Effects of chronic pesticide exposure on larval amphibians

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By

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ABSTRACT

The potential role of pesticides in amphibian declines has generated many studies of short-term, acute exposures. However, investigations of chronic exposures may more accurately reflect conditions experienced by natural populations. I examined effects of three insecticides on survival, growth, behavior, and development of several amphibian species (*Pseudacris triseriata* – the upland chorus frog, *Bufo americanus*- the American toad, *Rana pipiens*- the northern leopard frog, *Rana sphenocephala*- the southern leopard frog, and *Ambystoma jeffersonianum*- the Jefferson salamander). I made my protocol more biologically realistic by exposing both eggs and larvae at various stages of development and placing uncontaminated sediment in the bottom of aquaria to provide a natural route for detoxification. Egg masses and larval stages of three amphibian species were exposed to three concentrations of each pesticide based on previously determined LC50s (high = 0.5xLC50, medium = 0.1xLC50, low = 0.01xLC50). Acute testing ("LC50" determination) indicated that chlorpyrifos is most toxic and imidacloprid least toxic to amphibian larvae. Egg masses were placed in 15-L aquaria until hatching. High concentrations of carbaryl significantly reduced hatching success of *A. jeffersonianum* egg masses, however the percent of deformed hatchlings was not significantly different among treatments in any species. Larvae were maintained in 60-L aquaria from two weeks post-hatching through metamorphosis. Survival to metamorphosis was reduced in all species by high concentrations of pesticides. Because of significantly reduced survival in high concentrations of pesticide, analysis of sublethal effects on larvae were performed only on control, low, and medium concentrations. All species experienced a significant increase in time to metamorphosis in medium concentrations of pesticide. *Bufo americanus* larvae exhibited reduced growth in medium concentrations of chlorpyrifos and carbaryl, and *R. sphenocephala* displayed reduced growth in medium concentrations of all pesticides. Activity levels of larvae were not significantly affected by any pesticide treatment, and abnormal behavior (avoidance response) was only observed prior to death. Developmental abnormalities were observed in *R. sphenocephala*, but did not significantly differ among treatment groups. Water and sediment analysis indicates that these pesticides are rapidly absorbed by sediment. Decreased growth and maturation rate, altered behavior, and developmental deformities of amphibian larvae may decrease survival by increasing vulnerability to predation. Mortality of eggs and larvae in successive years due to pesticides may eventually lead to declines of amphibian populations.

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CHAPTER 1. Literature Review

Amphibian importance

Amphibians are a unique group of tetrapods with bi-phasic life history patterns. The anamniotic egg, which must remain in water (or in a moist environment) throughout the development of the embryo, ties amphibians strongly to water. Larval amphibians with indirect development need to live in water until metamorphosis has taken place (Zug 1993). Although many adults rely on resources available on land, their moist, scaleless skin is susceptible to desiccation.

Amphibians play vital roles in both aquatic and terrestrial ecosystems. Larval amphibians feed on algae, plant material, detritus, and bacteria (Zug 1993). Because tadpoles occur at high densities, have high rates of ingestion, and forage on a broad range of substances, they are a primary consumer in some ecosystems (McDiarmid and Altig 1999). Adults are carnivorous, feeding on a variety of small insects, worms, mollusks, and vertebrates (Zug 1993). They also serve as prey for larger vertebrates (Duellman and Trueb 1986). Amphibians may make up the majority of vertebrate biomass in a community, and their absence from the food web may also affect other trophic levels (Burton and Likens 1975).

Amphibian status

The observed decline of amphibians around the world has been well-documented (Barinaga 1990, Phillips 1990, Drost and Fellers 1996). For example, 17 Canadian amphibian species have documented losses of populations (Green 1997). The gastric brooding frog (*Ryeobatrachus silus*) in Australia has declined to the point of apparent extinction (Blaustein and Wake 1990). Corn and Fogelman (1984) found that six populations of leopard frogs (*Rana pipiens*) initially present at study sites in Colorado disappeared within ten years. Similarly, the mountain yellow-legged frog in the mountains of California has disappeared from 75 % of formerly inhabited sites (Bradford et al. 1994). Although habitat loss, drought, and human collection may be responsible, many scientists believe that these declines are an early signal of chemical pollution in the environment (Phillips 1990).

Proposed reasons for decline

Compounds that are commonly used because of low avian and mammalian toxicity may be very toxic to aquatic organisms (Hall and Henry 1992). Because amphibians have skin that is highly permeable, they are especially sensitive to environmental contamination. Their semi-aquatic lifestyle and dependence on water leave them vulnerable to both soil and water contaminants (Berrill et al. 1993). Furthermore, many larval amphibians are detritus feeders and may ingest chemicals in the substrate. Indirect effects of environmental pollution may leave amphibians more susceptible to disease, predation, and death from habitat degradation (Cooke 1971, Fioramonti et al. 1997, Green 1997).

Pollution from pesticides is a major cause of contamination in many amphibian habitats (Cooke 1972). Application of pesticides on agricultural fields and recreational areas is heaviest in spring and summer when breeding and larval development occur (Materna et al. 1995). Pesticides sprayed on crops or turf accumulate in temporary pools due to surface runoff or sediment transport and may result in very high water concentrations (Marian et al. 1983, Berrill et al. 1995). Concentrations of the insecticide fenitrothion have been found to reach 2.5 ppm in small shallow ponds (Berrill et al 1995). Mortality of green frog (*Rana clamitans*) and bullfrog (*Rana catesbeiana*) tadpoles has been reported at levels above 2.0 ppm (Ernst et al. 1991, Berrill et al. 1995).

Population effects of pesticides

Acute testing on many vertebrate species has been performed using a wide range of chemicals and LC50s for many pesticides are available for a few aquatic species (Saxena and Fisher 1981, Hudson et al. 1984, Herfenist et al. 1989). An LC50 is a bioassay-based statistical estimate of the concentration needed to kill 50% of experimental animals during a specified period of time (Hudson et al. 1984). LC50 values are often used as a convenient method for comparing relative toxicities of chemicals and serve as guidelines for chemical application (Hudson et al. 1984, Birge et al. 1995).

Although LC50s are performed routinely on fish, they are less commonly determined for amphibians, and application rates are often based upon LC50s for fish. Previous research has demonstrated that amphibian embryos and tadpoles may be more sensitive than fish to some toxicants (Herfenist et al. 1989, Hall and Henry 1992). Likewise, numerous investigators (Cooke 1972, Hall and Henry 1992, Berrill et al. 1995) have observed mortality of amphibian larvae in natural populations. McAlpine (1992) noted that mink frog (*Rana septentrionalis*) densities were negatively correlated with pesticide spraying in previous years. Increased mortality in successive years may eventually result in loss of entire populations over time.

Sublethal pesticide levels may cause developmental effects that leave amphibian embryos and larvae more vulnerable to predation. Developmental stages are subject to a wide range of toxicant-induced responses. Toxicants affect various biochemical and physiological pathways associated with gene action, cell differentiation, cell growth, and basic metabolism (Saxena and Fisher 1981). Although a small number of deformities in a population are expected to occur naturally (<2%), recent reports of abnormal levels of deformed frogs have caused concern about the effects of pesticides on embryonic and larval development of amphibians (LaClair et al. 1998). Developmental abnormalities of amphibian embryos and larvae in response to pesticide exposure have been well documented in the laboratory by many investigators (Bancroft and Prahlad 1973, Rzehak et al. 1977, Cooke 1981, Marchal-Segault and Ramade 1981, Elliot-Feeley and Armstrong 1982, Pawar and Katdare 1984, Fulton and Chambers 1985, Alvarez et al. 1995, Howe et al. 1998). Several field studies have demonstrated abnormal development of amphibians in pesticide contaminated areas (Cooke 1973, Bonin et al. 1997, Ouellet et al. 1997, Harris et al. 1998). Many of the deformities observed would likely compromise individual survival in the presence of a predator.

Exposure to sublethal levels of pesticides has been found to affect growth rate and time to metamorphosis of tadpoles (Cooke 1973, Marchal-Segault and Ramade 1981, Marian et al. 1983, Jung and Walker 1997, Fioramonti et al. 1997, Harris et al. 1998). Reduced growth and delayed time to metamorphosis of tadpoles may have negative effects on population persistence. Although amphibian larvae exhibit plasticity in size at metamorphosis and length of larval period, classic studies of amphibian metamorphosis support a minimum body size needed for metamorphosis (Wilbur and Collins 1973, Hensley 1993). Tadpoles of amphibian species that breed in temporary pools need to reach this minimum body size before ponds dry up. Delays in metamorphosis can increase competition and vulnerability to predation (Smith 1987). In addition, size of a tadpole at the time of metamorphosis affects the timing of first breeding, thus, decreased growth rate of larvae could negatively impact reproduction of adult frogs or decrease lifelong reproductive output (Marian et al. 1983).

Changes in tadpole behavior due to pesticide exposure may indirectly affect survival. Hall and Swineford (1981) reported that larvae of *Ambystoma opacum* showed abnormal behavior at toxaphene levels that were below lethal concentrations. Cooke (1971) found that tadpoles hyperactive from exposure to DDT were preyed upon more often by newts than tadpoles exhibiting normal behavior. Conversely, inactive tadpoles may avoid predation, but also encounter fewer resources needed for growth and compete less successfully when resources are scarce (Lawler 1989, Bridges 1997). In addition, smaller and less developed tadpoles are preferred prey over larger tadpoles (Brodie and Formanowicz 1983, Crossland 1998). Decreased foraging behavior may prolong the developmental period, increasing risk of temporary ponds drying up before metamorphosis. Abnormal predator avoidance response and altered swimming performance of tadpoles due to pesticide exposure also may increase risk of predation (Berrill and Bertram 1997, Bridges 1997, Raimondo et al. 1998).

Research needs

In spite of the extensive use of pesticides by humans, research into effects on amphibians is incomplete because most studies have focused on the short-term effects of acute testing (Sanders 1970, Materna et al. 1995). Although this may allow investigators to compare more easily the relative toxicity of chemicals, these studies have little relevance to the chronic, repeated exposure to chemicals that impact many amphibian populations. Acute tests only measure survival directly, however, low doses of chemicals may cause sublethal effects that indirectly affect survival.

Most studies have used only tadpoles (Cooke 1971, Hall and Swineford 1981, Materna et al. 1995). Because amphibians are thought to differ in susceptibility to toxins at different life history stages (Hall and Swineford 1981, Elliott-Feeley and Armstrong 1982, Herfenist et al. 1989), generalizations made for all amphibians are likely to be inaccurate when based only upon larval anurans. Differences in sensitivity to toxins among amphibian species have been noted by Herfenist et al. (1989) and Berrill et al. (1995), but few studies have addressed this issue.

Results of laboratory studies may not accurately predict the effects of pesticides on natural populations of amphibians. The degree of contamination in different environments can be influenced by the evenness of pesticide application, the solvent or pesticide carrier, and environmental components such as temperature, soil type, vegetative composition, and water quality parameters (Hudson et al. 1984). Routes by which animals contact the pesticide may be different in a laboratory setting than in the field. For instance, tadpoles in the laboratory may contact a pesticide mainly through dermal absorption whereas tadpoles in the field may be exposed to a pesticide by ingestion of contaminated plants and sediments (Hudson et al. 1984, Bauer-Dial and Dial 1995). Persistence of a pesticide may also differ in a natural environment compared to the laboratory because of sediment absorption and biotic interactions (Hudson et al. 1984).

Although studies of acute mortality are important, a study of the effects of chronic exposure on hatching success and larval development of several species gives a more accurate depiction of the conditions faced by natural populations of amphibians. Laboratory studies that include an environment similar to that encountered by amphibians in the field may make it easier to extrapolate the field hazard of a compound from laboratory data. In 1997, I initiated a study on the effects of three insecticides (carbaryl, chlorpyrifos, and imidacloprid) on hatching and larval development of amphibians. My objectives were to test the relative toxicity of these pesticides with three different genera of amphibians, and to develop a more complete and biologically realistic testing protocol.

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