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Impact of organophosphate pesticides on anurans: a mini review

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Organophosphate pesticides (OPs) are a group of widely used insecticides in agriculture and vector control. The primary mechanism of action of OPs is the inhibition of acetylcholinesterase (AChE), leading to the accumulation of acetylcholine and disruption of neural transmission. As acetylcholine acts as neurotransmitter in most animal groups, exposure to OPs has raised increasing ecological concerns due to their toxicity to non-target organisms. Among vertebrates, amphibians are particularly vulnerable to OP exposure given their dual life cycle, which makes them susceptible to contamination in both water and lands. The impairment of neural transmission by OPs can result in behavioral impairments such as abnormal swimming and decreased predator avoidance, ultimately reducing individual fitness and survival. Exposure to OPs also poses developmental risks, causing morphological abnormalities, delayed metamorphosis and reduced growth. Liver and muscle tissues exhibit histopathological changes, indicating systemic stress, while exposure to even low concentrations impairs immune function, increasing susceptibility to infection and reducing resistance against environmental stressors. This mini review synthesizes findings from peer-reviewed studies and reviews published in the last 10 years about the impact of OPs on amphibians, with special focus on anurans as the most studied group in this context. Despite the thematic evolution of ecotoxicology towards more ecology-focused studies, the fact that OPs are not emerging pesticides has somehow excluded them from this pattern. However, OP toxicity to anurans is still of concern, hence future research should prioritize field-based assessments, long-term studies, and species-specific sensitivity to better understand the ecological implications of OP exposure.

Key words: anurans; ecotoxicology; insecticides; pollutants; sub-lethal effects.

Amphibians hold a unique and vital role in ecosystems and are widely distributed across the globe, with the highest diversity occurring in tropical regions. The countries with the top amphibian species richness are Brazil (1188 species) (Segalla *et al.,* 2021), Colombia (836 species) (Roach *et al.,* 2020), and Ecuador (635 species) (Ortega-Andrade *et al.,* 2021). These nations are part of the Neotropics, which harbor nearly half of the world's amphibian species and are characterized by high levels of endemism (Koo *et al.,* 2013).

Amphibians are crucial for ecosystem health, functioning as both predators and prey in food webs. They control pest populations by consuming insects, while serving as a food source for various animals

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(WEST, 2018). Their role in pest management reduces reliance on chemical pesticides, thereby benefiting agriculture, human health, and energy transfer within ecosystems (HOCKING & BABBITT, 2014; WEST, 2018). Certain amphibian species burrow to escape harsh conditions, aiding ecosystem health by breaking down organic matter and enhancing soil fertility. Additionally, tadpoles support aquatic nutrient cycling by grazing on algae. Amphibians serve as nutrient conduits between terrestrial and aquatic environments, enriching soil and water through their nitrogen- and phosphorus-rich waste (HOCKING & BABBITT, 2014; WEST, 2018; ATKINSON *et al.*, 2021).

Amphibians are considered sentinel species due to their sensitivity to environmental changes. Their permeable skin makes them susceptible to pollutants and habitat destruction. Worldwide, amphibian populations are experiencing alarming declines, signaling broader ecological instability (BRÜHL *et al.*, 2013; BARRETO *et al.*, 2020). Factors such as climate change, habitat loss, diseases, invasive species, and pollution, particularly from pesticides, threaten their survival. Among the various pollutants, pesticides are particularly detrimental to amphibians, contributing significantly to individual mortality and population declines (BRÜHL *et al.*, 2013; GOESSENS *et al.*, 2022).

According to a recent report by LUEDTKE *et al.*, (2023) the global decline of amphibians continues to worsen, with 40.7% (2873 species) classified as globally threatened under IUCN Red List categories (Critically Endangered, Endangered, or Vulnerable). Habitat loss and degradation are the most frequently reported threats to endangered amphibians, with agriculture affecting 77% of species, timber and plant harvesting impacting 53%, and infrastructure development affecting 40%. Additionally, climate change and disease pose significant threats, each affecting 29% of species (WOMACK *et al.*, 2022; LUEDTKE *et al.*, 2023). In that review, pollution appears as an additional source for habitat loss and degradation, affecting by itself almost as many species as climate change (see Figure 2 in LUEDTKE *et al.*, 2023). The real extent of pollution as a threat to amphibian populations is, however, difficult to determine; for instance, after facing lethal exposure to environmental pollutants, amphibians may die away from the poisoning site, decompose rapidly, or be consumed by scavengers, resulting in only a small fraction of such fatalities being documented (BEASLEY, 2020).

The extent of pesticide risks to amphibians is not limited to the populations inhabiting farmland and using in-crop habitats. In fact, pesticides applied on crop fields are not confined to a limited area, but they reach off-crop, nearby areas, and sometimes travel long distances. Short-distance, off-crop pollution occurs through spray drift, and from runoff or erosion from treated soils (KAUR *et al.*, 2019). Long-distance travel may happen via atmospheric transport and further deposition in areas far beyond the point of pesticide application, acting those pesticides as transboundary pollutants. Deposition is particularly relevant in colder areas, and so mountain populations become particularly affected. Certain OPs like chlorpyrifos have been shown to travel long distances from their source (MACKAY *et al.*, 2014) and accumulate in tissues of aquatic organisms, thus increasing the chances of bioaccumulation as they move up the food chain (SIDHU *et al.*, 2019; HASAN *et al.*, 2022).

Like many other organisms, amphibians are vulnerable to pesticide exposure, with particular concern over the effects of OPs. In regions with heavy pesticide use, amphibians suffer from a range of adverse effects (GORDILLO *et al.*, 2024). Acute exposure to pesticides increases mortality, while sub-lethal exposure can cause long-term population declines in amphibians (ORTIZ-SANTALIESTRA *et al.*, 2017). A notable case is the decline of frog populations in California's mountains, attributed to airborne OP drift from agriculture in the San Joaquin Valley (SPARLING *et al.*, 2001; DAVIDSON, 2004). Such direct links, however, are uncommon, as amphibian declines often involve multiple interacting stressors that obscure the role of individual stressors. This review seeks to synthesize the available evidence on the effects of OPs on amphibians, emphasizing both well-established toxicological mechanisms and newer findings that hold relevance for conservation and ecological risk assessment.

ORGANOPHOSPHATE PESTICIDES AS PART OF THE GLOBAL PESTICIDE USAGE

The global trend in pesticide usage reflects a steady increase over the past few decades, driven by the need to enhance agricultural productivity and ensure food security amidst a growing population and climate change challenges. The extensive use of pesticides is largely driven by large-scale agricultural practices, particularly the cultivation of high-value cash crops such as soybeans, corn, and coffee (RAHMAN & CHIMA, 2018). From 1990 to 2022, the worldwide agricultural use of pesticides rose significantly, reaching 3.69 million metric tons in 2022 (STATISTA, 2024a) (Fig. 1). This growth is expected to continue, with forecasts indicating a slight increase to around 4.41 million metric tons by 2027 (STATISTA, 2024b). Developing countries have witnessed the most significant growth in pesticide application, mainly due to agricultural intensification and expansion of cultivated lands. Herbicides constitute the largest category of pesticides applied worldwide, accounting for approximately 47.5% of the total loads, followed by insecticides (29.5%), fungicides (17.5%), and other pesticide types (5.5%) (BONDAREVA & FEDOROVA, 2021).

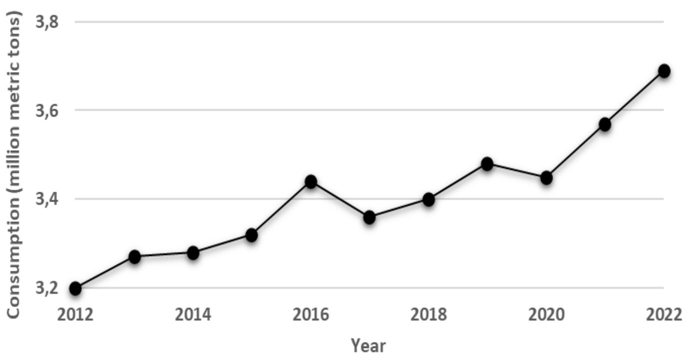

Figure 1: Global agricultural pesticide consumption between 2012 and 2022 (STATISTA, 2024a).

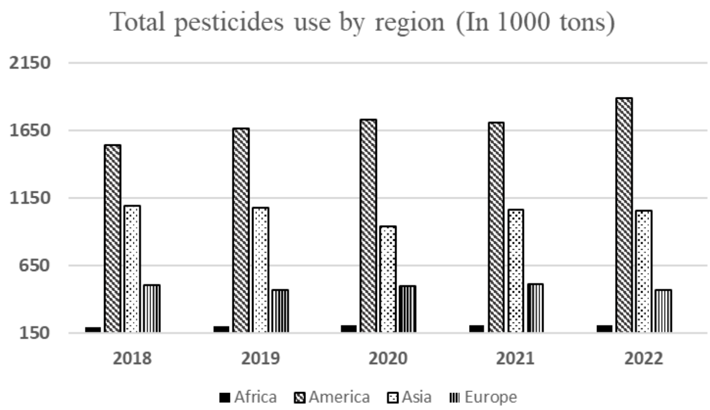

Figure 2: Global pesticide use between 2018 and 2022 in different regions (FAO, 2024).

OP use varies by region, with the Americas showing intensive usage, particularly in the United States, where they occupy a major share of the insecticide market (WISNUJATI, 2023). In southeast Asia, their use is also significant, driven by rapid agricultural development in countries like India and China (LEE *et al.*, 2022). Regulatory actions, such as the restriction on chlorpyrifos use in the United States of the European Union, have impacted OP market growth (BAKER *et al.*, 2024). There is a growing shift towards biopesticides and organic farming as consumers and farmers seek safer alternatives and major players in the market are focusing on developing more sustainable and less toxic formulations to address these concerns (MARRONE, 2019). The global pesticide used by region from 2018-2022 is shown in Figure 2.

Although herbicides and fungicides dominate global pesticide use, with compounds like glyphosate and atrazine being most common (SHARMA *et al.*, 2020), and despite many OP active ingredients being removed from pesticide markets in many countries, OPs still remain one of the most extensively used groups of pesticides. OPs became widely used all over the world from the 1970s and 1908s as an attractive alternative to the highly persistent organochlorine pesticides, which dominated the insecticide markets until those decades. Contrarily to organochlorines, OPs possess the ability to rapidly degrade under natural conditions such as sunlight, air, and soil (DHAS & SRIVASTAVA, 2010). In the last two decades, neonicotinoids and pyrethroids have increasingly replaced OPs, favored for their selectivity towards insect receptors and comparatively lower toxicity to vertebrates (SIMON-DELSO *et al.*, 2015). However, OPs remain extensively used in developing countries, particularly in Asia and Africa, where regulatory controls are limited and agriculture continues to depend heavily on these compounds (BALALI -MOOD *et al.*, 2012; SURATMAN *et al.*, 2015). In fact, global usage of OPs reached approximately 3.5 million tons in 2020 (CAMACHO-PÉREZ *et al.*, 2022), and OPs account for more than 40% of the global pesticide market (TESI *et al.*, 2025).

OPs primarily target acetylcholinesterase (AChE), the enzyme responsible for breaking down the neural transmitter acetylcholine (ACh) in synapses. By binding to and phosphorylating a serine residue at the active site of AChE, OPs prevent ACh hydrolysis, leading to excessive ACh levels. This causes a hyperstimulation of cholinergic receptors that results in the rapid death of insects, but also in severe effects in other organisms following acute exposure (ARONIADOU-ANDERJASKA *et al.*, 2023). OPs are mostly water soluble and disseminate easily into the environment via dissolution, abrasion and volatilization (WANG *et al.*, 2014). This property makes OPs to be commonly found in surface and groundwater due to agro-industrial activities (ALI *et al.*, 2018) and, despite the abovementioned low persistence, OPs show some potential to bioaccumulate in the environment (ZHANG *et al.*, 2010; ASCOLI-MORRETE *et al.*, 2022).

ACUTE TOXICITY OF ORGANOPHOSPHATES

Acute toxicity refers to the harmful effects that result from short-term exposure to a substance, leading to adverse health effects within a relatively short period, typically defined by hours to a few days. The most common methods for measuring acute toxicity include the determination of median lethal concentrations (LC_{50}) or doses (LD_{50}), which refer to the amount of substance that is lethal to 50% of a test population over a specific exposure period (CCOHS, 2018).

OPs exhibit acute toxicity in non-target organisms, including anurans (BERNAL-GONZÁLEZ *et al.*, 2023). Acute toxicity is affected by dose, exposure route, physical properties, environmental interactions with other chemicals, and also by receptor characteristics including species variations and individual factors like age and health status (SRIVASTAV *et al.*, 2017; MIKÓ *et al.*, 2021; ACQUARONI *et al.*, 2022). MAJUMDER (2024) indicated that technical grade (94% purity) chlorpyrifos was less toxic than one of its commercial formulations, containing 20% chlorpyrifos as emulsifiable concentrate, to a series of freshwater organisms including tadpoles of the Asian common toad (*Duttaphrynus melanostictus*). Moreover, there is often a distinct divergence in the effects observed between acute and chronic exposure, even when considering the same organism and test chemical (SRIVASTAV *et al.*, 2018). The main acute effects seen in anurans is the dose-dependent mortality rate (SRIVASTAV *et al.*, 2017); the acute toxicity (LC_{50}) values of organophosphate pesticides from recent studies conducted on amphibians is shown in Table 1.

Apart from mortality, acute exposure can have other consequences threatening survivability. Exposure to OPs during critical developmental stages can result in morphological deformities and behavioral anomalies (BARRETO *et al.*, 2020; SILVA *et al.*, 2020a; RAMADANI *et al.*, 2022). For example, acute exposure to chlorpyrifos in crab-eating frog (*Fejervarya limnocharis*) tadpoles caused marked behavioral impairments, including reduced swimming activity, loss of equilibrium, abnormal posture, decreased feeding, and erratic movements, with severity increasing at higher concentrations (RAMADANI *et al.*, 2022). Acute OP exposure can also cause biochemical disorders like hypocalcemia and hypophosphatemia; acute exposure to chlorpyrifos was shown to decrease serum calcium and phosphate levels in Indian skipper frogs

Table 1: Toxicity values, estimated as median lethal concentrations (LC_{50}) referred to levels in the exposure media, for several organophosphate pesticides extracted from studies conducted with anuran amphibians since 2010.

Substance	Exposure time	Species	Life stage	LC_{50} (mg/L)	Reference
Chlorpyrifos	24h	*Euflictis cyanophlyctis*	Adults	8.252	SRIVASTAV *et al.* (2017)
Chlorpyrifos	48h	*Duttaphrynus melanostictus*	Larvae	1.47	JAYAWARDENA *et al.* (2011)
Chlorpyrifos	48h	*Euflictis cyanophlyctis*	Adults	7.254	SRIVASTAV *et al.* (2017)
Chlorpyrifos	48h	*Fejervarya limnocharis*	Larvae	3.46	RAMADANI *et al.* (2022)
Chlorpyrifos	72h	*Euflictis cyanophlyctis*	Adults	6.247	SRIVASTAV *et al.* (2017)
Chlorpyrifos	96h	*Boana pulchella*	Larvae	0.98	BARRETO *et al.* (2020)
Chlorpyrifos	96h	*Duttaphrynus melanostictus*	Larvae	5.9	DAVID *et al.* (2018)
Chlorpyrifos	96h	*Euflictis cyanophlyctis*	Adults	4.993	SRIVASTAV *et al.* (2017)
Chlorpyrifos	96h	*Fejervarya limnocharis*	Larvae	2.86	RAMADANI *et al.* (2022)
Chlorpyrifos	96h	*Rana dalmatina*	Larvae	5.174	BERNABÒ *et al.* (2011b)
Chlorpyrifos	96h	*Rhinella arenarum*	Larvae	1.46	LIENDRO *et al.* (2015)
Dimethoate	48h	*Duttaphrynus melanostictus*	Larvae	8.89	JAYAWARDENA *et al.* (2011)
Dimethoate	504h	*Rhinella arenarum*	Embryos	16.38	ACQUARONI *et al.* (2022)
Dimethoate	504h	*Rhinella arenarum*	Larvae	12.82	ACQUARONI *et al.* (2022)
Diazinon	96h	*Sclerophrys regularis*	Adults	0.44	LAWRENCE & ISIOMA (2010)
Malathion	96h	*Duttaphrynus melanostictus*	Larvae	7.5	DAVID & KARTHEEK (2015)
Malathion	96h	*Euflictis cyanophlyctis*	Larvae	3.588	GIRI *et al.* (2012)
Temephos	48h	*Rhinella arenarum*	Larvae	16.79	JUNGES *et al.* (2017)
Temephos	48h	*Rhinella fernandezae*	Larvae	4.08	JUNGES *et al.* (2017)
Temephos	48h	*Physalaemus albonotatus*	Larvae	5.88	JUNGES *et al.* (2017)

(*Euphlyctis cyanophlyctis*) (SRIVASTAV *et al.*, 2018). Furthermore, reduced growth rates in tadpoles following acute exposure to OPs may limit their ability to reach metamorphosis, thus impacting population dynamics (SILVA *et al.*, 2020a).

CHRONIC EFFECTS OF ORGANOPHOSPHATE PESTICIDES

Various studies have emphasized the importance of assessing sub-lethal exposure when characterizing pesticide effects on amphibians. DAVID *et al.*, (2012) noted that prolonged exposure to environmentally relevant concentrations of OPs could lead to chronic health issues and altered behavioral responses, which could further threaten amphibian populations. Long-term exposure to OPs can lead to various sublethal effects in amphibians; for instance, chlorpyrifos has been shown to significantly affect the locomotor activity and morphology of amphibians at concentrations as low as 4 µg/L in the water medium (RAMADANI *et al.*, 2022). The observed effects are seen to increase in the presence of other stressors like temperature and UV (QUIROGA *et al.*, 2019; HENAO MUÑOZ *et al.*, 2020; HENAO *et al.*, 2022). These findings are particularly concerning as they highlight the sensitivity of amphibians to these chemicals even at low concentrations, which may not cause immediate lethality but can severely impair overall fitness and population. Below we review some of the main sublethal effects of OPs reported in amphibians, with emphasis on those studies published during the last ten years.

Biochemical and physiological implications

Organophosphate pesticides exert their primary toxic effects through well-characterized neurotoxic, oxidative, genotoxic, and endocrine-disrupting mechanisms that can manifest even at sublethal exposure levels. As already mentioned, amphibians are particularly vulnerable to these effects due to their permeable skin and aquatic larval stages, making them highly sensitive bioindicators of pesticide contamination (VENTURINO *et al.*, 2003).

***Effects on endocrine system*:** consistently with their mechanisms of action, OP exposure interferes with the neuroendocrine system of amphibians primarily through the inhibition of AChE (ATTADEMO *et al.*, 2015). Inhibition of AChE causes acetylcholine accumulation, leading to overstimulation of cholinergic receptors, disrupted nerve signaling, and impaired neuromuscular coordination. These disruptions extend beyond the nervous system, influencing hormonal regulation and physiological homeostasis. AChE is widely recognized as a sensitive biomarker for sublethal OP exposure in wildlife (VENTURINO *et al.*, 2003; SANTOS *et al.*, 2015). In *D. melanostictus*, exposure to chlorpyrifos significantly reduced AChE activity, resulting in decreased swimming performance which is a critical behavior for feeding, predator avoidance, and overall fitness (DAVID *et al.*, 2018; RUTKOSKI *et al.*, 2020; RAMADANI *et al.*, 2022). Chlorpyrifos also inhibits other esterases, including carboxylesterase (CbE) and butyrylcholinesterase (BChE), broadening its neurotoxic effects. In South American toad (*Rhinella arenarum*) tadpoles, both AChE and CbE activities were inhibited by chlorpyrifos in a tissue- and diet-dependent manner, suggesting intestinal detoxification modulation (ATTADEMO *et al.*, 2017). Moreover, mixtures with the herbicides 2,4-D and glyphosate intensified AChE, BChE, and CbE inhibition caused by chlorpyrifos (LAJMANOVICH *et al.*, 2015).

***Oxidative stress and cellular damage*:** A major downstream consequence of OP exposure is oxidative stress, resulting from both direct enzymatic activity and indirect interference with cellular metabolism (VENTURINO *et al.*, 2003). Pesticide metabolism frequently generates reactive oxygen species (ROS), depleting antioxidant reserves and disrupting redox homeostasis. Studies in Asiatic grass frog (*Rana chensinensis*) tadpoles exposed to trichlorfon revealed marked oxidative stress, characterized by an activation of the antioxidant defenses as shown by the elevated activities of superoxide dismutase and catalase across all tested concentrations (LI *et al.*, 2017). Glutathione transferase (GST) activity slightly increased as a response to trichlorfon exposure only at later stages, suggesting a delayed detoxification response. On the contrary, malondialdehyde levels unexpectedly decreased; this molecule usually occurs as a subproduct of lipid peroxidation, one of the main expressions of oxidative stress, which led the authors of that study to hypothesize a process of metabolic adaptation. Histological examination revealed hepatic swelling, cytoplasmic vacuolation, and nuclear necrosis, confirming hepatocellular damage (LI *et al.,* 2017). These oxidative disruptions can lead not only to lipid peroxidation, but also to immune suppression and compromised cellular function (BORKOVIĆ-MITIĆ *et al.,* 2016; SZUROCZKI *et al.,* 2019; AWKERMAN *et al.,* 2024).

Interactions between OPs and environmental stressors can further intensify or complicate these effects. SCHAVINSKI *et al.* (2022) reported that combined exposure of yellow-spotted tree frog (*Boana curupi*) tadpoles to trichlorfon and ultraviolet (UV) radiation produced complex oxidative responses. Individually, UV radiation and trichlorfon increased lipid peroxidation, protein carbonylation, and AChE activity while decreasing GST activity, indicating oxidative damage and impaired detoxification. Interestingly, co-exposure to UV radiation type B (UVB) and trichlorfon reduced mortality and DNA damage compared to UVB alone, suggesting antagonistic interactions possibly linked to enhanced DNA repair or suppression of apoptotic signaling (SCHAVINSKI *et al.,* 2022). Such findings illustrate the intricate interplay between pesticide exposure and environmental stressors in natural ecosystems.

Genotoxic effects and DNA damage: OP-induced oxidative stress often progresses to genotoxic damage, posing long-term risks to amphibian populations. Trichlorfon exposure in *R. chensinensis* caused significant increases in micronucleus (MN) formation in erythrocytes, with MN frequency and other nuclear abnormalities (e.g. lobed, notched nuclei) rising in a dose- and time-dependent manner (MA *et al.,* 2019). Similarly, chlorpyrifos exposure at environmentally relevant concentrations (0.4-1.0 μg/L) induced a dose-dependent rise in MN frequency, nuclear buds, and binucleated cells. The underlying mechanisms involve AChE inhibition and ROS-mediated DNA damage, reflecting the compound's pronounced genotoxic and cytotoxic potential (SILVA *et al.,* 2020b; HEREK *et al.,* 2021; RAMADANI *et al.,* 2022). The cumulative genetic damage from such exposures may persist across generations, reducing population resilience and potentially altering evolutionary trajectories. This genotoxic dimension underscores the far-reaching ecological consequences of even low-level OP contamination in amphibian habitats.

Endocrine disruption and thyroid dysfunction: OPs interfere with hormonal regulation, particularly thyroid hormone signaling, which governs amphibian metamorphosis (LEEMANS *et al.,* 2019). Amphibian metamorphosis depends critically on triiodothyronine (T3) and thyroxine (T4), making these stages highly sensitive to endocrine disrupting chemicals. WANG *et al.* (2025) demonstrated that chlorpyrifos exposure in African clawed frog (*Xenopus laevis*) tadpoles altered thyroid hormone homeostasis, accelerating early development but delaying later metamorphic stages. High concentrations (18 μg/L) reduced T3 and T4 levels by 28% and 39.4%, respectively. Molecular analyses revealed that chlorpyrifos competes with T3 for binding to thyroid receptor alpha, acting as a partial agonist that interferes with receptor activation. Co-exposure with T3 further reduced receptor activity, confirming direct receptor-level disruption and downstream gene expression alterations (WANG *et al.,* 2025). These hormonal perturbations culminated in abnormal metamorphic progression and reduced developmental success.

***Developmental toxicity*:** OPs exert profound developmental toxicity at embryonic and larval stages. Early exposure to chlorpyrifos caused neural and morphological abnormalities in amphibian embryos, including incomplete neural tube closure, fin shortening, tail curvature, and edema, with severity increasing over time as LC_{50} values decreased (SOTOMAYOR *et al.,* 2012; KHARKONGOR *et al.,* 2018). At the biochemical level, chlorpyrifos reduced ornithine decarboxylase activity and polyamine levels, which are vital molecules for cell division and tissue growth. The depletion of polyamines disrupted normal growth and differentiation, leading to developmental delays and malformations. These findings suggest that polyamine metabolism represents an early and sensitive biomarker of chlorpyrifos-induced developmental toxicity in amphibians (SOTOMAYOR *et al.,* 2012).

Morphological, histological and hematological abnormalities

OPs not only induce neurotoxic and developmental disturbances but also cause significant alterations in tissue structure and blood physiology of amphibians. These changes, though often sublethal, can impair vital functions such as respiration, osmoregulation, and immune defense, ultimately reducing survival and fitness. Assessing such morphological and hematological responses provides valuable insight into the systemic toxicity of OPs and their long-term consequences for anuran health and population stability.

***Morphological abnormalities*:** OPs pose significant developmental hazards to anuran embryos and larvae, inducing a range of morphological deformities that jeopardize tadpole survival and population stability. Exposure to chlorpyrifos during critical developmental stages resulted in severe malformations in *Physalaemus gracilis*, primarily affecting oral and intestinal structures, with deformity severity increasing at higher concentrations (RUTKOSKI *et al.,* 2020). Similarly, *D. melanostictus* embryos exposed to chlorpyrifos exhibited reduced body length and width, indicating impaired somatic growth (KHARKONGOR *et al.,* 2018). Such reductions in body size have ecological consequences, as smaller tadpoles experience reduced swimming efficiency and greater vulnerability to predation, negatively impacting survival and future reproductive potential (MONROE *et al.,* 2015).

The developmental deformities observed in OP-exposed amphibians largely stem from AChE inhibition, which leads to excessive acetylcholine accumulation and continuous muscle contraction, producing abnormal tail and trunk curvature (GHODAGERI & PANCHARATNA, 2011). Tail morphology, therefore, serves as a sensitive indicator of OP toxicity, with acephate exposure causing pronounced tail deformities that impair swimming and feeding performance. Endocrine interference may further contribute to delayed limb emergence and prolonged metamorphosis, suggesting disruption of thyroid hormone signaling pathways essential for growth and differentiation. Apart from external malformations, OP exposure alters brain development, with reduced total brain size and regional shrinkage of the optic tectum and telencephalon (McCLELLAND *et al.*, 2018), disrupting sensory and motor coordination and ultimately compromising behavioral and ecological fitness.

***Gonadal abnormalities*:** Studies directly investigating the reproductive effects of pesticides on amphibians are relatively scarce, and most of these studies focus on herbicides (HAYES *et al.*, 2006, 2010) and fungicides (POULSEN *et al.*, 2015; SVARTZ *et al.*, 2016). OPs have also been shown to disrupt amphibian reproductive development through endocrine-related mechanisms. In agile frogs (*Rana dalmatina*), chronic exposure to low concentrations of chlorpyrifos (0.025-0.05 mg/L) throughout larval development did not impair survival or metamorphic success but caused clear gonadal abnormalities. Histological analyses revealed the presence of testicular oocytes in males, indicating intersex development and impaired spermatogenesis, whereas controls exhibited normal gonads (BERNABÒ *et al.*, 2011a). Although the overall sex ratio remained unchanged, the reduced proportion of males with histologically normal testes indicates that chlorpyrifos acts as an endocrine disruptor, compromising male reproductive capacity (BERNABÒ *et al.*, 2011a). Such subtle but heritable impairments highlight the long-term, population-level risks of chronic pesticide exposure in natural amphibian habitats. Gonadal abnormalities have now been observed across a wide range of species, including mammals, birds and fish, highlighting the urgent need for integrated research on the long-term effects of endocrine-disrupting chemicals on biodiversity (GARCÊS *et al.*, 2020).

***Hematological and histological alterations*:** In addition to developmental and reproductive toxicity, OP exposure induces significant hematological alterations that undermine physiological performance and immune competence. Sublethal chlorpyrifos exposure in amphibian tadpoles led to marked reductions in lymphocytes, monocytes, and basophils, accompanied by increased neutrophil and eosinophil counts (SILVA *et al.*, 2020b). This characteristic pattern of neutrophilia coupled with lymphopenia represents a generalized stress response and immunosuppression, making affected individuals more vulnerable to infections and environmental stressors.

The hematotoxic effects of OPs extend to erythropoiesis. Exposure to malathion caused a significant decline in total erythrocyte count and hemoglobin concentration, resulting in acute hemolytic anemia in *F. limnocharis* (KUNDU *et al.*, 2011). Similar effects were observed in adult *D. melanostictus*, where reduced hemoglobin levels compromised oxygen transport and overall aerobic capacity (MAHANANDA & MOHANTY, 2012).

OP exposure induces pronounced histopathological damage in tadpoles, affecting multiple organ systems. Liver tissues in exposed individuals commonly exhibit sinusoidal congestion, cytoplasmic vacuolation, nuclear fragmentation, oedema, and severe degeneration at high concentrations (BANDARA *et al.*, 2012). Gill structures also deteriorate, showing lamellar distortion, epithelial thickening, altered vascularization, and reduced respiratory efficiency. Chlorpyrifos further disrupts tail musculature, causing fiber atrophy, enlarged myotomal spaces, and misaligned muscle bundles. Digestive impairments are equally significant; OP exposure leads to epidermal dissolution, vacuolation, serosal degeneration, necrosis, and epithelial rupture, ultimately compromising nutrient absorption and hindering normal development (KUNDU *et al.*, 2011).

Behavioral implications

Behavior serves as an ecologically relevant and highly sensitive indicator of pesticide toxicity in amphibians, offering early warnings of sublethal impacts before severe physiological damage manifests (COHN & MACPHAIL, 1996). OP exposure frequently disrupts locomotion, feeding, and predator avoidance, thereby reducing individual fitness and revealing toxicity even at non-lethal concentrations across multiple anuran species (JUNGES *et al.*, 2017). Such behavioral endpoints provide critical insight into population-level risks, as even subtle impairments can compromise survival and reproductive success.

***Locomotor and activity alterations*:** Chlorpyrifos, one of the most widely studied OPs, has been shown to affect tadpole locomotor performance in a concentration- and duration-dependent manner. In *R. dalmatina* tadpoles, chronic exposure to 5 μg/L chlorpyrifos increased swimming trajectory length by over 20% compared to controls, and reduced body mass at metamorphosis by about 7%, whereas lower concentrations (0.5 μg/L) or acute exposure produced no measurable effects on locomotion, development or survival (MIKÓ *et al.*, 2021). These results indicate that agile frogs exhibit tolerance to environmentally realistic chlorpyrifos levels, but repeated exposure to elevated concentrations can alter energy allocation and activity patterns in ways that heighten predation risk and reduce long-term fitness.

***Predator avoidance and escape response*:** Predator avoidance behavior is an essential survival mechanism in tadpoles, and OP exposure has been shown to compromise this critical response. In the Argentine horned frog (*Ceratophrys ornata*), OP exposure impaired the detection and reaction to predator-derived chemical cues, leading to diminished escape responses and reduced swimming activity in the presence of predators (COSTA *et al.*, 2021). Consequently, predator capture success and prey consumption were significantly higher in pesticide-exposed individuals compared to unexposed controls (COSTA *et al.*, 2021; MCCLELLAND & WOODLEY, 2022). These findings highlight that even sublethal OP concentrations can profoundly disrupt antipredator strategies, increasing vulnerability to predation in natural habitats.

***Abnormal behavioral manifestations*:** A range of abnormal behavioral patterns have been reported following OP exposure, including loss of equilibrium, reduced feeding activity, and altered locomotion (DENOËL *et al.*, 2012; DAVID & KARTHEEK, 2015; CURI *et al.*, 2022; SAMOjeden et al., 2022; Bernal-González et al., 2023). The loss of fright response, normally characterized by an immediate halt in movement upon disturbance, is consistently observed among exposed tadpoles. Other motor anomalies include circular swimming, staggered movement, and whirling locomotion (David & Kartheek, 2015). These behavioral abnormalities are primarily attributed to the neurotoxic mechanism of OPs, inhibition of AChE, which can disrupt neuromuscular coordination (Peltzer *et al.*, 2013; Rutkoski *et al.*, 2020). The resulting muscle spasms and tail contortions impair effective swimming mechanics, further reducing the ability to evade predators or forage efficiently. Structural alterations in the brain, as observed by McClelland & Woodley (2022), may underlie some of these behavioral deficits, linking neuroanatomical damage with functional impairment.

***Bioacoustic disruption as a novel endpoint*:** An unusual yet highly informative behavioral endpoint involves the disruption of underwater acoustic signaling in *C. ornata*. Tadpoles of this species naturally emit acoustic signals during conspecific interactions, but exposure to chlorpyrifos significantly altered call structure, reducing duration and pulse number while increasing dominant frequency, especially under chronic exposure (Salgado Costa *et al.*, 2018). These bioacoustic alterations appeared at concentrations similar to those inducing early behavioral impairments, preceding severe physiological damage or mortality, thus representing a sensitive and ecologically meaningful biomarker of sublethal OP exposure.

***Recovery and reversibility of behavioral effects*:** The reversibility of OP-induced behavioral changes remains an important area of investigation in ecotoxicology. In *C. ornata* tadpoles, most behavioral impairments caused by 96-hour chlorpyrifos exposure were reversible after 72 hours in pesticide-free water, with normal swimming activity and prey consumption restored and no delayed mortality or morphological abnormalities detected (Rimoldi *et al.*, 2023). These findings suggest that many acute behavioral effects represent transient neurofunctional disruptions rather than permanent damage. However, not all endpoints recovered equally; while locomotor functions returned to normal, acoustic alterations persisted after exposure, suggesting that vocal behavior is a more sensitive and longer-lasting indicator of chlorpyrifos toxicity (Rimoldi *et al.*, 2023). The findings imply that OP-induced behavioral disruptions in amphibians may not always cause permanent damage, but recovery depends on the type and severity of the affected behavioral endpoint.

Knowledge Gaps and New Paths

Despite significant advancements in understanding the effects of OPs on anuran populations, numerous gaps remain in the current body of knowledge. Interactions between combinations of OPs can produce synergistic effects that exacerbate toxicity. Research indicates that pesticides, when combined with other stressors, can exert greater toxicity than individual components (Henao *et al.*, 2022), highlighting potential cumulative effects on anurans in agricultural ecosystems. More studies are needed to understand the impact of pesticides on amphibians. Most studies have focused primarily on chlorpyrifos, while other OPs such as malathion, parathion, and diazinon remain comparatively underexplored. Further research on these compounds is needed to provide a more comprehensive understanding of OP impacts on amphibians.

Another important gap lies in the field of histopathology; most studies give limited attention to histopathological assessments. Expanding research in this area is essential to better understand tissue-level alterations caused by pesticide exposure in amphibians. Genotoxic studies are an emerging field within toxicology, with technological advances enabling new approaches to assess pesticide effects. Further research in this field is essential to enhance our understanding of pesticide-induced genetic damage and its implications for environmental and human health.

Endocrine disrupting chemicals primarily disrupt the normal functioning of hormones by effectively binding to, among others, estrogen or androgen receptors (TABB & BLUMBERG, 2006). These substances can interact with multiple hormone receptors, including estrogen, androgen, and estrogen-related receptors, acting as agonists or antagonists. They also disrupt hormone synthesis, transport, metabolism, and elimination, reducing hormone levels. Anuran amphibians are key indicator species for detecting endocrine disrupting chemicals in aquatic ecosystems (LUTZ & KLOAS, 1999). Thyroid hormone-driven metamorphosis is a primary target for endocrine disruption research. Estrogen and other hormones also influence this process, making larval tissue changes valuable indicators for assessing endocrine disrupting effects on amphibian development (MIYATA & OSE, 2012). Thyroid hormones and their receptors, thyroid receptors α and β, are pivotal in regulating amphibian metamorphosis, which is a critical phase involving extensive morphological and physiological remodeling. Acting as nuclear transcription factors, these receptors control gene expression for neural differentiation, skeletal restructuring, and tissue reabsorption. Thyroid hormone-mediated signaling ensures precise temporal coordination of these transformations. Although OPs are well known for neurotoxicity via AChE inhibition, their endocrine disrupting effects on thyroid receptor pathways remain poorly explored, with emerging evidence indicating potential interference in thyroid receptor-mediated transcription during anuran development.

An important yet understudied aspect of OP toxicity in amphibians is the potential for recovery or reversibility following exposure. Understanding post-exposure recovery provides valuable insight into the resilience of anuran species and helps establish ecologically relevant pesticide thresholds for safe agricultural use. As mentioned above, in *C. ornata* tadpoles, most behavioral impairments from a 96-hour chlorpyrifos exposure were reversed after 72 hours in pesticide-free water (RIMOLDI *et al.*, 2023). Similarly, *B. pulchella* tadpoles showed partial recovery after removal from contaminated environments, though certain genotoxic and cellular alterations persisted (PÉREZ-IGLESIAS *et al.*, 2018). Such studies underscore the need to integrate recovery assessments into toxicity evaluations to refine pesticide regulation and environmental risk management.

With this review, we stress the importance of extensive research to understand the implications of OP exposure and to establish evidence-based conservation measures to protect anurans from these substances, especially in those regions where their use is still widespread. Future research projects could address these critical gaps in our knowledge of the impact of OPs on anuran populations and their broader ecological consequences.

CONCLUSION

Amphibians are undergoing an unprecedented global decline, with more than 43% of species encountering a population decrease. This concerning tendency renders their conservation an immediate priority. Amphibians are essential for sustaining ecological equilibrium by managing insect populations, promoting nutrient cycling, and regulating water quality. Their loss would have cascading effects on ecosystems, directly affecting both predators and prey reliant on them. This change would destabilize food webs and undermine critical ecological services, including the regulation of disease-carrying insects.

Chemical pesticides represent a significant threat to amphibians, causing substantial sublethal effects, especially during crucial developmental phases. Numerous anuran species exhibit significant vulnerability to pesticide exposure, particularly during their breeding seasons, which align with periods of heightened pesticide application. Contaminated food and water supplies aggravate these hazards, impacting both adult individuals and their larvae. Exposure during larval stages can diminish hatching success, decrease survival rates, and elevate the probability of abnormalities. Moreover, pesticide toxicity modifies eating behavior, inhibits the immune system, and heightens predation risk, all of which can lead to population decreases. Since behavior integrates various physiological systems, it serves as a sensitive indicator of pesticide toxicity.

Addressing these risks needs appropriate pesticide management systems. Educating farmers on appropriate pesticide usage and promoting alternatives such as biopesticides and natural product-based treatments are essential measures. A full examination of pesticide hazards and benefits is crucial for environmental protection. Integrated Pest Management (IPM) offers a sustainable method by combining targeted, low-toxicity pesticides with alternative strategies to reduce harm to amphibians and biodiversity in general. By adopting IPM, farmers, foresters, and other stakeholders can limit the harmful effects of pesticides while preserving agricultural output, creating a balance between pest control and ecosystem health.

In addition to enhanced pesticide management, tougher regulation and enforcement are necessary to defend both environmental and public health, especially in developing countries. Given the broad dissemination of pesticides, thorough hazardous monitoring should be conducted, particularly in protected regions and biodiversity hotspots. Compliance with national and international pesticide standards must be strictly enforced, with active engagement from users to assure conformity and limit ecological harm.

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