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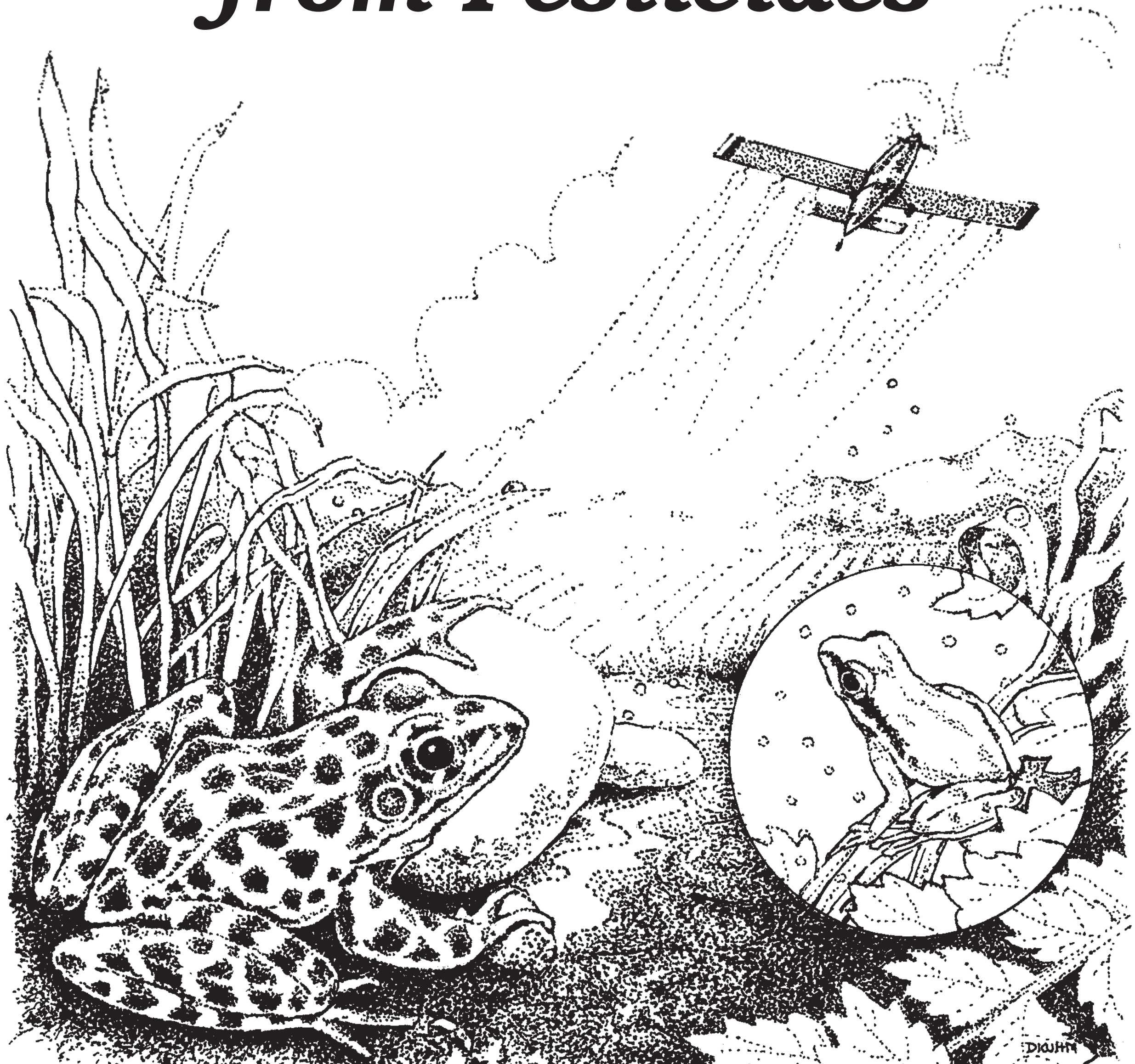
Protecting Amphibians from Pesticides



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B · I · R · C

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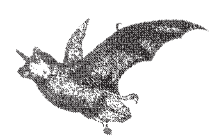

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Pesticides and Amphibian Decline

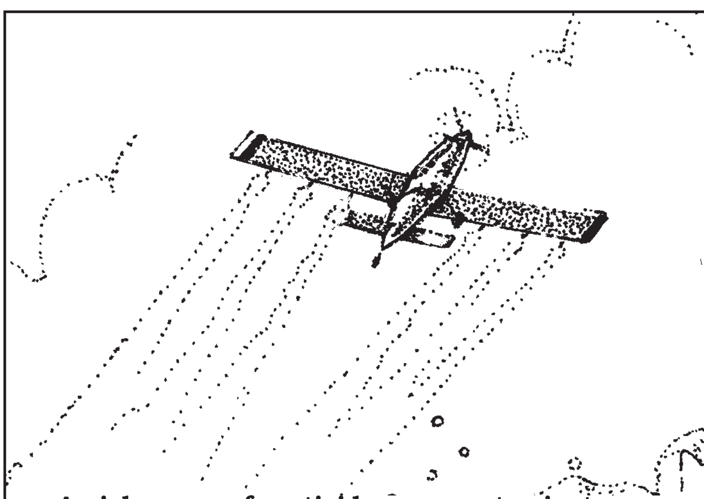
By William Quarles

Since 1970, there has been a widespread worldwide decline of amphibians. The decline has accelerated over the last 20 years. About 32% of species are threatened, 43% are seeing population declines, and many have gone extinct (Stuart et al. 2004). Probable causes include habitat destruction, global warming, disease, pollution, and pesticides. Interactions among causes such as global warming, disease, and pesticide contamination are likely (Hayes et al. 2010; Collins and Crump 2009). This article concentrates on the effects of pesticides. The second article in this publication describes pesticide alternatives that protect amphibians.

Scale of Use

In the U.S. about 1.1 billion lbs (500 million kg) of pesticide active ingredients were used in 2007. This is about 20% of the amount used in the whole world. About 80% is used in agriculture, the rest includes 9% home and garden, and 11% industrial, government, and miscellaneous. About 47% of the total is herbicides, 8% insecticides, and 6% fungicides. The latter figure does not include 181 million lbs (82.3 million kg) of sulfur and oil used as fungicides. Urban use is significant, as pesticides are used in 78 million households (EPA 2011).

Use of herbicides such as glyphosate has exploded with plantings of GMO Roundup Ready corn and soybeans. Application of glyphosate surged from 25 million lbs (11.4 million kg) in 1996 to 180 million lbs (81.8 million kg) in 2007 due to plantings of GMOs. A relatively new problem is associated with GMO soybeans. Roundup Ready soybeans are susceptible to diseases such as soybean rust caused by *Phakopsora pachyrhizi*. Fungicide applications in soybeans, corn, and wheat have increased from 2% to 25% of planted acreage since 2005 (Belden et al. 2010).



Aerial sprays of pesticides can contaminate water and are blown for miles by the wind.

Drawing by Diane Kuhn

Much of the U.S. pesticide use is in California. About 603 million lbs (274 million kg) of pesticide active ingredients were sold in California in 2012, including about 22 million lbs (10 million kg) of glyphosate, and at least 45 million lbs (20.5 million kg) of fungicides, including chlorthalonil, pyraclostrobin, mancozeb, and sulfur. Insecticides included about 2 million lbs (0.9 million kg) of chlorpyrifos and malathion, and more than a million pounds of pyrethroids, mostly bifenthrin and permethrin (DPR 2012).

Pesticides are applied in vast quantities both in agriculture and in urban areas. Amphibians are exposed through direct overspray, pesticide drift, rainfall, and runoff into water bodies. They are exposed to pesticides at all life stages, in the water and on land. Both exposure pathways can lead to population reductions (Todd et al. 2011).

Pesticides are Killing Amphibians

Amphibians are sensitive to most classes of pesticides including insecticides, fungicides, and herbicides. Acute and chronic toxicity kills them outright. Direct oversprays of many pesticides cause 100% mortality. Sublethal effects such as delayed metamorphosis, increased predation, reduced size, reproductive problems, deformities, and depressed immune systems can also lead to destruction (Mann et al. 2009; Wagner et al. 2013; Christin et al. 2013; Hayes et al. 2010).

Exposures tend to be seasonal and surge with each application (Mann et al. 2009). Toxic concentrations of active ingredients are often found in the environment (See Table 1). Formulations can be 400x more toxic than the active ingredient. Pesticide mixtures found in the real world can have additive toxic effects (Relyea 2004; 2009; Hayes et al. 2006).

Sublethal effects are very important. Atrazine at water concentrations of 0.1 parts-per-billion (ppb) is an endocrine disruptor. Concentrations of 100 ppb and more are often found in the environment. Atrazine and other endocrine disruptors interfere with reproduction and can reduce amphibian populations (Hayes et al. 2002; 2003).

Atrazine (21 ppb) and other pesticides can depress amphibian immune systems. For example, Christin et al. (2003; 2004) found that environmentally relevant mixtures of atrazine, metribuzine, endosulfan, lindane, aldicarb, and dieldrin were able to depress the immune systems of juvenile leopard frogs, *R. pipiens*; and African clawed frogs, *Xenopus laevis*. In a laboratory study sublethal doses of DDT (990 ng/g), malathion (990 ng/g) and dieldrin (50 ng/g) were able to depress the immune system of *R. pipiens*. These concentrations have been found in wild frogs (Gilbertson et al. 2003). When *R. pipiens* was fed DDT and dieldrin, tissue concentrations of 2.1 ppb dieldrin and 75 ppb DDT caused immune depression (Albert et al. 2007).

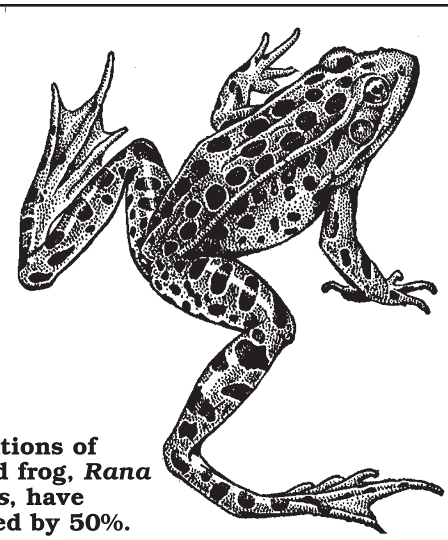
Weak Immune Systems

Weakened immune systems make amphibians more susceptible to ranavirus, trematodes, and possibly chytrid fungus (Christin et al. 2013; Rohr et al. 2008a). Chytrid fungus, *Batrachochytrium dendrobatidis* (Bd), is an especially important factor in amphibian decline (Collins and Crump 2009).

Toxicity depends on the species. We are losing species such as the mountain yellow legged frog, *Rana muscosa*, that is susceptible to pesticides and disease, and are retaining hardier species, such as the Pacific chorus frog, *Pseudacris regilla*. But even hardy species thought to be stable are showing signs of decline. Since 2002, surveys of ponds and other habitats in the U.S. show an average occupancy decrease of about 4% a year (Sparling and Fellers 2009; Adams et al. 2013).

Pesticides also interfere with the normal balance of community ecology. Zooplankton, phytoplankton, predators, and the amphibian food supply can be disturbed. Effects are complex and not well understood (Boone and Semlitsch 2002; Relyea and Hoverman 2008). Pesticides may also be changing the microbial distribution in amphibian habitats and accelerating the evolution of virulent pathogens (Harris et al. 2009; Fisher et al. 2012; McMahon et al. 2012).

Amphibians can be found nearly everywhere. They live in forests, swamps, ditches, meadowlands, suburbia, cities, and agricultural lands (Smyth 1962).



Populations of leopard frog, *Rana pipiens*, have declined by 50%.

Drawing from Stebbins 1954

Pesticides also can be found nearly everywhere, and amphibian exposures can be considerable. Even pristine areas are contaminated (Smalling et al. 2013; Gilliom et al. 2007).

Their highly permeable skin makes dermal absorption of pesticides likely at every exposure. Up to 83% of an applied pesticide can be absorbed through dorsal or ventral skin. Up to 46% can be absorbed through the legs (Bruhl et al. 2011). Glyphosate absorption is 26 times faster in amphibians than mammals (Wagner et al. 2013). Frogs shed their skin about every two weeks and eat it for nutrition. Any pesticide residues left on the skin are probably absorbed (Smyth 1962).

Pesticides are killing amphibians in the water where they breed and develop, in terrestrial areas where adults live, and during overland migrations to aquatic areas (Bruhl et al. 2013).

Exposure to Pesticides in Water

Many freshwater sources are near agricultural lands, and the water is contaminated. The United States Geologic Survey (USGS) has found that 97% of streams in urban and agricultural areas, and 65% of streams in pristine areas are contaminated with pesticides. About 83% of urban streams and 57% of agricultural streams are contaminated with enough pesticide to be hazardous to aquatic life. Up to 10% of these streams are hazardous to human health (Gilliom 2007; Gilliom et al. 2007; USGS 2014ab).

Many frogs lay their eggs in water and develop as tadpoles (see Box A). So eggs and tadpoles can be exposed to pesticides dissolved in water. Also, sediment concentrations of pesticides are at least 1000x greater than water concentrations (Smalling et al. 2013). Tadpoles can be exposed while feeding in contaminated sediments, and adults of species that hibernate in sediments are at increased risk of toxic effects (Smyth 1962; Smalling et al. 2015).

Exposure to Pesticides on Land

Adult and juveniles are exposed on land through aerial sprays for mosquitoes, forestry and agricultural pests, drift, and dermal absorption from soil and plants. Because the EPA does not require amphibian toxicity tests for pesticide registration, there are large data gaps. These data gaps are being closed by independent scientists. One experiment tested label spray rates of 7 pesticides on adults of the common frog species, *Rana temporaria*. Mortality ranged from 40-100%. Perhaps most surprising was the lethal effects of fungicides. Two fungicides caused 100% mortality within one hour, others showed 40-60% mortality. Three products caused 40% mortality after 7 days after 10% label rate exposure (Bruhl et al. 2013). Direct overspraying of terrestrial life stages of several frog species with Roundup at label rates resulted in an average 79% mortality (Relyea 2005a).

Van Meter et al. (2014) tested pesticide absorption from soil with 5 pesticides and 7 adult frog species. Atrazine showed highest absorption and bioaccumulation, although skins were generally more permeable to

Table 1. Environmentally Relevant and Toxic Water Concentrations

Pesticide	Environmentally Relevant Concentration*	Toxic Water Concentrations	Species**	Reference
Chlorpyrifos	0.3 ppb to 4 ppb	0.8 ppb lethal effects	*R. boylii*	Giesy and Solomon 2014; Sparling and Fellers 2009
Chlorpyrifos	161,000 ppb	in sediments	-----------	Sparling and Fellers 2009
Cypermethrin	0.6 ppb	1 ppb adverse effects, 10 ppb 100% mortality	*R. arvalis*	Greulich and Pflugmacher 2003
Endosulfan	10 ppb to 1700 ppb	10 ppb lethal effects	*R. dalmatina*	Lavorato et al. 2013; Smalling et al. 2013
Endosulfan	48,700 ppb	in sediments	-----------	Sparling and Fellers 2009
Propiconazole	74 ppb	74 ppb Stratego***, 40% mortality	*B. cognatus*	Hooser et al. 2012; Belden et al. 2010
Atrazine	100 ppb	0.1 ppb endocrine	*R. pipiens*	Hayes et al. 2003; Rohr and McCoy 2010
Atrazine	100 ppb	21 ppb immune depression	*R. pipiens juveniles*	Bishop et al. 2010, Christin et al. 2004
Pyraclostrobin	150 ppb	15 ppb Headline*** 100% mortality	*B. cognatus*	Hooser et al. 2012; Belden et al. 2010
Chlorthalonil	164 ppb	0.164 ppb 86% mortality	*R. sphenocephala*	McMahon et al. 2012; 2011
Imidacloprid	240 ppb	50 ppb, genetic damage	*R. nigromaculata*	Bonmatin et al. 2015; Gibbons et al. 2015
Imidacloprid	240 ppb	9000 ppb lethal	*Acris crepitans*	Bonmatin; Ade et al. 2010
Glyphosate	2,000 ppb	570 ppb Roundup Weather Max*** 80% lethal	*H. versicolor*	Wagner et al. 2013
Glyphosate	19,000 ppb	in sediments	------------	Howe et al. 2004

*Refers to concentration in surface water unless otherwise specified.
**Refers to tadpoles unless otherwise specified.
***Refers to concentration of formulation.

fipronil. Water solubility and soil partition coefficients were good predictors of dermal absorption. Maximum label rates were applied to soil, and resulting tissue concentrations ranged from 0.019 to 14.6 µg/g (ppm) over an 8 hour period. Immune suppression can occur at tissue concentrations 300-7300 times lower (Gilbertson et al. 2003; Albert et al. 2007).

Fragmentation of Habitat

Amphibians dwelling in terrestrial areas often migrate across agricultural lands toward breeding areas in water and can be exposed dermally to direct pesticide oversprays and by contact with contaminated plants and soil. This “habitat split” between aquatic breeding areas and terrestrial habitat can reduce amphibian species diversity. It can explain why most of amphibian declines are associated with species with aquatic larvae (Rubbo and Kiesecker 2005; Todd et al. 2011; Becker et al. 2007).

Environmentally Relevant Concentrations

Pesticide concentrations detected frequently in the environment are called environmentally relevant amounts. Most often, water concentrations are measured. This article estimates effects on amphibians in the wild by comparing toxic water concentrations found in the laboratory with environmentally relevant amounts found in surface water. This is a very conservative approach. By the time a water measurement is made in the environment, pesticide levels may have dropped, dispersing into amphibian tissues and into sediments. Field studies show that tissue and sediment concentrations can be 2500 to 5000 times higher than water concentrations (Smalling et al. 2013; Smalling et al. 2015).

Most laboratory studies do not measure tissue concentrations. Water concentrations are measured over time periods of 24 to 96 hours. As we see in Table 1, environmentally relevant water concentrations often exceed toxic concentrations found in the laboratory, so amphibians are often exposed to toxic amounts.

Drawing from Stebbins 1954

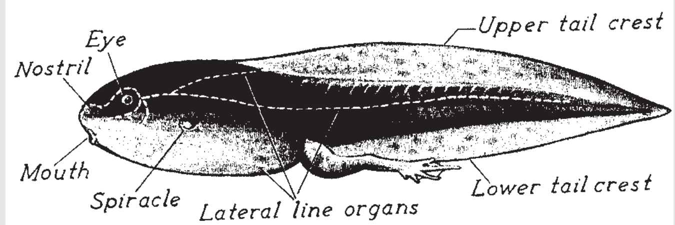
The Great Plains toad, *Bufo cognatus*, is sensitive to fungicides.

Box A. Amphibian Biology

Amphibians include frogs, toads, newts, salamanders, and caecilians. They have been on earth for 350 million years. Amphibians are marked by diversity. Caecilians are wormlike creatures with no legs, salamanders have tails and legs, frogs have legs and generally no tails. There is a great diversity of size. Adult frogs can vary from the 7.7 mm (1/4 in) *Paedophryne amauensis*, to goliath frogs, *Conraua goliath*, more than 35 cm (14.2 in) inches long weighing 3 kg (6.6 lbs) (Attenborough 2008: Smyth 1962).

The Amphibia Class includes the Orders Caudata (salamanders), Salientia (frogs and toads), and Gymnophiona (caecilians). The Salientia Order includes families of tree frogs (Hylidae), aquatic frogs (Ranidae), and toads (Bufoidae). So a toad is one kind of frog. Generally toads have short snouts, dry warty skins and short hind legs good for walking. Skins often contain bioactive chemicals including poisons. Most frogs shed their skins and eat them about every two weeks. Frogs of Ranidae and Hylidae have bulging eyes, moist skin, and large back legs for hopping. Habitat determines frog morphology. Aquatic frogs have webbed feet, tree frogs have round toe pads that help with climbing (Stebbins 1954).

Amphibians live just about everywhere. Forests, meadows, ditches, swamps, and your backyard. Most frogs lay and fertilize their eggs in water. Eggs protected by jelly can be found on top the water, down in sediment, on aquatic plants, or deposited in shallows near the water's edge. Eggs hatch into mostly herbivorous tadpoles that undergo metamorphosis to the adult forms. Metamorphosis can be in days or the tadpole stage can last for years. Adult frogs are carnivorous, and their major food supply is insects, especially mosquitoes.



Eggs hatch into aquatic tadpoles that change into adult frogs.

Drawing from Smyth 1962

Frogs fertilize eggs as they are laid. Salamanders carry fertilized eggs in their bodies, which they then lay in water, usually moving water. They are generally silent and hard to find. Frogs are vocal, especially during mating season. Some frogs are reproductively prolific laying 5,000 to 30,000 eggs, others may lay as few as 5 eggs.

Unlike birds and mammals, amphibians cannot regulate their body temperature. Their bodies equilibrate to ambient temperatures. Adult frogs breathe through their lungs, but can also absorb oxygen through their skin, making it possible to spend long times underwater. In cold weather, they hibernate, burrowing into soil or sediment to escape the effects of cold (Smyth 1962; Attenborough 2008).

Pesticide effects in the environment are complex, and interacting factors such as temperature and biological factors may make environmental concentrations either more or less toxic. To compensate for these factors, pesticides are often studied in mesocosms. Mesocosms are large tanks supplied with tadpoles, zooplankton, phytoplankton, leaves, aquatic plants, and predators. They are meant to simulate environmental exposures (Relyea 2005c).

Looking at pesticides one at a time is also a conservative approach. Mixtures of pesticides are often found in the environment, and these can have at least additive toxic effects (Relyea 2004; 2009; Hayes et al. 2006). [Note: Concentrations in this article are often given in micrograms (µg)/liter or nanograms (ng)/gram. A microgram is one-millionth of a gram, a nanogram is one-billionth of a gram. So, 1 µg/liter and 1 ng/gram both equal 1 part-per-billion (ppb).]

Insecticides Deadly

Low water concentrations (1 ppb) of insecticides can be directly lethal to amphibians (Sparling and Fellers 2009). Amphibian decline was first noticed about 20 years after DDT was introduced. DDT and other organochlorine insecticides were the first pesticides to be used extensively after World War II. DDT use started during the war, but the golden age was during the 1950s and 1960s. DDT was banned in 1972 because of its persistence, bioaccumulation, and toxic effects on wildlife (NPIC 2000). Hordes of amphibians were killed when DDT was applied as an aerial spray for forest insects or mosquitoes (Goodrum et al. 1949). Other organochlorines such as dieldrin, endosulfan, and chlordane are more acutely toxic to amphibians than DDT (Jones et al 2009).

Though most organochlorines have been banned, endosulfan is still in use. Environmentally relevant concentrations of endosulfan can kill amphibians (Sparling and Fellers 2009). Concentrations of 10 µg to 1.7 mg/liter (10-1700 ppb) are often found in surface waters (Lavorato et al. 2013). Concentrations of 10 µg/liter (10 ppb) cause high mortality, decreased growth, and malformations in *R. dalmatina* tadpoles (Smalling et al. 2013). There is a lag effect after exposure. Leopard frogs, *Rana pipiens*, showed no mortality after 4 days, but 97% mortality after 8 days when exposed to 60 µg/liter (60 ppb) (Jones et al. 2009). Residues of organochlorines are found in amphibian tissues, even in pristine locations (Smalling et al. 2013). See Box B for more on insecticides.

Herbicides Lethal

Herbicides such as atrazine and glyphosate can kill amphibians. Atrazine is banned in Europe, but 74-78 million lbs (33.6 to 35.5 million kg) of atrazine were used in 2007 in the U.S. (EPA 2011). Atrazine is not

Box B. Other Insecticides

Organophosphates (OPs) replaced organochlorines and were predominantly used in the 1970s through the 1990s (EPA 2011). Chlorpyrifos, malathion and other OPs are still used in agriculture. Some frog species are more sensitive than others. The chlorpyrifos LC50 of a declining species such as *R. boylii* is 66.5 µg/liter (66.5 ppb). Lethal effects are seen at 0.8 µg/liter (0.8 ppb). Species not declining, such as *P. regilla* are more resistant (365 µg/liter) (Sparling and Fellers 2009). Some of these toxicities were measured over a period of 30 days. For OPs, the longer the exposure, the greater the toxicity. The OPs kill by inhibiting the neuroenzyme acetylcholinesterase, and oxidation products of the OPs are 10 to 100 times more toxic to the enzyme (Sparling and Fellers 2007).

Environmentally relevant amounts of chlorpyrifos vary greatly. In areas near agriculture, water concentrations of 0.01 to 0.3 µg/liter (ppb) are often seen. In surface water, maximum concentrations are 0.33 to 3.96 µg/liter (ppb). Undoubtedly, amphibians are subjected to larger transient pulses (Schuytema and Nebeker 1996; Giesy and Solomon 2014). Chlorpyrifos concentrates in tissues and sediments. Concentrations of 161,000 ppb have been found in sediments of California mountain lakes (Sparling and Fellers 2009).

Pyrethroids

Natural pyrethrins have been used for more than 100 years. Allethrin, the first synthetic pyrethroid to be developed, was introduced in 1949. Other successful first generation products, such as resmethrin, were sold in the 1960s. But permethrin, the first synthetic pyrethroid to see extensive use in agriculture, was commercialized in 1979 (Zalom et al. 2005; NPIC 2009). Use of synthetic pyrethroids accelerated during the 1980s. They are currently major use insecticides in structural pest control, landscapes and agriculture. Pyrethroids used as perimeter sprays in structural pest control contaminate water. Runoff eventually ends up in sediments, causing toxicity to water creatures such as *Hyalella azteca*. Most problematic in California is bifenthrin (Quarles 2012).

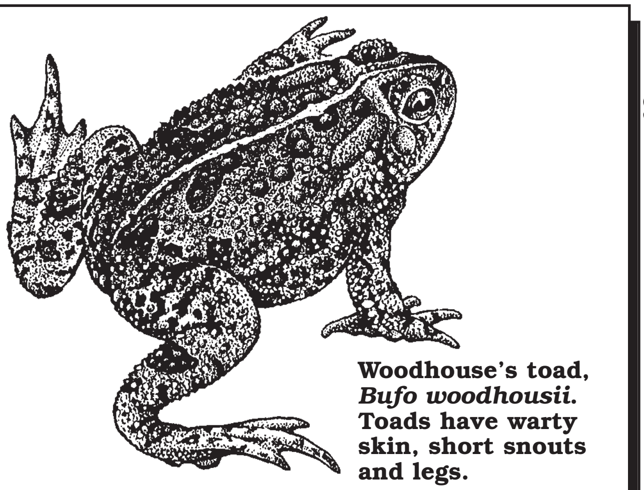
Concentrations of 3 µg/liter (3 ppb) of cis-cypermethrin are lethal within 24 hrs to *R. pipiens* adults. The 48 hr LC50 to *R. temporaria* tadpoles is 6.5 µg/liter, but adverse effects occur at 1 µg/liter (1 ppb), which is near an environmentally relevant concentration. When label rates of alpha-cypermethrin (15 g/ha) were applied to a field, concentration in adjacent ditches were 0.6 µg/liter (0.6 ppb). Concentrations of 10 µg/liter (10 ppb) are 100% lethal to *R. arvalis* tadpoles. Concentrations of 1 µg/liter (1 ppb) decreased hatching rate, lengthened time to metamorphosis, caused deformities and behavioral abnormalities. Concentrations that are sublethal during acute exposure may become lethal during chronic exposure (Gruelich and Pflugmacher 2003).

Neonicotinoids and Fipronil

Fipronil and the neonicotinoids imidacloprid, clothianidin, and thiamethoxam are less acutely toxic to amphibians than organochlorines, organophosphates and pyrethroids. The 96 hr LC50 of fipronil to *X. laevis* tadpoles is 850 µg/liter (Overmeyer et al. 2007). The LC50 of imidacloprid to *P. trisierra* is 194 mg/liter. But imidacloprid is genotoxic at 50 µg/liter (50 ppb) to *R. nigromaculata* (Gibbons et al. 2015). Some species are more sensitive than others. About 77% of cricket frog tadpoles, *Acris crepitans*, were killed by exposures of 9 mg/liter (9000 ppb) imidacloprid, while green frogs, *Rana clamitans*, showed no mortality (Ade et al. 2010).

Imidacloprid depresses the immune system of bees, fish, and probably amphibians (Mason et al. 2012). Exposure to neonicotinoids and fipronil is widespread (Quarles 2014). Neonicotinoids were found in 23% to 75% of water samples in corn and soybean regions. Maximum concentrations ranged from 42 to 257 ng/liter. Neonicotinoid average concentrations of 100-400 ng/liter, frequent detections at 1000 ng/liter (imidacloprid), and maximum concentrations 46-44,000 ng/liter have been found (Hladik et al. 2014).

Environmentally relevant concentrations of imidacloprid show a large variation. Initial concentrations in rice fields are 240 µg/liter (240 ppb). Runoff from concrete in urban situations can be 3,297 µg/liter (Bonmatin et al. 2015).



Woodhouse's toad, *Bufo woodhousii*. Toads have warty skin, short snouts and legs.

Drawing from Stebbins 1954

Pacific tree frog, *Hyla regilla*. Tree frogs have round toe pads to help with climbing.

Drawing from Stebbins 1954

very toxic to adult amphibians, but at concentrations of 0.1 ppb it is an endrocrine disruptor in the leopard frog, *R. pipiens*, in the African clawed frog, *Xenopus laevis*, and probably many other species. Exposure leads to feminization of males and gonadal abnormalities. Atrazine is often found in the environment above these concentrations, especially in areas where corn is produced. Concentrations of 250 µg/liter (250 ppb) have been found in agricultural wetlands, and 57 µg/liter (57 ppb) in ponds. In fact, levels of atrazine up to 0.40 ppb are found in rainwater (Hall et al. 1993; Bishop et al. 2010; Hayes et al. 2002; Hayes 2004; Storrs and Kiesecker 2004). EPA software predictions for current application rates are 100 ppb (Rohr and McCoy 2010).

Atrazine is water soluble, persistent, mobile and can travel 1000 km (600 mi) in rainwater. Feminized males have low fertility, and feminized males have been identified in fields where atrazine is used. So, atrazine can lead to amphibian decline through reduced reproduction success (Hayes et al. 2010). Atrazine at levels seen in drinking water (3 ppb) caused reduced survival of tadpoles of four frog species: spring peepers, *Pseudacris crucifer*; American toads, *B. americanus*; green frogs, *R. clamitans*, and wood frogs, *R. sylvatica* (Storrs and Kiesecker 2004). Atrazine (21 ppb in water) also can depress amphibian immune systems, leading to increased problems with ranavirus, trematode infections, and possibly chytrid fungus (Bishop et al. 2010; Sifkarovski et al. 2014; Rohr et al. 2008a). A meta-analysis of about 125 studies found atrazine exposure led to reduced size, reduced antipredator defenses, depressed immune systems, and hormonal problems (Rohr and McCoy 2010). An industry funded study found possible negative effects of atrazine were not supported by the published data (Solomon et al. 2008).

Roundup and Glyphosate

Application of glyphosate surged from 25 million lbs (11.4 million kg) in 1996 to 180 million lbs (81.8 million kg) in 2007 due to plantings of Roundup Ready GMOs. Glyphosate is an acid, and it is formulated as a salt, so concentrations are often expressed as acid equivalents (ae). Expected environmental concentrations of glyphosate vary between 0.17 mg ae/liter in surface water near soybean fields to 7.6 mg ae/liter with direct overspray of a flooded field (Wagner et al. 2013). Worst case scenarios for surface water range from 1.7 to 5.2 mg ae/liter (Annett et al. 2014). Measured concentrations in ponds range from 0.09 to 1.7 mg ae/liter and in sediments from 0.26 to 19 mg ae/liter (Howe et al. 2004). So, 2 mg ae/liter (2000 ppb) is a reasonable estimate of environmentally relevant amounts of glyphosate and its formulations.

Exposure to 0.57 mg/liter (570 ppb) of Roundup WeatherMAX killed 80% of gray tree frog, *Hyla versicolor* tadpoles. This is well below environmentally possible concentrations. Relyea and Jones (2009) tested Roundup Original Max on 9 tadpole species. They found the 96 hour LC50 ranged from 0.8 to 2 mg ae/liter. Average LC50 of representative formulations for 37 species of tadpoles is 2 mg ae/liter (2000 ppb). Toxic effects increase with pH (Wagner et al. 2013).

Glyphosate formulations are more toxic than glyphosate. The major toxic component of Roundup is polyethoxylated alkylamine (POEA). The LC50 of glyphosate is about 100 mg ae/liter, whereas the 24 hr LC50 of Roundup Original containing POEA to the Pacific chorus frog, *Pseudacris regilla*, is 0.23 mg ae/liter. The Roundup Original formulation is more than 400x more toxic than the active ingredient (Wagner et al. 2013).

POEA formulations at environmentally relevant concentrations were toxic to tadpoles of several common frog species. *R. sylvatica* was the most sensitive. POEA formulations (1.6 mg ae/liter) decreased size and tail length, increased gonadal abnormalities, decreased snout vent length and decrease in rate of development. POEA by itself showed similar toxicity (Howe et al. 2004).

Roundup Oversprays

The Roundup formulation can be extremely toxic with direct oversprays. Over a period of three weeks, direct oversprays at maximum label rates of artificial outdoor ponds (mescosms) resulted in 96-100% mortality of three larval amphibian species, the American toad, *B. americanus*; the leopard frog, *R. pipiens*; and the gray tree frog; *H. versicolor*. Direct overspraying of terrestrial life stages at maximum label rates resulted in an average 79% mortality after one day (Relyea 2005a). Single large applications of Roundup Original Max applied earlier in amphibian development had greater effects than smaller, repeated doses. The formulation tended to stratify near the water surface (Jones et al. 2010).

When Roundup, 2,4-D, malathion, and carbaryl (Sevin) were applied by overspray to outdoor mesocosms at maximum label rates, Roundup caused more mortality to tadpoles than the other pesticides over a two week period (Relyea 2005c). Predatory stress made Roundup more toxic (Relyea 2005b). Increased competition for food made Roundup Original Max more toxic to tadpoles of bullfrogs, *R. catesbeiana*, but there was no competition effect for tadpoles of gray treefrogs, *H. versicolor* or green frogs, *R. clamitans* (Jones et al. 2011).

Toxic effects of glyphosate formulations are hotly contested by industry researchers. They argue that the pesticide dissipates into vegetation, soil and sediment, reducing aquatic toxicity (Relyea 2011). Environmental effects are more pronounced in shallow ponds and ephemeral pools than in larger bodies of water where the pesticide can dissipate (Wagner et al. 2013; Edge et al. 2012). But concentrations in sediments (260-19,000 ppb) may be toxic to hibernating frogs (Smyth 1962; Howe et al. 2004).

Fungicides a Problem

Perhaps due to global warming, fungi are expanding their ranges and becoming more virulent. Fungi normally are more of a problem for plant diseases such as potato late blight. But serious pathogenic fungus attacks on animals are increasing. *Geomyces destructans* has killed about 6 million bats, and the chytrid

Box C. Fungicides Cause Mortality

Fungicides have been found toxic to amphibians in the laboratory and in simulated natural settings. For instance, the fungicide chlorthalonil at environmentally relevant concentrations of 164 µg/liter (164 ppb) killed amphibians (tadpoles of *Osteopilus* sp. and *Rana* sp.), gastropods, zooplankton, algae and a macrophyte in simulated field conditions. Only insects and crayfish showed no significant mortality. Just 1/1000 of the environmentally relevant concentration was 86% lethal to tadpoles of the southern leopard frog, *Rana sphenocepala*. Chlorthalonil depressed immune systems and elevated corticosterone levels (McMahon et al. 2011). About 7-9 million lbs (3.2 to 4.1 million kg) of chlorthalonil was used in the U.S. in 2007 (EPA 2011).

Other experiments have confirmed the extreme toxicity of chlorthalonil to amphibians, and amphibian die offs were documented after application to cranberries in the field. Chlorthalonil is broadspectrum as it inhibits respiration (McMahon et al. 2011; 2012). It is synergistic with insecticides, causing the death of bees (Zhu et al. 2014; Quarles 2011; Quarles 2014).

Fungicides are everywhere, even in pristine environments. They are more prevalent in tropical areas where chytrid fungus and decline are more pronounced (Smalling et al. 2013; Ghose et al. 2014). Chlorthalonil is one of the most frequently used pesticides in Central America, and it is found in mountain areas where major declines are occurring (McMahon et al. 2013a).

Fungicide Toxicity

Environmentally relevant concentrations of pyraclostrobin are 150 µg/liter (150 ppb). Relevant concentrations of trifloxystrobin and propiconazole are 74 µg/liter (74 ppb), and of azoxystrobin, 44 µg/liter (44 ppb) (Hooser et al. 2012). Concentrations of pyraclostrobin (Headline) as low as 15 µg/liter (1/10 label rates) were 100% lethal to *Bufo cognatus* tadpoles. Label rates killed about 65% of adults. Label rates (74 µg/liter) of propiconazole and trifloxystrobin (Stratego) killed about 40% of tadpoles (Belden et al. 2010). There were also chronic effects. Headline at 1.7 µg/liter (1.7 ppb) increased development rates by 5 days (Hartman et al. 2014).

Environmentally relevant concentrations (2 and 11 µg/liter) of the fungicide fenpropiomorph led to smaller body size, delayed metamorphosis, and up to 93% *R. temporaria* tadpole mortality (Teplitsky et al. 2005).

fungus *Batrachochytrium dendrobatidis* (Bd) attacks and kills amphibians. Humans have been attacked by *Cryptococcus gatii* in the northwest U.S. *Aspergillus sydowii* is attacking sea animals, and fish are dying from *Aphanomyces* spp. (Fisher et al. 2012).

There has been a dramatic increase in fungicide use. Fungicides applied on 89 million ha (219.8 million acres) of U.S. corn, soybean and wheat increased from 2% of acreage in 2005 to 25-30% in 2009. Fungicides are being applied preventatively and for emerging diseases such as soybean rust, *Phakopsora pachyrhizi*; and other diseases (Belden et al. 2010). For more on fungicides, see Box C.

Agricultural Pesticides

Amphibians are exposed to pesticides in agricultural situations. Smalling et al. (2015) investigating wetlands near agricultural sites in Iowa found 32 pesticides in the water, 17 in sediments, and 22 in tissues of chorus frogs, *Pseudacris maculata*; and leopard frogs, *Lithobates (Rana) pipiens*. The most common pesticides in the water were the herbicides atrazine, metolachlor, and glyphosate. Average atrazine water concentrations (0.2 µg/liter; 0.2 ppb) were large enough to cause reproductive abnormalities.

Fourteen pesticides were found in sediments, and the most frequent were prometon (herbicide) and metalalaxyl (fungicide). About 17 pesticides were found in frog tissues: 8 fungicides, 4 herbicides, and five insecticides. The most frequently detected were the fungicides fluoxastrobin and pyraclostrobin; the herbicide metolachlor, and the insecticide bifenthrin. Maximum whole frog concentrations were 470 µg/kg (470 ppb) wet weight (Smalling et al. 2015). [Dieldrin is toxic at tissue concentrations of 2-50 ppb. Pyraclostrobin (Headline) is 100% lethal to *Bufo cognatus* tadpoles at water concentrations of 15 ppb (Gilbertson et al. 2003; Albert et al. 2007; Belden et al. 2010).]

Deformed Frogs

There is less biodiversity and fewer amphibians near agricultural sites than in adjacent nonagricultural habitats (Allran and Karasov 2001). Christin et al. (2013) found that adult leopard frogs, *Rana pipiens*, were smaller in size and weight in areas where agricultural chemicals were applied. The frog's immune system was also affected. Other studies have found endocrine effects causing reproductive problems. Leopard frog populations declined by 50% during the 1980s (Christin et al. 2013; Hayes 2004).

Exposure to pesticides in the laboratory causes deformities, and the general consensus is that amphibian deformities in the U.S. are occurring at increasing frequencies (Johnson et al. 2010). Deformities such as extra legs and missing eyes are often found in amphibians exposed to agricultural chemicals (Collins and Crump 2009). Kiesecker (2002) found pesticides depressed immune systems, encouraging trematode infections. Rohr et al. (2008a) found that atrazine depresses amphibian immune systems, making them more susceptible to trematode infection. Trematodes are known to cause deformity. Abnormalities make amphibians more vulnerable to predators, causing population declines (Collins and Crump 2009).

A meta analysis of 48 studies found environmental pollutants including pesticides caused increased mortality, smaller size, and a 535% increase in abnormalities in amphibians (Egea-Serrano et al. 2012). Another meta analysis of 111 studies found pesticides and fertilizers negatively impacted amphibian growth and survival (Baker et al. 2013).

Death in the Mountains

Pesticides can be blown from agricultural areas to pristine locations. Pesticide concentrations in mountain soils of Costa Rica are larger than in lowland areas of the country. Pesticides found such as endosulfan, dacthal, and chlorthalonil, are especially toxic to amphibians. Several amphibian species, such as the golden toad, *Bufo periglenes*; and the harlequin frog, *Atelopus varius*, have gone extinct in these mountain areas (Collins and Crump 2009; Daly et al. 2007).

There has been a 95% population decline of the California frogs *Rana muscosa* and *R. sierrae* in the Sierra Nevada. Other frogs such as *R. boylii* are also in decline. Sites of decline are related to the total amount of pesticide use upwind from the site in California's Central Valley (Davidson 2004).

Amphibian decline in California was first noticed about 1970. Applications of DDT and organochlorines in the Central Valley were blown by the wind up to the Sierra Nevadas, and frogs started to die. Declines continued when organochlorines were replaced by organophosphates. Both of these insecticide classes are extremely toxic to amphibians. Surviving frogs are more resistant to their effects. Chlorpyrifos is about 5x more toxic to the vanishing frog, *Rana boylii* (LC50 66.5 µg/liter), than to the surviving frog, *Pseudacris regilla* (LC50 365 µg/liter). Endosulfan is 28x more toxic to *R. boylii* (LC 50 0.55 µg/liter) than to *P. regilla* (LC50 15.6µg/liter) (Sparling et al. 2001; Sparling and Fellers 2009).

Chlorpyrifos and Endosulfan

Pesticide use in the Central Valley is intense. In 2005, 371 different pesticides were applied in the Sacramento Valley. About 5.9 million kg (13 million lbs) of active ingredients were applied to the San Joaquin Valley in 1995 (Davidson et al. 2012; Sparling et al. 2001).

As a result, at mountain lakes pesticides have been found in the water, sediments, and in frog tissues. Concentrations of chlorpyrifos and endosulfan of 4-12 ng/liter (ppt, parts per trillion) have been found in lakes, chlorpyrifos concentrations of 13 ng/g (13 ppb) and endosulfan 22 ng/g (22 ppb) have been found in *P. regilla* tissues. Chlorpyrifos concentrations of 161 mg/kg (161,000 ppb) and endosulfan concentrations of 48.7 mg/kg (48,700 ppb) have been found in sediments (Sparling and Fellers 2009). According to Sparling and Fellers (2009), "Exposure to chlorpyrifos and endosulfan poses serious risk to amphibians in the Sierra Nevada Mountains."

Amphibian cholinesterase levels are depressed in areas of amphibian decline. About 60-80% of *Hyla (Pseudacris) regilla* populations in affected areas had >50% depression of cholinesterase enzyme compared to 9-17% depression in less affected areas (Sparling et al. 2001).

The greatest *R. muscosa* decline is found in sites closest to the Valley where historical exposure was likely greatest. Most frequent pesticides detected are chlorpyrifos, endosulfan, chlordane, nonachlor, dacthal, and DDE (Bradford et al. 2011).

At one problem site where frogs had disappeared, efforts to restore the population failed because the healthy frogs introduced to the site died. Dying frogs at the site had larger concentrations of pesticides than the same species obtained from other high elevation sites (Fellers et al. 2004).

Pesticide residues have also been discovered in the Cascades where the Cascades frog, *R. cascadae* is in decline. The most frequently detected residues were endosulfan, dacthal, chlorpyrifos, PCB, chlordane, and nonachlor. Chlorpyrifos, dacthal (herbicide), and endosulfan are still in use (Davidson et al. 2012).

Other Pesticides

Smalling et al. (2013) found 9 pesticides, including 4 fungicides, in tissues of the Pacific chorus frog, *P. regilla*, at high elevations in California's Sierra Nevada. The sites were downwind from application points in the Central Valley. The most frequent in tissues were the fungicides pyraclostrobin and tebuconazole and the herbicide simazine. Maximum tissue concentrations ranged from 64 to 363 µg/kg (ppb) wet weight. [Toxic tissue concentrations of dieldrin are 2-50 ppb. Lethal water concentrations of pyraclostrobin (Headline) are 15 µg/liter (15 ppb), and the toxic water concentration of atrazine, a triazine related to simazine is 0.1 ppb (Hayes et al. 2003; Belden et al. 2010; Gilbertson et al. 2003).] Degradation products of DDT were also frequently observed. Six pesticides were also found in water (0.2 to 67 ng/liter), and seven pesticides in sediment with concentrations ranging from 2.5 to 430 µg/kg (ppb) dry weight (Smalling et al. 2013). Tissue concentrations did not correlate with amounts in water or sediment, so bioconcentration may have occurred.

Other factors in Sierra Nevada amphibian declines are fish predation and infection with chytrid fungus (Davidson and Knapp 2007; Fellers et al. 2001).

Emergence of Virulent Chytrid Fungus

The chytrid fungus, *Batrachochytrium dendrobatidis* (Bd), is one of the causes of amphibian decline (Berger et al. 1998; Fisher et al. 2009). It infects keratin on amphibian skin, thickening it and forming sporangia. Sporangia release zoospores into the water that subsequently infect amphibians or crayfish alternate hosts. The fungus may also release toxic chemicals (Rosenblum et al. 2010; McMahon et al. 2013b).

Some species, such as *Rana muscosa*, are extremely susceptible. Laboratory and field infections lead to high mortality. Others, such as the American bullfrog, *Rana catesbeiana*, and the African clawed frog, *Xenopus laevis*, are resistant (Rachowitz et al. 2006). Daszak et al. (2003) see a continuum of effects from infections with no mortality to mass die offs and declines, possibly assisted by cofactors.

One of the mysteries of Bd is its sudden emergence. It was not noticed until 1998, and was not named until 1999. One theory is that it was present all the time, but suddenly became more virulent due to a weakened host or changing environmental conditions that made virulent strains more fit (Rachowitz et al. 2005). Research on archive collections show that the fungus was present on frogs in California in 1928 (Huss et al. 2013). Archive collections from the U.S. and Canada show it was just as prevalent in the 1960s as the 1990s. So it has likely been around awhile. But earlier it was not associated with mortality (Ouellet et al. 2005).

Strains Differ in Virulence

Strains isolated from the environment differ in virulence, and one virulent strain shows signs of genetic recombination (Farrer et al. 2011). So the chytrid fungus is still evolving. Sites in the Sierra Nevadas show presence of several different genotypes (Morgan et al. 2007). Global warming may have encouraged emergence of virulence (Pounds et al. 2006), although it is hard to establish a causal link (Rohr et al. 2008b).

One idea that has not been explored is that pesticides may have encouraged increased virulence of the fungus. Exposure to antibiotics is known to encourage microbial mutations and production of pathogens (Sci. Amer. 2007; Hussein and Bollinger 2005). Exposure of chytrid fungus to agricultural pesticides is likely in both agricultural and pristine areas. Fungicides are everywhere, even in pristine environments. They are more prevalent in tropical areas where chytrid fungus and decline are more pronounced (Ghose et al. 2014).

In the laboratory, pesticides such as atrazine and chlorthalonil reduce infections of chyrtrid fungus on tadpoles of *Osteopilus septentrionalis*, and kill the fungus in culture. Amounts used were environmentally relevant with chlorthalonil (0.017 to 1.7 µg/liter) and atrazine (0.016 to 106 µg/liter) (McMahon et al. 2013a). Glyphosate formulations can also kill the fungus (Hanlon et al. 2012).

When a fungus is exposed to lethal chemicals, this can be a stimulus for production of more resistant and possibly more virulent strains. More virulent strains of pathogens may have a competitive advantage (de Roode et al. 2005). Some fungi have obviously been affected. For instance, *Aspergillus fumigatus*, which infects humans, is resistant to agricultural azole fungicides (Verweij et al. 2007).

Effects of pesticides on community ecology may be a factor. Organophosphates kill water fleas, *Daphne* spp., that feed on the infective zoospheres of Bd (Relyea and Hoverman 2008). Zoospheres can live for 7 weeks, plenty of time for exposure to pesticides and predators (Fisher et al. 2009).

Another idea is that pesticides may have depressed amphibian immune systems, making them more susceptible (Gilbertson et al. 2003; Rohr et al. 2008a; Kiesecker 2011). Skin peptide defenses of *R. boylii* against Bd are depressed by carbaryl (Davidson et al. 2007). Other experiments, using tadpoles, which are not normally killed by the fungus, (McMahon et al. 2013a; Buck et al. 2012; Gaietto et al. 2014), or juveniles of species resistant to the fungus (Paetow et al. 2012; Edge et al. 2013) have not been able to confirm the hypothesis.

Conclusion

Environmentally relevant concentrations of pesticides are killing amphibians. Low concentrations are causing direct mortality, reproductive problems, and increased susceptibility to pathogens. Pesticides are a major cause of amphibian decline.

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Protecting Amphibians from Pesticides

By William Quarles

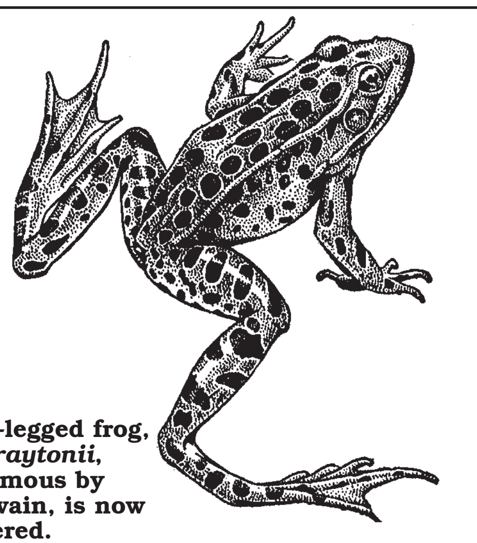
Pesticides, along with habitat destruction, global warming and diseases are causing amphibian decline and destruction (Quarles 2007a; Stuart et al. 2004; Collins and Crump 2009). Amphibians are sensitive to all classes of pesticides including insecticides, fungicides, and herbicides. A review of the literature (see first article) shows that herbicides such as glyphosate and atrazine, insecticides such as endosulfan and chlorpyrifos, and fungicides such as chlorthalonil and pyraclostrobin are likely having an impact.

Glyphosate and atrazine are overused. Together, they represent about 30% of all conventional pesticides applied in the U.S. (EPA 2011). Atrazine has been banned in Europe because it contaminates groundwater. Environmentally relevant water concentrations in the U.S. are 100 ppb; concentrations up to 3 ppb are allowed in drinking water; and it causes hormonal disruption in amphibians at 0.1 ppb (Hayes et al. 2003; Rohr and McCoy 2010). Glyphosate itself may not be causing problems, but Roundup® formulations containing POEA are especially toxic (Wagner et al. 2013). Removing atrazine from the market, and restricting the most toxic formulations of glyphosate could help protect amphibians.

Endosulfan is one of the few organochlorine insecticides left on the market. DDT and most of its relatives have been banned due to toxicity and persistence. Environmentally relevant water concentrations of endosulfan can be lethal. Restricting the use of endosulfan could help protect amphibians. Amounts of chlorpyrifos in surface waters are low, but toxic amounts can accumulate in frog tissues and sediments (Sparling and Fellers 2009). A reduction of chlorpyrifos use could help protect amphibians.

Amphibians could also benefit from fewer applications of chlorthalonil. Chlorthalonil is extremely toxic to amphibians and is also synergistic with other pesticides, contributing to honey bee decline and colony collapse disorder (McMahon et al. 2011; Zhu et al. 2014; Quarles 2011).

Because the EPA does not require amphibian toxicity testing to register a pesticide, there are many data gaps, especially for new products. The EPA should require amphibian testing before pesticide registration, and sensitive species should be used to document the worst case scenarios.



The red-legged frog, *Rana draytonii*, made famous by Mark Twain, is now endangered.

Drawing from Stebbins 1954

How to Protect Amphibians

Most pesticides are used in agriculture. Less pesticide would be used if IPM methods including monitoring, crop rotation, windbreaks, conservation biocontrol, intercropping and other techniques were adopted (Flint and Roberts 1988; Gray 2011; Morandin et al. 2011; Liebman et al. 2008; Long et al. 1998; Quarles 2014). But monocultures of GMOs are firmly entrenched in U.S. agriculture, and they have the support of U.S. government regulators (Quarles 2012a). We can encourage change by voicing our opinions in public discourse and buying organic food.

Insecticides are often used for mosquito control. We can help amphibians by asking our mosquito control districts to use larval control methods with biopesticides such as *Bacillus thuringiensis israelensis* (BTI) (Quarles 2001a). In our backyard gardens, we can treat water with BTI Mosquito Dunks (see Resources). That way, we can maintain water sources for amphibians without breeding mosquitoes. Encouraging frogs has an added benefit of mosquito biocontrol, as mosquitoes are the favorite food of many adult frogs (Smyth 1962).

We can make an impact on the 66 million lbs (30 million kg) of pesticide active ingredients used in the home and garden. By using alternatives to herbicides, fungicides and insecticides in our gardens, we can help amphibian recovery in urban and suburban areas (Quarles 2001bc; 2004ab). (see below)

By carefully choosing plants, and crafting habitat, we can make our gardens wildlife refuges. Not only can we encourage amphibians, we can garden for birds, bees, butterflies and other wildlife. These wild creatures are just as important as plants for garden aesthetics (Quarles 2014; Olkowski et al. 2013).

To save space, many IPM techniques are described here, but details are not given. References, however, have been provided. Many of these publications are available at the BIRC website, www.birc.org.

Backyard Water Garden

We can establish backyard water gardens full of aquatic plants (Fowler 2013). These gardens can provide water for birds, bees, beneficial insects, and amphibians. On a larger scale, we can help restore wetlands in our immediate area. Restoring wetlands can help preserve amphibians, and also birds and other wildlife will benefit. If you want to help, many resources are available, including a publication of the EPA and NOAA called *Wetland Creation, Conservation and Enhancement*. This publication has a list of organizations working on wetland conservation (NOAA 2001).

Herbicides should be avoided. Cultural methods such as hoeing and mulching can help control weeds.

Drawing by Diane Kuhn

Stop Using Herbicides

About 43 million lbs (19.5 million kg) of herbicide active ingredients each year are used in home lawns and gardens (EPA 2011). Most of the herbicides are applied for weeds on lawns. Today there are about 58 million lawns covering 25 million acres (10.1 million ha) (Steinberg 2006).

One way to reduce herbicides is to replace your lawn with native plants and alternative landscaping. This approach is outlined in the BIRC publication *Rethinking the American Lawn* (Quarles 2009c). Lawns can be sheet mulched, and replaced with low maintenance ornamentals. To sheet mulch, mow or knock down vegetation. Add compost and manure, then cover with sheets of cardboard. Then add another layer of compost and organic mulch. Holes are made in the mulch, so that plants can be established (Quarles 2008).

If you need to keep your lawn, you can use organic methods and alternative herbicides that have less of an impact on amphibians (Quarles 2003a; 2009a). Alternative herbicides include soap, corn gluten meal, vinegar, essential oils, iron chelates, and a number of new microbial formulations (Quarles 2010).

In the garden, weeds can be eliminated by changes in landscape design, cultural methods such as mulching, mechanical methods such as hoeing, and alternative herbicides as a last resort (Quarles 2008; Quarles 2003b). In some situations, flaming or thermal weed control may be appropriate. Flaming is most effective for broadleaf annual weeds and is less effective for perennials and grasses. Groundsel, *Senecio vulgaris*; and lambsquarters, *Chenopodium album*, are most susceptible (Quarles 2004b).

Landscape Design

Weeds can be designed out of ornamental beds by proper choice of plants. Groundcovers, trees, and shrubs can be used to shade the ground so weeds will not grow. Newly planted shrub beds can be seeded with fast-growing annuals such as sweet alyssum, *Lobularia maritima*; farewell-to-spring, *Clarkia amoena*; and scarlet flax, *Linum grandiflorum* var. *rubrum* to smother and crowd out weeds. Fast-growing groundcovers can be used along pathways and in areas hard to access and cultivate (Daar 1995).

In landscapes, intelligent use of irrigation water can minimize weeds. Drip irrigation allows water to be delivered only to ornamentals, not to weeds. Pruning and shaping plants to increase their vigor will help reduce weed germination and growth (Elmore 1993).

Flowers can be chosen that suppress weeds. For instance, sunflowers are allelopathic, and root exudates tend to suppress competing weeds. Shrubs such as manzanita, *Arctostaphylos glauca*; *Salvia leucophylla*; *S. apiana*; *S. millifera*, and *Artemisia californica* will suppress annual weeds. Weeds can also be suppressed in new plantings by landscape fabrics or other mulches (see below)(Quarles 2003b).

Native Plants

A good weed control strategy is to remove weeds by cultivation and replant with hardy native species adapted to the site. California alone has 5,000 species of native plants belonging to more than 1100 genera, and other states also have a rich botanical bounty. Native plants are generally very hardy, and hardy plants compete well against aggressive exotic weeds. In California, some lawns have been entirely replaced by plantings of California poppy, *Eschscholzia californica*, and other native plants.

Sheet mulching can be used to eliminate a lawn.

Photo by Geoff Hall, Sentient Landscape

For groundcovers and borders to crowd out invasive weeds, California has 31 native *Clarkia* spp. These free-flowering annuals bloom from late spring and into summer. They can be grown from seed and will thrive in sunny areas. Especially striking is red ribbons Clarkia, *C. concinna*. Other good native annual ground covers are blue lips, *Collinsia grandiflora*; California gilia, *G. achilleaefolia*; tidy tips, *Layia* spp.; and baby blue eyes, *Nemophila menziesii* (Schmidt 1980). Some of these plants also encourage beneficial insects (Quarles and Grossman 2002).

Drought-resistant plants such as native fremontia, *Fremontia californicum*; California lilacs, *Ceanothus* spp.; sages, *Salvia* spp., buckwheats, *Eriogonum* spp.; and manzanitas, *Arctostaphylos* spp. can be planted to crowd out invasive weeds. Difficult areas such as slopes can be covered with coyote bush, *Baccharis pilularis*, a plant that grows quickly with or without water and is considered to be indestructible. This genus secretes substances that suppresses weeds growing around it (Schmidt 1980; Jarvis et al. 1985). Native grasses and forbs have been established on many California roadways, reducing herbicide applications (Quarles 2003c).

California perennials that can be used to combat weeds include one of the earliest spring flowers, grand hound's tongue, *Cynoglossum grande*. One of the 76 species of wild California buckwheat, *Eriogonum* spp. can be combined with other hardy perennials such as yellow yarrow, *Eriophyllum confertiflorum*; coyote mint, *Monardella villosa*; and fragrant sage, *Salvia clevelandii* (Schmidt 1980).

Reduce Insecticide Use

About 14 million lbs (6.4 million kg) of insecticide active ingredients are used in the home and garden market (EPA 2011). Some of these are for insects on lawns (Quarles 2006a). Again lawn replacement will solve the problem (Quarles 2009c). Your garden should have plenty of plant diversity. Variety discourages accumulation of specialist insects that rely one or few plant species. Variety encourages insect generalists that are easier to control (Quarles and Grossman 2002). Using natives and companion plantings can also reduce insect problems (Riotte 1998).

California poppy, *Eschscholzia californica*

Photo by Br. Alfred Brousseau

Tidy tips, *Layia platyglossa*

Photo by Gary Monroe

If you can establish a few resident frogs in your garden, they can help with insect biocontrol. Most of their diet is composed of insects (Smyth 1962).

Beneficial insects can be encouraged by providing insectary plants that provide pollen and nectar. Insectary plants used to conserve beneficial insects include native annual wildflowers such as California poppy, *Eschscholzia californica*; buckwheat, *Eriogonum*; tansy leaf, *Phacelia tanacetifolia*; umbelliferous herbs such as coriander, chervil, and fennel, garden flowers such as sweet alyssum, *Lobularia maritima*; yarrow, *Achillea millefolium*; baby blue eyes, *Nemophila* and tidy tips, *Layia platyglossa* (Quarles and Grossman 2002). Sweet alyssum and phacelia have so much pollen, they are planted in organic lettuce fields to attract syrphid flies for aphid control (Chaney 2007).

Perennials such as California lilac, *Ceanothus* spp.; yarrow, *Achillea millefolium*; coyote bush, *Baccharis pilularis*; and perennial grasses are also good food sources. These plantings have something in bloom all year, so beneficials have a constant food supply (Long et al. 1998).

If cultural methods and biocontrols should fail, biopesticides can be used as a last resort. Common insect problems on ornamentals and in vegetable gardens such as aphids, whiteflies, thrips, and caterpillars can be treated with insecticidal soap, neem and other biopesticides, such as *Chromobacterium* (Grandevo®), *Bacillus thuringiensis* (BT), *Beauveria bassiana*, and *Metarhizium anisopliae* (Quarles 2013). (see Resources)

Perimeter sprays for ant control are a major source of water contamination in urban areas. Sprays of insecticides are applied on foundations and on adjacent soil completely around a structure. We can protect amphibians by switching from pesticide sprays to IPM methods, including sanitation, exclusion, habitat management, and ant baits. Vegetation should be pruned back away from structures, and watering should be minimized. Use of sticky barriers on trees can keep ants away from honeydew food sources produced by pest insects. Ant baits have low toxicity, and unlike pesticide sprays, will not kill beneficial insects or contaminate water (Quarles 2007b; Quarles 2012b).

Resources

Alternative Fungicides

Bacillus subtilis (Serenade®)—**Bayer Crop Science**, PO Box 4913; 8400 Hawthorn Rd., Kansas City, MO 64120; 816/242-2000, Fax 816/242-2659; www.cropscience.bayer.com
Bicarbonate—**BioWorks Inc.,** see below
Horticultural Oil—**Brandt Consolidated,** 2935 South Koke Mill Rd., Springfield, IL 62711; 800/442-9821, 217/547-5800. Fax 217/547-5841; www.brandt.co
Reynoutria (Regalia®)— **Marrone Bio Innovations,** 2121 Second St., Suite B-107, Davis, CA 95618; 877/664-4476, 530/750-2800; www.marronebioinnovations.com
Trichoderma harzianum (Plant Shield®)— **BioWorks Inc.**, 100 Rawson Road, Suite 205, Victor, NY 14564; 800/877-9443, 585/924-4362, Fax 800/903-2377; www.bioworksinc.com
Soap (Concern®)—**Woodstream**, 69 N. Locust St., Lititz, PA 17543-0327; 800/800-1819, 717/626-2125, Fax 717/626-1912; www.woodstreampro.com

Alternative Herbicides

Acetic acid (20% vinegar)(Weed Pharm®)—**Pharm Solutions,** 2023 East Sim's Way #358, Pt. Townsend, WA 98368, 805/927-7500. Fax 805/927-7501; www.pharmsolutions.com
Clove oil (Burn Out®)—**St. Gabriel Organics**, 14044 Litchfield Rd., Orange, VA 22960; 800/801-0061, 540/672-0866, Fax 540/672-0052; www.stgl.us
Clove oil (Matran® II)—**EcoSmart Technologies**, 20 Mansell Court E, Ste 375, Roswell, GA 30076; 877/723-3545, Fax 615/261-7301; www.ecosmart.com
Clove oil and cinnamon oil (Weed Zap®)—**JH Biotech Inc.,** 4951 Olivas Park Drive, Ventura, CA 93003; 800/428-3493; 805/650-8933; Fax 805/650-8942; www.jhbiotech.com
Corn gluten meal—**Gardens Alive**, 5100 Schenley Place, Lawrenceburg, IN 47026; 513/354-1482, Fax 812/537-8660; www.gardensalive.com
d-limonene (GreenMatch®)—see Brandt Consolidated above
Iron Chelate (Iron-X)—Gardens Alive, see above
Phoma macrostoma (Phoma)—**Scotts Company**, 14111 Scottslawn Rd., Marysville, OH 43041; 800/221-1760, 937/642-6402, Fax 937/644-5533; www.scotts.com

Alternative Insecticides

Ant Bait (Terro®)—**Woodstream**, see below
Ant Bait (Maxforce®)—**Bayer ES (Environmental Science)**, 2 TW Alexander Drive, Research Triangle Park, NC 27709; 800/331-2867; www.backedbybayer.com
Ant Bait (Advion®)—**Dupont Professional Products,** CRP Bdlg. 705, Contra Rd., Wilmington, DE 19805; 888/638-7668, www.proproducts.dupont.com
Bacillus thuringiensis (BT)—Gardens Alive, see above
Beauveria (Botanigard®)—**BioWorks Inc.**, 100 Rawson Road, Suite 205, Victor, NY 14564; 800/877-9443, 585/924-4362, Fax 800/903-2377; www.bioworksinc.com
BTI (Mosquito Dunks®)—**Summit Chemical Co.**, 235 S. Kresson St., Baltimore, MD 21224-2616; 800/227-8664, 410/522-0661, Fax 410/522-0833; www.summitchemical.com
Chromobacterium (Grandevo®)—Marrone. see above
Metarhizium (MET-52®)—**Novozymes Biologicals**, 77 Perry Chapel Church Rd., Franklinton, NC 27525; 919/494-3000, Fax 919/494-3450; www.novozymes.com
Neem-(BioNeem®)—**Woodstream,** see below
Soap (Safer®)—**Woodstream**, 69 N. Locust St., Lititz, PA 17543-0327; 800/800-1819, 717/626-2125, Fax 717/626-1912; www.woodstreampro.com

Organizations

Amphibian and Reptile Conservancy (ARC), www.amphibianandreptileconservancy.org
Audubon Society, www.audubon.org
Bay Friendly Gardening, www.bayfriendlycoalition.org
California Native Plant Society, www.cnps.org
Humane Society Backyard Sanctuary Program, www.humanesociety.org
National Wildlife Federation, www.nwf.org
National Reptile and Amphibian Advisory Council (NRAAC), www.nraac.org
National Resources Defense Council, www.nrdc.org
Nature Conservancy, www.nature.org
Save the Frogs, www.savethefrogs.com
Sierra Club, www.sierraclub.org
Society for Conservation Biology, www.conbio.org
Partners in Amphibian and Reptile Conservation (PARC), www.parcplace.org

Reduce Fungicide Use

About 7 million lbs (3.2 million kg) of fungicide active ingredients are used in the home and garden market. Another 19 million lbs (8.6 million kg) are used on commercial landscapes (EPA 2011). Some of the fungicides are used on turfgrass, others are used for ornamental plants. Again, fungicide applications can be reduced by replacing your lawn.

Diseases can be controlled on ornamental plants by having the right plant in the right place, by choosing resistant species, and by following proper cultural methods. The proper amount of water should be used. Trees should be watered at the dripline, not at the trunk. The site should have good drainage, and watering should be done in the morning. Drip irrigation causes fewer problems with diseases. Composts and compost teas will help prevent diseases. Quick release fertilizers that encourage soil pathogens and pollute water should be avoided (Quarles 2001bc; 2005; 2013).

Spraying foliage with dilute solutions of potassium hydrogen phosphate and other materials will cause plants to produce molecular defenses against disease. This is called induced systemic resistance (Quarles 2002).

Diseases can be prevented by induced resistance, and by alternative fungicides such as biorationals and microbials. Sprays of 1% bicarbonate (1 Tbsp/gallon water), or 1% oil (3 Tbsp/gallon water) can protect against powdery mildew. *Bacillus subtilis* (Serenade®) or extract of giant knotweed, *Reynoutria sachalinensis* (Regalia®) can protect against many diseases without killing amphibians. Biorationals such as neem oil


Applications of compost tea can help reduce disease.

Photo Harvard Facilities Maintenance

sprays are also useful (Quarles 2005; Quarles 2002; 2004a; 2009b). (see Resources)

Professional Landscape Maintenance

Techniques that work for the backyard gardener will also work for professionals working on commercial landscapes. Implementation of these techniques in a landscape IPM program can reduce pesticide use by more than 85%(Quarles 2004c).

Most of the pesticides used on landscapes are either herbicides or fungicides (EPA 2011). For those of us who work on landscape design and maintenance, we can help amphibians by choosing plants resistant to disease, and making sure plants in the same area have similar watering and fertilization needs. If possible, turfgrass should be replaced. Possibilities are mowed pathways in native meadowgrasses and wildflowers; mowed, gravel, or flagstone pathways integrated with planting beds of ornamentals, spices, or vegetables (Quarles 2004b; Quarles 2009c).

If removal of turfgrass is not an option, organic methods, proper choice of turfgrass species, and least-toxic herbicides can help protect amphibians (Quarles 2009a; Quarles 2011). Hardy allelopathic turfgrass such as mixtures of tall fescue, *Festuca arundinacea*; and perennial ryegrass, *Lolium perenne*, will help suppress weeds. Do not use potassium rich fertilizers that encourage dandelions. Infrequent, deep watering is best for weed control (Quarles 2005).

Herbicides in planting beds can be avoided by mulching and establishing low growing groundcover (Quarles 2003b; Quarles 2004b; Quarles 2008). Landscape fabrics of permeable plastic can add to weed protection. Organic mulches of bark, sawdust, straw, or rice hulls four inches (10.2 cm) deep are effective for many weeds. Combinations of landscape fabrics and organic mulches provide the best protection. Mulches should also be used around trees to prevent weeds (Quarles 2004b).

Fungicides can be avoided by using resistant species, proper sunlight and watering, and alternative fungicides such as essential oils, giant knotweed extract, microbials such as Serenade® and Plant Shield®. (Quarles 2006b; Quarles 2009b; Quarles 2013).

Use of composts and slow release natural fertilizers will keep waterways free of fertilizer pollution. Composts can be produced onsite from prunings and trimmings. Grass clippings are good fertilizer (Quarles 2004c).

Conclusion

We can protect amphibians by providing them with habitat and by reducing pesticide use. Backyard gardens with resources for amphibians, birds, butterflies, bees and beneficial insects are aesthetically pleasing and can help prevent the relentless slide of amphibians toward extinction.

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