common toad [64].

Glyphosate is the most widely used herbicide in the world with application in agriculture, forestry, industrial weed control, garden and aquatic environments [72]. In microcosms, glyphosate (herbicide) caused no statistic difference among treatments for larval body size, embryonic development and swimming performance [72]. Mortality related to glyphosate was less than 50% in microcosms, so no calculation of LC₅₀ was possible [72]. In laboratory experiments, glyphosate at high sublethal concentrations is responsible for a decrease in tadpole body size in four anuran species *E. pustulosus*, *R. humboldti*, *R. marina* and *H. crepitans* [72]. In *R. humboldti*, glyphosate generates a significant delay in embryonic development [72]. Glyphosate is a bladder and liver toxicant added to its ability to disrupt aromatase activity [56].

Atrazine consistently affected male gonadal morphology in amphibians [77]. Malathion exhibited reliable and dramatic effects on the morphology (bent) of *Xenopus* [82]. Alpha-cypermethrin has many sublethal effects on amphibians; this compound generated axial anomaly, visceral oedema, deformation of the mouth and tail malformation in tadpoles of the variable green toad [66]. Chlorothalonil created lesions on the tails of the amphibians *Smilisca baudinii* and *Isthmohyla pseudopuma* [58].

Dimethoate (insecticide) has been reported to elucidate morphological changes and disruption the metamorphosis process in amphibians of the species *Rhinella arenarum* [67]. This compound is therefore a good contributor to amphibian population decline as it impairs growth.

3.3.3. Behavioural Changes

Endosulfan and diazinon pesticides created behavioural changes in *Bufo regularis*, because of their neurotoxic effects. Both compounds generated the following symptoms: hyperactivity, loss of coordination, erratic swimming, unusual retention of water, prolonged and motionless lying down on the aquarium bottom. In addition, to these, diazinon caused skin discoloration, reddening of the snout and the protrusion of the intestine from the anus [61].

Abnormal behavioural responses were also observed with Atrazine in tadpoles and the response was positively correlated with the concentration gradient [71]. Some of these adverse behavioural effects included reduced reaction to stimuli, short swimming distance and loss of equilibrium induced by copper sulphate in tadpoles of the green toad, *Bufo viridis* [15]. Atrazine reduced amphibian water-conserving behaviours, which increased their rate of water loss. Amphibians are extremely susceptible to desiccation; thus atrazine-induced changes in water conserving behaviours would be expected to increase mortality risk [77].

Abnormal avoidance responses were recorded in tadpoles of *P. bibroni* exposed to diazinon [62]. Malathion reduced swimming frequency in *Xenopus* [82]. Shortening of the swimming distance and immobility are the two behavioural changes caused by alpha-cypermethrin on the variable green toad [66]. Decreased activity of tadpoles in response to sublethal doses of carbaryl have been reported in many anurans species [57].

Fenitrothion at doses between 5 and 9ppm paralyzes tadpoles, making them lack a normal avoidance response in wood and leopard frogs. American toads and spotted salamanders are less sensitive to fenitrothion; doses of this compound between 2 and 5 ppm caused abnormal avoidance response in a few tadpoles [68].

The compound dimethoate caused abnormal behaviour in amphibians of the species *Rhinella arenarum* [65]. Behavioural manipulation may make the amphibian, vulnerable to predators and parasites. Parasites are known to cause serious problem in wildlife conservation [83] along with other covariates of species endangerment such as habitat loss, climate change, pollution, competition, invasive species.

3.3.4. Bioconcentration and Carcinogenic Effects

Current-use pesticides especially fungicides are accumulating in *Pseudacris regilla* (pacific chorus frog). Nine pesticides and three pesticide degradates were detected in males *P. regilla*. The fungicides (pyraclostrobin and tebuconazole) and the herbicide (Simazine) were frequently detected: this is the first field study reporting the occurrence of those three pesticides in frog tissues [84].

Pesticides bioaccumulate in amphibian tissues and disrupt the endocrine system. The compound 2, 2', 4, 4', 5-pentabromodiphenyl ether (Penta-BDE) is easily up taken by juvenile stages of *Xenopus tropicalis* [85].

3.3.5. Histological Changes

Copper sulphate (0.1 mg/L) caused oedema with *B. viridis* larvae. This oedema brought about increase distance between organs such as the medulla oblongata, notochord, pronephric tubules, liver, stomach and intestine. This fungicide generated deformations in the epithelial cells of the stomach, intestines and pronephric tubules. Deformation was observed in the somites of the tadpoles that were exposed to 0.05 and 0.1 mg/L of Copper Sulphate [15]. Histological abnormalities caused by copper sulphate included tissue deformation observed through a cross-section of the liver as a result of degeneration of hepatocytes and increase intercellular areas. These same deformations were observed in the epithelia of the pronephric tubules and somites, in addition to poor development [15].

Trends or statistically significant alterations in at least one aspect of general gonadal morphology are associated with atrazine exposure; those effects include discontinuous and multiple testes, sexually ambiguous gonadal tissue, Testicular Ovarian Follicles (TOFs), altered Gonadal Somatic Index (GSI), expanded testicular lobules, and spermatogenic tubule diameter [77].

3.3.6. Diseases

Parasites and diseases have been reported as factors of biodiversity decline [83]. Chytridiomycosis is one of the most virulent diseases of amphibians caused by *Batrachochytrium dendrobatidis* (the amphibian chytrid fungus); this disease has been associated with amphibian population decline [18].

Pollutants increase the susceptibility of amphibians to parasites. In this light, synergistic effects of trematode infection and exposure to chemical contaminants (atrazine, malathion and esfenvalerate) were reported [86]. Trematode-mediated limb deformities at amphibians natural breeding sites were a function of proximity to agricultural runoffs [86]. Studies done with atrazine have revealed that this pesticide was associated with an increase in infection end points in amphibians, consistently reduced immune functioning of amphibians, and this compound elevated trematode, nematode, viral, and bacterial infections [77].

Anuran species such as *Ammirana galamensis*, *Ptychadena bibroni*, *Ptychadena pumilio*, *Sclerophrys pentoni*, *Sclerophrys maculata*, *Sclerophrys regularis*, *Sclerophrys xeros*, and *Xenopus fischbergi*, have been reported to be common hosts of the trematode *Mesocoelium* with prevalence up to 86% documented in the amphibian *Sclerophrys maculata* [87].

Parasites such as the trematode *Ribeiroia* are able to bring about pelvic limbs malformations in anurans [88]. A mixture of atrazine, methribuzine, endosulfan, lindane, dieldrin and aldicarb caused immunosuppression in *X. laevis* and *Rana pipiens* [89].

Atrazine, malathion and esfenvalerate in high concentration significantly reduced eosinophil count of the wood frogs, *Rana sylvatica*. Exposure to these pesticides had dramatic effects on cercarial encystment: more cercariae encysted under high pesticide conditions; the implications being limb deformities: polymelia, amelia and polydactyly [86].

Sublethal exposure of *Rana clamitans* to atrazine, glyphosate, carbaryl and malathion, increases the susceptibility of this anuran to infection of the trematode *Echinostoma trivolvis* [90].

The impact of pollutants on amphibians is moderately to highly negative [74]. Parasites (Trematode infection) have a synergistic effect with high pesticide concentration [86, 89] the outcome being limb deformities in amphibians [86]. Variation in sensitivity to pollutants is generally independent of phylogeny [74]. Unfortunately in a country like Cameroon, studies on the impact of pollutants on amphibians are very rare; moreover, pesticide importation, distribution and use are done under conditions that are far from ideal [91] and regulation on pesticide use is still at the embryonic stage [7]. Pesticide users are unfortunately not conscious of the implications of their actions. In the Fako Division (South-West, Cameroon) some pesticide users discard empty pesticide containers in water (11%) or in the bush (7%), a practice that will increase pesticide residues in the environment [92]. Amphibian vulnerability to pollutants also depends on the presence of prey and competitors [20] this stresses the need to carry out studies at the community level in order to evaluate other covariates. Amphibians are less susceptible to herbicides as compared to other pesticide families [20, 92] but as herbicides reduce periphyton population, amphibians populations will suffer from indirect effects [92].

4. REDUCTION OF POLLUTANTS EFFECTS ON AMPHIBIANS

Monitoring amphibians' populations is the first and most important step in this process. Many techniques can be implemented for that purpose: call surveys, egg mass count and nocturnal road surveys, and assessment of land use and water physicochemical quality [93]. The purpose of the monitoring exercise is to map Key Biodiversity Areas (KBAs) all over the bio-geographical regions using biological and geophysical data [94]. Frogs of genus *Phrynobatrachus*, endemic to sub-Saharan Africa are threatened by a number of factors bringing about a gradual population decline [95]. Another Cameroonian frog *Arthroleptis palava* (Anura: Arthroleptidae) may also be facing population decline [96]. Amphibian conservation can be done via the protection of genetic resources, cleaning habitat, sustainable harvesting, captive breeding, cloning and reintroduction programmes.

4.1. Protecting the Genetic Resources of Amphibians

Good amphibian conservation research programs should be implemented in order to maintain their genetic resources; applied reproductive technologies and bio-banking are emerging tools for this purpose [97, 98]. Bio-

banking and especially cryopreservation has been reported to be an adequate solution to amphibian population decline related to anthropogenic pressure, climate fluctuation and diseases [99].

4.2. Cleaning Up Habitats

This is done by preventing pollutants from reaching their habitats or protecting their habitats. This will prevent them from predators. Prevention is the best way to avoid the occurrence of diseases in the ecosystem; but the disease is already established, control measures should be implemented for its eradication. Habitats can be cleaned by removing pollutants with phytoremediation techniques, i.e., use of plants and their associated microbes for environmental clean-up [100-102].

4.3. Sustainable Harvesting

Amphibians are used as tools for biological experiments as well as they are consumed by populations. Endangered amphibian species such as *Mantella milotympanum* are edible in Madagascar are collected for overseas export [103]. Frogs have been reported to be a good source of protein in southeast Nigeria, mainly consumed by farmers and teachers [104]. *Conraua goliath*, *C. robusta* and *Trichobatrachus robustus*, three endemic amphibians of Mt. Nlonako (Cameroon) are consumed by local population [17]. The anuran species, *Conraua goliath* in particular is widely hunted for human consumption, hence it currently deserves exceptional protection in Cameroon [105].

Many amphibian and reptiles species are exploited for pests and food worldwide [103, 106]. Community outreach stands as an important strategy to educate masses on amphibian preservation by a sustainable harvesting of species used as pet and food [107, 108] and even other living organisms whose reduction in diversity may affect amphibians [109].

4.4. Captive Breeding, Cloning and Reintroduction

The *ex-situ* conservation is a short-term or even the only solution in establishing conservation assurance of species at risk of extinction [110]. Amphibians (eggs and adults) can be cultured in the laboratory: Eggs masses are cut into clusters of about 10-20 eggs and placed in a 200mL aquarium containing dechlorinated water: the surface area should be large enough to allow gaseous exchange [111]. For adult amphibians, 4-5 individuals can be placed in an aquarium of 40cm x 50cm x 30cm, to study behaviour, adult advertisement call and spawning [95]. Cloning is another technique that can be implemented for amphibian preservation [112, 113]. Cloning is asexual reproduction implying the production of identical copies of a biological entity [114]. Cloning includes gene cloning or recombinant DNA technology, therapeutic cloning and reproductive cloning. The purpose of cloning and captive breeding is the re-introduction of species in the wild; reintroduction of amphibians have been done with success [115, 116] despite constraints such as lack of immunity to diseases due to inbreeding, loss of ability for escape from predators and habitat loss [117].

On the long run, apart from amphibians, pollutants are very risky for the aquatic environment. Insecticides in particular exhibit a very high aquatic toxicity. In an assessment of chemical pollution related to pesticides in freshwater using the *Pesticide Risks in the tropics to Man, Environment and Trade* (PRIMET) model, five pesticides (cypermethrin, lambda-cyhalothrin, cadusafos, malathion and ethoprophos) exhibited very high aquatic risk [49]. Insecticides in particular exhibit a very high risk as compared to other pesticide families [7, 118, 119]. Signs observed in aquatic life in general and amphibians in particular are early warnings of human health implications. Pesticides are among the major poisoning agents recorded in patients referred to the Buea and Limbe Regional Hospital in the south-west region of Cameroon [120, 121]. In men, exposure to pesticides is associated with sperm quality, DNA damage, sperm aneuploidy or diploidy [122] pesticides have been reported to seriously lower men's reproductive capacities in Djutitsa, West Cameroon [91] as well as kidney and liver functions of farmers in the Buea municipality, South-West Cameroon [123].

5. CONCLUSION

Pesticides are necessary for agriculture production but have negative effects on non-target organisms. Agrochemicals have lethal and sub-lethal effects on amphibians. Responses recorded at the individual level make the prediction of what will happen in a complex environment very complicated. Further studies are needed under laboratory, semi-field (microcosms and mesocosm) and field conditions. Farmers should be trained on pesticides application, population should be trained on proper aquatic resources management and protection, and stakeholders should be provided with relevant information from researchers for proper environmental management scheme. The regulation on the importation, distribution and use of agrochemicals should be reinforced.

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