VERMONT
PUBLIC
INTEREST
RESEARCH
GROUP

64 Main Street
Montpelier
Vermont
05602

Tel: 802.223.5221
Fax: 802.223.6855

vpirg@vpirg.org
www.vpirg.org

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THE FATE OF FROGS

A Closer Look at Frog Deformities

A Guide for the Public, Policymakers, News Media and Researchers

By Bryan M. Pfeiffer

What Is VPIRG?

The Vermont Public Interest Research Group is the state's largest environmental and consumer watchdog organization with 20,000 members statewide. Founded in 1972, VPIRG combines research, organizing and advocacy campaigns to ensure policies are made in the public's interest. We rely on the generosity of individuals for two-thirds of our budget. If you find this report helpful and informative, please consider sending a donation to: VPIRG, 64 Main Street, Montpelier, VT 05602, or visit our website at www.vpirg.org. Tax deductible donations may be made to the Vermont Public Interest Research Education Fund at the same address.

VPIRG Staff

Dave Rapaport
Executive Director
drap@vpirg.org

Jenny Carter
Policy Director
(802) 229-2235
jenny@vpirg.org

Chris Donnelly
Development Director
(802) 223-4098
chris@vpirg.org

Sarah O'Brien
Environmental Health Advocate
(802) 223-6383
sarah@vpirg.org

Melinda Herrick
Development Associate
(802) 223-8418
melinda@vpirg.org

Anthony Pollina
Senior Policy Advisor
(802) 223-0765
anthony@vpirg.org

Linda Catling
Major Donor Coordinator
(802) 229-2138
linda@vpirg.org

Les Snow
Administrative Director
(802) 223-5221
les@vpirg.org

Ben Davis
Outreach Director
(802) 223-4097
ben@vpirg.org

About the Author

Bryan Pfeiffer is a veteran journalist, naturalist and former environmental chemist. For 14 years he covered politics, nature, environment and health care policy for two newspapers, the Rutland Herald and The Times Argus, whose combined circulation spans two-thirds of Vermont. Before his career in journalism, he worked as an air quality chemist at an environmental consulting firm in Ann Arbor, Michigan. Mr. Pfeiffer has a B.S. in Chemistry with Honors from the University of Michigan. His essays and articles have appeared in *The New York Times, Field & Stream, The Progressive, Vermont Magazine* and numerous other publications.

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Preface

The din of spring peepers in a cattail marsh, the snore of a leopard frog along a riverbank, the comical clucking of wood frogs in a backyard pond – these are the ancient sounds of places where water mingles with land. Few other creatures, with the obvious exception of birds and crickets, announce their presence so clearly to humans. Even so, a healthy chorus of grunts and trills and peeps doesn't tell an entire story. Amphibian populations worldwide are declining under the pressures of habitat loss, toxic substances and other factors not yet well understood.

But equally disturbing is that frogs across North America are turning up with deformities – extra, missing, partial or malformed limbs or digits; missing, misplaced or abnormal eyes; abnormal bones and organs; or retained tails. It's a grotesque signal that something is wrong in aquatic habitats.

Frog deformities burst into the public awareness in 1995, after students on a field trip in Minnesota found disturbing numbers of abnormal frogs in a farm pond. Soon after, Vermont children playing along the shores of Lake Champlain similarly discovered large numbers of malformed frogs. VPIRG and state researchers quickly went to work to learn the extent of the problem and its causes.

Since then, deformed frogs have captured the attention of the news media, policy makers, scientists and the public. Among the suspected causes are agricultural chemicals and other toxic contaminants, increased levels of ultraviolet radiation, native and introduced predators, and a naturally occurring flatworm called a trematode.

Most recently, a study from California unleashed a rash of news stories suggesting that trematodes were indeed the cause. Many Americans might have felt relief in the notion that frog deformities were a fact of nature. Unfortunately, as this report concludes, far too much research suggests the causes are not entirely natural. Many factors can cause deformities in frogs,

including parasites and aquatic predators such as fish and insects, but the research also implicates agricultural chemicals, including some used around the home.

This report, based largely on peer-reviewed scientific literature, summarizes the current state of knowledge on frog deformities. It draws on expertise from researchers in state and federal governments, universities and private institutions. It is a guide for anyone who wants to know more about what frogs are telling us, and ultimately for anyone who cares about biodiversity and environmental health, including human health.

It is already well established that the use and release of toxic substances and the destruction of wildlife habitats pose tremendous risks and consequences for wildlife, human health and the environment. Frog deformities arise against a backdrop of concern about endocrine disrupters – chemicals, including pesticides and other pollutants, that interfere with the body's own hormones and, by extension, everything from behavior to reproductive success. Because hormones also help guide human and animal development, these chemicals pose the greatest threats in the earliest phases of life – the very phase that deformities begin to appear in frogs.

No one has yet established a link between frog deformities and human disorders. Although the search for causes of deformities continues, there is already enough evidence to warrant urgent action. Amphibians are unwittingly revealing to us a threat so compelling that we must respond with a new, precautionary approach. It is embodied in a doctrine gaining worldwide support called the Precautionary Principle: Where an activity raises threats of harm to the environment or human health, precautionary measures should be taken even if some cause-and-effect relationships are not fully established scientifically. Those precautionary measures might include: advance notification of pesticide use, increased support for farmers moving away from pesticide-intensive agriculture, buffer zones between pesticide use and water, and prohibiting the use of pesticides on school grounds.

Thirty-seven years ago, Rachel Carson, writing *Silent Spring*, concluded that agricultural chemicals, particularly DDT, were responsible for a troubling decline in American songbirds. Before it was banned, DDT nearly wiped out the Bald Eagle, Peregrine Falcon and Osprey by causing females to lay eggs with dangerously thin shells. Told through the song of birds, Carson's seminal work generated an awareness of the hazards we humans perpetrate on wildlife and wild places. Perhaps now, through the sounds of frogs, Carson's legacy can continue.

Dave Rapaport
VPIRG Executive Director

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Executive Summary

By now the images are no longer surprising, even if they remain grotesque and disturbing to look at: frogs with missing or extra legs, missing or misplaced eyes and other strange deformities.

Ever since children in Minnesota and Vermont discovered large numbers of abnormal frogs in the mid 1990s, researchers have scrambled for answers in what has become an alarming environmental issue. Speculation was rampant, with little research to back it up. But in the last two years there has emerged new research, some of it still unpublished, that begins to close in on potential causes. This report, based largely on scientific literature, summarizes new insights into what is known and unknown about frog deformities.

A breakthrough in the search for causes came in April, when the journal *Science* published research demonstrating that a parasitic flatworm, called a trematode, could induce a range of deformities in Pacific treefrogs. Although the study was front-page news, even the principal researcher agrees that the case is hardly closed on the causes of frog deformities.

While any number of factors may be responsible, the research is converging on several potential causes, acting singly or together: parasite infestation and disease, increased levels of predation, ultraviolet radiation and natural or anthropogenic (introduced by humans) chemicals, including pesticides and other toxic contaminants.

Growing Evidence Implicates Chemical Contaminants

- Martin Ouellet, a herpetologist at McGill University who has analyzed more than 40,000 frogs, concludes agricultural chemicals are a likely cause of limb deformities. In habitats exposed to pesticides, Ouellet found rates of frog deformities averaging between 10 and 20 percent versus no more than 1 percent in similar sites not exposed to pesticides. "All my hot spots for deformities are always in the sites subjected to pesticides," says Ouellet, "and nothing in the controls."

- New evidence from Minnesota and Vermont suggests that both naturally occurring and synthetic chemicals are responsible for limb deformities in frogs there. In one experiment, researchers isolated a diverse mixture of chemicals, including several pesticides, from a pond in Minnesota with deformed frogs. Eight compounds induced various eye, mouth, gut, or facial deformities in developing frog embryos after four days. Two induced limb deformities.

- Another suspect is a class of synthetic and natural compounds called retinoids, which are known to cause birth defects. Retinoids are compounds derived from vitamin A. They include retinoic acid, a hormone that at the cellular level regulates the development of vertebrates – from frogs to birds to humans. While plants can produce compounds that mimic retinoids, researchers at the University of California at Irvine are also looking at insecticides and other toxic compounds that may act as retinoid mimics.

- Researchers suspect that one or more chemicals in the frog's environment may act as endocrine disrupters, interfering with the normal developmental processes from egg to tadpole to adult frog. There is already a well-established concern about similar problems in humans – a concern that chemicals in the environment can disrupt normal hormonal function in humans and cause fertility problems, birth defects, developmental disabilities and cancer. Research, supported in part by VPIRG, suggests that some compounds associated with frog deformities have "marked antithyroid activity," and therefore impair normal development.

Parasites and Predators

- The lead researcher establishing a clear link between trematode parasites and frog deformities says the case is hardly closed. "I see our research as a piece of the puzzle – finally we know the cause for some of the limb deformities in amphibians," said Pieter Johnson, a recent graduate of Stanford University. "That other causes are also at work is a given and must remain a priority in light of the potential human health threat. Why amphibian deformity reports are on the rise remains a front-and-center issue. Even if parasites or some other 'natural' agent is the proximate cause for deformities, some other factor, possibly of anthropogenic origin, could be elevating parasitism or reducing tadpoles' resistance to infection."

- Other research, still unpublished, bolsters the case for trematodes, even though they cannot explain all the deformities seen in the field. One proponent of trematodes as a cause, Stanley Sessions of Hartwick College in Oneonta, N.Y., says northern leopard frogs can wipe out evidence of trematode infestation after the limb deformity has occurred, which could explain why some deformed frogs don't contain the flatworms. Sessions also cites unpublished research suggesting that an introduced fish species, as well as predatory insects and even other amphibians can cause a range of leg injuries that appear on the surface to be deformities.

Ultraviolet Radiation

- One theory is that frog deformities may be due to increased levels of harmful ultraviolet radiation striking the earth due to a thinning of the stratospheric ozone layer. Research conducted by the US Environmental Protection Agency found that approximately 50 percent of frogs held under UV light in the laboratory for 24 days developed hind limb malformations.

Conclusions

At its core, the search for the cause or causes of frog deformities is certainly about frogs. But it's unquestionably about human beings as well. Frogs are predators and prey; they're vocal and covert; and they're a symbol of healthy wetlands. But grossly deformed limbs or eyes should also alert us that something has gone terribly wrong with the basic mechanisms of development. For the early events in the life of an embryo – cells signaling one another and arranging themselves to grow into organs and limbs – are similar in frogs and in humans.

It would be premature to conclude that environmental contaminants are causing frog deformities and posing direct threats to humans. But it would be premature – even foolish – to conclude that they are not. The emerging evidence about chemicals and deformities, as well as the nation's history of approving and using substances that harm wildlife, warrants a more careful approach to their approval and a decreasing reliance on their use.

Foremost is the need to adopt a more cautious approach about our use of chemicals. It is embodied in a doctrine known as the Precautionary Principle. Where an activity raises threats of harm to the environment or human health, precautionary measures should be taken even if some cause-and-effect relationships are not fully established scientifically. Accordingly, whenever pesticides or other toxins are introduced into the environment, the least toxic alternatives must be pursued as a first step. In Vermont, a number of policy initiatives modeled on the Precautionary Principle are under consideration in the Legislature:

- Establishment of uniform buffers between pesticide applications and surface waters;
- Elimination of pesticides and other toxic materials from school buildings and grounds; and
- Establishment of a pesticide reporting system to give policy makers and public health officials hard data with which to guide decision-making and research, and to give the public the right to know about toxic materials introduced into their neighborhoods.

William Souder, the author of a forthcoming book on frog deformities, titled "A Plague of Frogs: The Race for a Solution" (Hyperion), put the concern about chemicals this way in a recent commentary:

"What is certain at this point is that development is the most sensitive stage of life. Fleeting exposures to vanishingly small doses of powerful teratogens can have gross consequences for the maturing organism. We don't know if chemicals in the environment are responsible for any of the frog deformities, but the mere possibility is a chilling thought."

Frog Biology 101

Frogs are like People

> *"These foul and loathsome animals are abhorrent because of their cold body, pale color, cartilaginous skeleton, filthy skin, fierce aspect, calculating eye, offensive voice, squalid habitation, and terrible venom; and so their Creator has not exerted his powers to make many of them."*
>
> - Carl Linnaeus

Carl Linnaeus, one of the greatest biologists of the 18th Century, didn't always get it right. And he couldn't be more wrong about frogs. Ever since their ancestors crawled from ancient seas 350 million years ago, amphibians have been lead actors in the long drama of life on earth. The first vertebrates to walk the land, they've managed to survive dinosaurs, meteors, the uplifting of mountains and the rise of the human race. One of their basic features came long before humans showed up with the same trait about 3 million years ago: four limbs.

Yet beside our shared features, frogs are more like people than Linnaeus ever could have imagined. At their cellular level, frogs and *Homo sapiens*, indeed all vertebrates, develop limbs and other organs using similar compounds that signal cells how and when to develop into bones and organs, flesh and blood. Genes don't fulfill their destiny on their own. They require signals from compounds in the developing embryo. These compounds, and the way they work in cells, give frogs and humans a shared history – and perhaps a shared vulnerability. So when frogs turn up with high rates of grotesque abnormalities – extra, missing, partial or malformed limbs or digits; missing, misplaced or abnormal eyes; abnormal bones and organs; or retained tails – it's not only bad news for frogs, it warrants concern about humans and the environment as well.

Their lowly status in Linnaeus' day notwithstanding, frogs have plenty that humans lack, including the ability to assimilate air through lungs and skin alike. That permeable skin allows amphibians essentially to "breathe" the water around them – including its natural and unnatural contaminants. Their intimate contact with water, along with their complex life cycles in sediments, water and land, make frogs sensitive indicators, or "sentinel species" for aquatic environments. When something's wrong with the water, something is wrong with frogs.

Global Amphibian Decline

By now it is clear something has gone terribly wrong with frogs. For one thing, amphibian populations are declining around the world. In some places, entire populations have vanished – even from relatively pristine locations. According to the North American Reporting Center for Amphibian Malformations[1]: Two species of gastric brooding frogs, native to Australia, have not been seen since the early 1980s. The golden toad of Costa Rica was last seen in 1989. The red-legged frog is no longer present in entire counties and valleys on the North American Pacific coast, where it was once quite common.

Widely reported explanations for amphibian mortality or decline, documented on six continents, include[2, 3]:

- Direct habitat loss due to the filling or destruction of wetlands;
- Introduction of non-native predators, usually fish;
- Increased ultraviolet (UV) radiation resulting from atmospheric ozone depletion;
- Global warming and altered weather patterns;
- Disease resulting from a compromised immune system;
- Acid rain; and
- Toxic pollutants, even in trace amounts, including mercury and other metals, agricultural pesticides and fertilizers, industrial chemicals and other compounds.

These factors can act singly or in concert (synergistically). Loss of habitat is the single most effective way to reduce amphibian populations[4]. But also drawing much of the attention from researchers are environmental contaminants. They can interfere with amphibian development and growth in any number of ways[2]: making young vulnerable to pathogenic organisms and disease, retarding growth and development, hampering the ability to avoid predators, impairing reproduction, or directly causing mortality.

In many cases, the actual causes of amphibian decline remain unknown. But the scientific literature readily reveals that many pesticides or their metabolites or other human-made compounds are capable of causing amphibian death in the laboratory or decline in the wild. For example, the use of the pesticide DDT in the forests of Oregon caused mortality in a population of western spotted frogs *(Rana pretiosa)*[5]. The herbicide atrazine also influenced the growth of Tiger salamanders collected from a prairie wetland in North Dakota and raised in the laboratory[6]. And the local extinction of amphibians in parks and wildlife reserves on the north shore of Lake Erie was correlated to the degree of site contamination with chlorinated pesticides[7].

Disturbing Deformities

Also disturbing, however, is the evidence of a surge in frog deformities. It burst into the public awareness after students from New Country School in Le Sueur, Minnesota, on a nature trip in 1995, found grossly deformed frogs in a farm pond near Henderson – frogs with extra limbs, missing limbs and other strange abnormalities. Vermont soon after became known as a hot spot for deformities when children, playing on the shores of Lake Champlain, discovered large numbers of deformed frogs in the fall of 1996.

The findings mobilized researchers and sparked tremendous speculation about causes. Like frogs themselves, members of the news media jumped from one explanation to another. One of the last words on the subject, drawing a huge amount of attention, was that a common and naturally occurring parasite, a flatworm called a trematode, burrows into developing tadpoles and causes their legs to grow abnormally[8].

"I'm totally convinced from the literature survey that trauma, parasites and pesticides are all three able to cause deformities. In no way is there one single cause for deformities. There are many causes, and it depends on where you are working.

Martin Ouellet
Herpetologist
McGill University

The trematode research, published in April of 1999 in the journal *Science*, offered direct, unambiguous evidence that a "natural" cause might be responsible for a range of deformities seen in Pacific tree frogs (*Hyla regilla*) at certain sites in California. As a result, some politicians and members of the public believed the problem was solved, that trematodes were the answer to widespread frog deformities.

Unfortunately, that view is misguided. While trematodes are certainly a factor, they cannot always be blamed for frog deformities. Trematodes did not appear to cause limb deformities in samples of Vermont and Minnesota frogs analyzed specifically for parasites[9,10]. And even where trematodes are clearly a cause, some non-natural factor – perhaps excess nutrients or pollutants – could be favoring parasitism or reducing tadpoles' resistance to infection[35].

The Search for Answers

One thing is certain. No one has solved frog deformities – at least not yet. The malformed frog discoveries in Minnesota sparked a frenzy of new research about causes over the last several years. But now the rush of public speculation has eased somewhat. The scientists have left the glow of the headlines and television cameras, and have returned to the ponds and laboratories to continue the long, tedious process of research. While any number of factors may be responsible for frog deformities, the research is converging on several potential causes, acting singly or together: parasite infestation and disease, increased levels of predation, a depletion of essential minerals in pond water, UV radiation and natural or anthropogenic (introduced by humans) chemicals, including pesticides and other toxic contaminants.

One prominent herpetologist, Martin Ouellet of McGill University, who along with his colleagues has examined more than 40,000 amphibians, has found a strong link to agricultural chemicals[4]. But Ouellet, who has also analyzed some 400 research papers for a chapter he contributed to a book on amphibians, also emphasizes there is no single explanation for deformities. “I’m totally convinced from the literature survey that trauma, parasites and pesticides are all three able to cause deformities,” Ouellet says. “In no way is there one single cause for deformities. There are many causes, and it depends on where you are working. ... It’s very dangerous to generalize about what is causing deformities.”

What We Know
Something's in the Water

> *"I'll risk forty dollars that he can outjump any frog in Calaveras County."*
>
> - Mark Twain
> The Celebrated Jumping Frog of Calaveras County

That synthetic chemicals can cause death, produce genetic mutations, disrupt normal embryonic development and induce congenital deformities (birth defects) is no longer subject to debate. DDT thinned the egg shells of Bald Eagles, Peregrine Falcons and Osprey, driving them to the federal list of endangered species. The drug thalidomide, used as a sleeping pill and to treat morning sickness during pregnancy, caused the births of thousands of deformed babies[11]. Not only did thalidomide severely affect the growth of fetal arms, legs, hands and feet, it also put the fetus at risk for defects to the eye, ear, heart, genitals, kidneys, digestive tract and nervous system.

In many ways, frogs are no different than birds and humans. The scientific literature is replete with experiments on amphibians demonstrating that chemical agents disrupt normal embryonic development. This body of research forms the basis for the field of teratology[12] – the study of the effects that drugs, medications, chemicals and other exposures may have on the developing fetus. Indeed, much of the current knowledge about normal limb development is derived from inducing deformities in laboratory animals, including frogs, by exposing them to chemicals in the laboratory[12,13].

Frogs as Guinea Pigs

Researchers studying developmental abnormalities in the lab use a standardized test known as FETAX, Frog Embryo Teratogenesis Assay – *Xenopus.* FETAX is an established method for measuring the toxicity of chemicals and other substances. In the procedure, embryos of the South African clawed frog (*Xenopus laevis*), the laboratory rat of amphibian toxicology, are exposed to a solution of the test substance for a specific period of time and examined for consequent malformations. The African clawed frog is chosen not only for its versatility in the lab, but because its developmental reaction can be readily measured and extrapolated to other species, including humans[14]. Using FETAX and direct field observation, researchers learned that amphibian embryos were sensitive indicators for environmental contaminants and problems[14]:

- Frog embryos exposed in the laboratory to the herbicide diuron, a weed-killer, exhibited reduced growth and limb deformities[15].
- Tiger salamanders eggs collected from a prairie wetland in North Dakota and exposed to the herbicide atrazine in the laboratory transformed at a smaller size than in the absence of the contaminant; smaller size is associated with lower survival[16].
- The insecticide Abate (44.6% temephos), widely used to control mosquitoes in National Wildlife Refuges, reduced the growth of gray tree frogs (*Hyla versicolor*) when it was sprayed in a pond at the Patuxent Wildlife Research Center in Maryland[17].

- Diazinon and dithane DG caused frog mortality, deformities or growth inhibition in frogs at concentrations of the pesticides seen in the environment[18].

Yet much of the research on frogs and chemicals wasn't about searching for the cause or causes of limb deformities in the wild. It was instead to determine whether frogs were reliable indicators of environmental degradation. As one study[19] that produced deformed frogs in the lab concluded: "The current tests confirm the teratogenic, toxic and bioaccumulative potential of dieldrin in frogs and demonstrate the usefulness of these organisms in assessing potential environmental hazards."

To be sure, deformed amphibians have been found in wetlands long before FETAX and long before Mark Twain canonized one particularly great jumper from Caleveras County in American literature. Amphibian deformities date back in the scientific literature to the late 1700s[20]. Herpetologists (the scientists who study reptiles and amphibians) generally believe the "normal" or "background" rate of deformity is between zero and 2 percent[4,12]. But suddenly, populations are turning up with huge percentages of deformities. Some researchers believe this is a recent, rapid onset – one not explained simply by more people out there looking at more frogs[21,22]. Others disagree completely, including Ouellet, and point to peer-reviewed literature from decades ago, in which scientists reported wetlands with high rates of deformed frogs[4]. Decades ago, herpetologists tended not to work in damaged or altered habitats, so they were less inclined to see deformed frogs.

While there may be no way to compare rates today with rates decades ago, many scientists – not all – believe the rates of deformities are now higher. In any event, deformed frogs have been reported in the last few years from 44 states and four Canadian provinces[23].

The recent surge in reports of limb deformities in the wild turned frogs from guinea pigs to legitimate subjects in their own right. Even so, it's one thing to induce frog deformities in the

laboratory; it's another to determine what exactly causes them in the field. Teams of scientists, working for universities, private institutions and state and federal governments, are now studying limb deformities in the lab to learn what might be causing them in the wild.

New Evidence Implicates Chemical Contaminants

Some of the strongest evidence implicating agricultural chemicals in frog deformities comes from Quebec, where Martin Ouellet of McGill University and his colleagues have since 1992 examined habitats near agricultural lands subject to pesticide applications. In his soon-to-be-published work, which involved more than 40,000 frogs from more than 100 sites in Quebec, Ouellet concludes agriculture chemicals are a likely cause of limb deformities[4]. The link Ouellet found between agriculture sites and deformities is strong. That's not to say other factors – trematodes or predatory fish and aquatic insects, for example – can't cause deformities. Ouellet believes they do. But they can't explain all the deformities.

In habitats exposed to pesticides, Ouellet found rates of frog deformities averaged between 10 and 20 percent versus no more than 1 percent in similar sites not exposed to pesticides (preliminary and unpublished data). Even if predators such as fish and insects are causing deformities, Ouellet says they exist in lower concentrations in wetlands exposed to pesticides – the very locations where deformities are highest. That points to pesticides as a cause.

> ***"All my hot spots for deformities are always in the sites subjected to pesticides, and nothing in the controls."***
>
> Martin Ouellet
> Herpetologist
> McGill University

"All my hot spots for deformities are always in the sites subjected to pesticides," says Ouellet[4], "and nothing in the controls."

The Environmental Protection Agency estimates that 2.2 billion pounds of pesticides were used in the United States in 1995. The term pesticide refers to herbicides, insecticides, fungicides, rodenticides, molluscicides, nematicides, fumigants, and other miscellaneous pesticides. Humans and wildlife have been widely exposed to pesticides from contamination of food and water supplies. Exposure also results from offsite dispersal of pesticides via drift, dust, fog, rain and snow.

Pesticide formulations include active ingredients, and so-called "inerts" – compounds added to enhance application or increase toxicity. The composition of inerts is largely unknown to the public because most are protected as trade secrets. What is known is that most are poisonous in their own right, and many are regulated as hazardous materials.

The growing knowledge about adverse health effects of older pesticides has led to some of them being phased out and replaced with new, more potent compounds. Little is known about the long-term human and environmental health effects of these modern pesticides.

Further clouding the issue of health effects of pesticides is the creation of breakdown metabolites as pesticides degrade. While metabolites are generally not scrutinized, many are highly toxic and long lived in the environment. The persistence of some, such as DDE, a metabolite of DDT, is measured in decades.

'A Mixture of Chemicals'

New evidence from Minnesota and Vermont strongly supports the hypothesis that both naturally occurring and human-introduced chemicals are responsible for limb deformities in frogs there. Researchers working for the states of Minnesota and Vermont as well as the National Institute of Environmental Health Sciences first demonstrated that pond water and sediments collected from various sites of deformities in Minnesota and Vermont had the ability to induce a variety of abnormalities, including deformed limbs, in the South African clawed frog in the lab[24]. Frogs exposed to pond water from Minnesota sites, for example, had deformity rates of 12 percent, compared to 2.3 percent in control sites. The study suggested that unknown substances – not trematodes – in the pond water or sediments were causing the deformities.

©Minnesota Pollution Control Agency

In the next round of experiments[25], researchers reported progress in linking the deformities to a mixture of both naturally occurring and human-made compounds. From water and sediments taken from sites in Minnesota with deformed frogs, the team isolated and identified a diverse mixture of chemicals, including several pesticides. A few of those compounds were subsequently tested using FETAX for their ability to induce deformities. Eight compounds induced various eye, mouth, gut or facial deformities in developing frog embryos after four days: aldoxycarb, diphenylamine, desisopropyl atrazine, propylthiourea, maneb, permethrin, nickel chloride and Bisphenol A. Aldoxycarb, atrazine, maneb and permethrin are pesticides. Propylthiourea and maneb induced limb deformities after 30 days of exposure. Maneb, a fungicide use to control diseases on food crops, is a probable human carcinogen[26].

"Results from these studies suggest that a mixture of chemicals, including naturally occurring compounds, pesticides, and anthropogenic organic compounds, were primarily responsible for the effects observed in *X. laevis* [clawed frog]," the researchers wrote in the journal *Environmental Toxicology and Chemistry*. "Although these studies do not rule out the role of the previously mentioned factors such as parasites and UV radiation, they support the hypothesis that

both naturally occurring and man-made chemicals may contribute to the incidence of malformed frogs in the field in certain locales."

Retinoids

Another suspect in the search for a cause is a class of synthetic and natural compounds called retinoids, which are known to cause birth defects. To understand how retinoids might play a role in frog deformities requires a quick lesson in how retinoids work miracles in vertebrate development.

Retinoids are compounds derived from vitamin A. They include retinoic acid, a hormone that at the cellular level regulates the development of vertebrates – from frogs to birds to humans. Without retinoic acid, a cell's DNA can't direct development from embryo to adult. Rather than working directly with DNA to control development through the expression of genes, retinoic acid interacts with highly specific molecules in cells called retinoic acid receptors. Like a master key that fits different locks, retinoic acid binds with various receptor molecules in cells, unlocking their potential to signal DNA to guide development.

Internally, and in normal quantities, retinoic acid is critical to vertebrate development, including limb development. But exposure to abnormally high or low levels of retinoids, particularly retinoic acid, is known to induce birth defects. The acne cream Accutane, which includes a retinoid compound, causes birth defects. A pregnant women who takes Accutane even for a short time is at great risk of having a baby with severe facial birth defects, malformed thymus glands, and mental retardation[27].

A team of researchers has concluded that only one class of chemicals has been shown in experiments to induce all of the types of limb malformations observed in frogs across North America – the retinoids[12]. They suspect retinoids or retinoid mimics in water or sediments cause limb deformities. Natural plant compounds can mimic retinoids. So can pesticides or their byproducts.

Writing in the *Journal of Experimental Zoology*[12], David M. Gardiner of the University of California at Irvine and David M. Hoppe of the University of Minnesota analyzed the spectrum of deformities in mink frogs (*Rana septentrionalis*) collected from a site in Minnesota. They included missing, truncated or extra limbs, as well as bones that grew in unusual triangular shapes, what the researchers called a "bony triangle." Looking through the published research, Gardiner and Hoppe found evidence that retinoids can induce the formation of these bony triangles in many vertebrates, including frogs, chickens, mice and humans. It strengthened their belief that environmental retinoids have a role in deformities.

"Given that the molecules involved in the developmental signaling pathways are conserved structurally and functionally among all vertebrates," Gardiner and Hoppe wrote, "a retinoid that disrupts the development of mink frogs in Minnesota will likely have adverse effects on the development of other vertebrates, including humans."

From the Lab to the Lily Pad

Retinoids have not been proven to cause frog deformities in the field. One challenge for researchers now is to isolate and identify retinoids or their mimics from wetlands with deformed frogs. One lead is the insecticide methoprene, introduced in the late 1970s and routinely applied to wetlands and other habitats across the United States for mosquito and flea control. A growth

regulator, methoprene mimics a natural juvenile hormone in insects, preventing pupae from molting and developing into the adult stage. It is applied to wetlands when insects are in larval stages – a time that often coincides with early amphibian development. Since methoprene interferes with a hormone only insects have, it was not believed to cause problems in vertebrates. Indeed, the research suggests methoprene causes little mortality or malformations in vertebrates. But methoprene readily decays when it comes in contact with sunlight and microorganisms. And its decay products mimic retinoids and cause deformities in frogs exposed in the lab[28].

Using FETAX to expose African clawed frogs to methoprene in the lab, researchers at California's Scripps Research Institute and Oklahoma State University found that the pesticide at concentrations of 15 parts per million induced little mortality or malformation. But a sample of methoprene exposed to sunlight for a few hours caused malformations at concentrations as low as 7.5 parts per million – a two-fold increase in effective concentration. At this concentration, 18 percent of frogs exposed died and 12 percent were malformed, as compared to 4 percent and zero percent respectively for non-metabolized methoprene. The researchers cited another study[29] showing that methoprene's decay products bind to a retinoic acid receptor in cells by mimicking the atomic structure of natural retinoic acids. The deformities observed in this experiment included reduced embryo lengths, corkscrew-shaped guts and severe eye defects. Since FETAX does not expose the embryos for more than four days, the researchers could not reproduce the kinds of hind limb deformities seen in the wild. At the very least, they concluded that looking at a pesticide may not be enough to assess its hazards, that the decay products can be even more toxic.

"This study provides an example of an insecticide which is rapidly converted to more detrimental materials," the authors wrote in *Environmental Science & Technology.* "In so doing, this result suggests that criteria for analyzing the environmental impact of a chemical should be extended to consider carefully the products of its metabolism and environmental degradation in order to minimize future impact."

The researchers also pointed out that their study did not account for the effects of multiple treatments of the insecticide, improper over-application as well as potential synergism with other

chemicals. Methoprene is added to flea sprays used on pets, they reported, and has a wide range of agriculture applications, including as an additive to cattle feeds, use on grazing areas, certain grains and tobacco.

Endocrine Disruption and Humans

No matter what induces frogs to grow extra or deformed limbs – UV radiation, chemicals or trematodes – many researchers believe the cause or causes disrupt – at the cellular level – the complex mechanisms of embryo development. Researchers suspect that one or more chemicals in the frog's environment may act as endocrine disrupters, interfering with the normal developmental processes from egg to tadpole to adult frog[30].

There is already a well-established concern about similar problems in humans – a concern that chemicals in the environment can disrupt normal hormonal function in humans and cause fertility problems, birth defects, developmental disabilities and cancer[31]. The chemicals are called endocrine disrupters.

Endocrine disrupters are substances that have the potential to cause problems by acting like hormones in human and animal systems. And hormones are critical to life itself. As one writer put it[32]: "In all vertebrate species, hormones act as chemical messengers and as switches, turning on and off bodily systems that control growth, development, learning and behavior. Hormones start affecting every animal shortly after it begins life as a fertilized egg. Hormones control growth and development prior to birth or hatching, and hormones continue to influence behavior throughout life. Hormones tell bears when to hibernate, tell salmon when to return to their spawning grounds, and cause women to menstruate every 28 days or so. Hormones profoundly affect the nervous system, the reproductive system, and the immune system."

Humans are constantly exposed to endocrine disrupting substances – from food, air, water, soil, household products. But they pose the greatest hazards in the earliest stages of life. For a fetus to develop according to its genetic blueprint, the right hormone message has to arrive at the right place in the right amount at the right time. During this phase of life, hormone disrupters – even in minute quantities – can alter or damage these exquisitely sensitive developmental mechanisms. Disturbingly, little research has examined the effects of chemical contaminants at these exceedingly low exposure levels that can occur in the environment or in-utero.

The list of individual chemicals identified as having endocrine-like activity is long. It includes industrial chemicals such as PCBs, as well as a wide variety of pesticides, including herbicides and fungicides. For the most part, these chemicals can accumulate in the environment or in animal tissue and compete with naturally occurring hormones (such as estrogen, testosterone, and thyroxine) for specific binding sites in cells.

Douglas Fort is a veteran and often-published researcher in developmental biology at the Department of Zoology at Oklahoma State University. Fort and his colleagues are pursuing new evidence that certain chemicals induce deformities by disrupting the activity of the thyroid gland. In a new study[33], partly funded by VPIRG, Fort and his colleagues induced deformities in African clawed frogs by exposing them to two sulfonylurea herbicides, nicosulfuron and sulfometuron methyl, both of which are used in agricultural settings for weed and grass control. In this and a previous study, Fort and his colleagues suspected that the chemicals, perhaps acting with others in the pesticide mix, are disrupting the normal functioning of the "thyroid axis" – the complex system of organs and cellular compounds with critical roles in vertebrate development.

The thyroid gland is a key component of the endocrine system in humans and amphibians. It makes and stores hormones that help regulate heart rate, blood pressure, body temperature, and the rate at which food is converted into energy. The hormones also have critical roles in growth and development. Thyroid impairment has profound implications for the development of frogs in the wild. The thyroid axis at least partially controls resorption of the tadpole tail, development of limbs and maturation of the juvenile skin to that of an adult frog[30]. Fort's research for VPIRG found that exposure to the sulfonylureas inhibited a frog's metamorphosis from tadpole to adult, perhaps prolonging the exposure to a number of deleterious effects.

"What you learn about frog development tells you a lot about human development."

David Gardiner
Developmental Biology Center
University of California at Irvine

A frog that doesn't develop properly almost certainly won't survive. It may be less able to escape predators or survive the winter. And Fort suspects improperly developed skin may make a frog more vulnerable to the ill-effects of UV radiation. Maneb and propylthiourea, which induced limb deformities in earlier research, have "marked antithyroid activity" in clawed frogs, Fort reported[25]. The results on thyroid impairment in Minnesota frogs are even more intriguing because of concerns about human exposure to a class of pesticides called thiocarbamates and reports of thyroid cancer rates among humans in Minnesota[25].

Moreover, Fort and his colleagues surmised that antithyroid activity and retinoids may have a joint role in causing the deformities. A "direct biochemical link may exist and warrants further study," they wrote[30].

Gardiner, the researcher investigating whether chemicals in water, sediments or food act as retinoids and induce deformities, says the search for answers involves compounds not only important in frog development but in the development of humans as well. "What you learn about frog development tells you a lot about human development," he says.

Parasites

The latest and biggest splash in the search for causes of deformities came earlier this year from a tiny parasitic flatworm called a trematode. Writing in the April 30 edition of the journal *Science*[9], Pieter Johnson, a recent graduate of Stanford University, induced limb deformities by exposing tadpoles of the Pacific treefrog to various concentrations of a living trematode whose genus is *Ribeiroia*. Of those surviving metamorphosis to frogs, 85 percent had severe limb deformities. None of the surviving tadpoles in a control group (not exposed to the trematode) developed abnormalities. Many researchers, even those pursuing other causes, agreed Johnson's work was extremely important because it established a clear link between trematodes and limb deformities seen in the wild.

Johnson's research was bolstered by an accompanying article in *Science* from Stanley Sessions of Hartwick College in Oneonta, N.Y., who in 1990 induced extra legs and other deformities in amphibians by implanting beads, which mimic the effects of parasite infestation, in their developing limbs[34]. Sessions is an active proponent of the trematode parasite theory, discounting other causes such as UV radiation and chemicals, particularly retinoids, which he says tend to cause bilateral deformities – occurring on each hind limb – contrary to what is often seen in the

field. Sessions says too many researchers are unwilling to accept the likelihood that the cause of some deformities might be predators or natural parasites.

"I think that basic natural history has been neglected, has sort of been pushed to the side to make room for the important environmental problems that we see all around us, many of which are very serious," Session says[9]. "But we push aside natural history at our peril."

Johnson is not ready to declare the case closed on deformities. Recognizing his was one experiment on one frog species, Johnson and his co-authors wrote that their findings call for "further investigation of parasite infection as a cause of amphibian deformities." (Johnson continued his work in Minnesota and western states in the summer of 1999.) The researchers went on to say that if the trematode *Ribeiroia* is a factor in the widespread reports of deformities, it could be due to an increase in populations of snails. Trematodes begin their life cycles as eggs inside pond snails, where they hatch and emerge as swimming organisms, able to find and attach themselves near a tadpole's cloaca, close to where the hind limbs develop. Natural or anthropogenic (human-induced) changes in the environment could cause snail populations to increase, they wrote, adding that organic pollution and the removal of snail predators have both been shown to increase snail abundance and the incidence of parasite infection.

"I see our research as a piece of the puzzle – finally we know the cause for some of the limb deformities in amphibians," Johnson said[35]. "That other causes are also at work is a given and must remain a priority in light of the potential human health threat. Why amphibian deformity reports are on the rise remains a front-and-center issue. Even if parasites or some other 'natural' agent is the proximate cause for deformities, some other factor, possibly of anthropogenic origin, could be elevating parasitism or reducing tadpoles' resistance to infection."

Breaking News

Some members of the news media didn't recognize the subtleties of Johnson's research. Many newspapers and broadcasters practically declared the search for deformities in frogs over, or at least left Americans believing so. "Parasite Deforming Frogs – Studies Say Pollution Isn't Causing Amphibian Abnormalities," ABC News declared on its web site[36]. "Worms Fingered as Root of Frog Deformities," said *Chemical & Engineering News*[37].

One problem is that parasites can't explain all the frog deformities. In 1996, Sessions examined 19 deformed northern leopard frogs (*Rana pipiens*) from four sites in Vermont and found no link between trematode infestation and the deformities[38,9]. In 1997 normal and malformed frogs from five Vermont sites were sent to the federal government's National Wildlife Health Center (NWHC) for detailed examinations. Although the sample size was small, "No association could be found" between trematodes and the malformations, according to Carol Meteyer of NWHC, which is in Madison, Wisconsin[38].

Researchers elsewhere, particularly in Minnesota, have said trematodes do not explain deformities there. The Minnesota Pollution Control Agency (MPCA), working with the National Institute of Environmental Health Sciences, has been active in research into limb deformities. Indeed, Minnesota reacted swiftly to the *Science* articles. After studying hundreds of frogs from Minnesota, parasitologists found no correlation between deformities and parasites[10]. Normal and abnormal frogs alike can be heavily, lightly or not at all infected with trematodes, MPCA said. The MPCA and its research partners reiterated that evidence continues to build that the cause of Minnesota's frog deformities "is most likely one or more chemical contaminants in the water."

"Contrary to some recent news reports, *the case is far from closed*," MPCA said[10].

Sessions, however, is preparing to publish new research of his own – and is reviewing other soon-to-be published work – that enhances the case for trematodes or parasites. "It turns out that trematodes are just custom-designed for doing this kind of thing," he says. "They seem to be adapted to infecting frogs and some frogs have adaptive mechanisms against them." Not only do trematodes cause deformities by mechanically rearranging cells that go on to develop into limbs, he explains, they also secrete a substance that biochemically enhances the growth rate of cells, which helps them grow additional limbs.

As for the deformed frogs found without parasite infections, such as those sampled from Vermont and Minnesota, Sessions offers an explanation: After repeating Johnson's work with northern leopard frogs, Sessions says he found frogs with trematode-induced deformities but no trematodes. Preliminary evidence is that northern leopard frogs have stronger immune responses to the parasites – they can kill them off after the damage is done. "It's like a frog riddled with bullets and someone removed all the bullets," he says.

Predators

Although trematodes can induce truncated or deformed limbs, which are often found in the field[8], Sessions agrees that parasites cannot account for all the deformities seen in the wild. He attributes those cases to predators – fish, insects or even frogs that nip or nibble on developing limbs. Other researchers, including Ouellet of McGill, agree that predators deserve serious consideration as a cause for some – not all – deformities. Sessions says new research he has reviewed suggests that an introduced fish species, a stickleback, attacks tadpoles mercilessly. They're not big enough to kill maturing tadpoles, he says, but instead nip off limbs, which has the potential to explain deformities in the field. Sessions says he has also personally observed (but not published) evidence of bullfrog tadpoles attacking one another and causing limb and eye deformities.

Sessions also says tadpoles at certain stages of limb development can regenerate limbs lost to predators. They lose the ability to do so as they approach metamorphosis into adult frogs. Instead, in response to a lost limb, they regenerate what Sessions called a "cartilaginous spike," which appears to be a truncated or deformed limb.

Sessions says he has an "open mind" on theories other than parasites and predators. "There may be chemicals involved," he says, "but you can bet your bottom dollar that parasites are involved."

UV Radiation

A decade ago, the National Institutes of Health issued a broad warning about the health hazards of ultraviolet radiation. It began: "It is ingrained in humans to love light and, indeed, since mankind's first wanderings from the caves, worship of the sun has been a fundamental tenet that many societies hold even to the present[39]."

It is now well ingrained in humans – and in scientific literature – that sunlight can cause everything from sunburn to skin cancer. One major concern is the well-documented thinning of the earth atmospheric ozone layer, caused in part by chlorofluorocarbons (CFCs), chemicals used in numerous commercial products, including aerosols and refrigerants. It is also evident from the

scientific literature that UV radiation can cause deformities in amphibians. One researcher from the Environmental Protection Agency explained it this way[40]:

- Corresponding with the apparent increase in frog deformities is a clearly documented increase in the intensity of UV light, particularly UV-B (wavelength of 290-330 nm.) reaching the earth's surface.
- Some of the largest increases in UV occur in late spring and early summer, which coincides with reproduction and the critical window of development of amphibian species in northern latitudes.
- UV might explain the random nature of some deformities – in locations ranging from agricultural sites to protected state forests.

To test UV radiation's effects on frogs, including a possible link to methoprene, EPA's National Health and Environmental Effects Research Laboratory exposed northern leopard frog embryos to different concentrations of methoprene, both in the presence and absence of UV light[41].

The highest methoprene concentrations induced "profound and lethal" developmental effects, with and without UV light, with all developing embryos dying within 12 to 16 days. Methoprene at the four lowest concentrations did not induce abnormalities, either in the presence or absence of UV light. But a high percentage, approximately half, of developing tadpoles held under UV light for more than 24 days developed hind limb malformations. They were usually bilateral, which is not common in the field, and included missing limb segments or reduced digits.

Irrespective of methoprene exposure, a high percentage (approximately 50 percent) of frogs held under UV light for 24 days developed hindlimb malformations.

Although the researchers emphasized that much additional work needed to be done to fully assess the significance of their results, they concluded UV light warranted further investigation. And they suspected that frog deformities could not be pinned on any one single factor.

"In considering the fact that a variety of chemical and nonchemical stressors can cause both extra and missing limbs and digits in amphibians, its is perhaps naïve to hypothesize that the broad range of malformation observed in wild anurans can plausibly be attributed to a single environmental stressor," they wrote. "It seems more likely that multiple factors acting via different mechanisms, perhaps including natural causes [trematodes], are responsible for the malformations."

Where To Go From Here
Conclusions

If 10 to 20 percent of the newborns in an American community were developmentally deformed, if even 1 percent were deformed, a hunt for answers would ensue. Soil would be dug and tested. The community's water supply would be scrutinized for the presence of toxic chemicals. Similarly, in the case of deformed frogs, many researchers suspect one cause is chemical contaminants in water or sediments. While it hardly rules out other causes, such as UV light, trematode parasites and predators, the body of research is also pointing at humans.

Wetlands are under assault. We are filling or draining them at an alarming rate. The Canadian herpetologist Martin Ouellet finds deformed frogs in a pond one year, only to return the next to find no pond at all – and no frogs at all. Anyone who cares about amphibians and their role in the web of life, must protect their habitats as well. Yet even when those habitats persist, they can be repositories for toxic chemical pollutants, including pesticides and fertilizers.

To be sure, the research shows trematode parasites and predators can cause limb deformities. And the case for trematodes could get even stronger with new results from Minnesota. But a large body of evidence, as well as the concern and devotion of researchers across the continent looking at other causes, indicates the case is hardly closed on frog deformities. As one researcher put it, "Pesticides are designed to kill things." It is already well established that pesticides and other chemicals in use today can cause cancer, birth defects and other diseases in humans. And the nation's record on pesticides is hardly cause for comfort. DDT, Alar and more recently methyl parathion are some of the widely used pesticides banned after they caused health problems in humans or wildlife.

It is certainly premature to conclude chemicals are causing frog deformities. But it would be premature – even foolish – to conclude that they are not. The emerging evidence about chemicals and deformities, as well as our history with pesticides, warrant a more precautionary approach to their approval and a decreasing reliance on their use.

When something as alarming as frog deformities suggests ongoing harm to the environment or human health, the Precautionary Principle dictates action even if some cause-and-effect relationships are not fully established scientifically. VPIRG believes the potential consequences of inaction are tremendous when compared to the modest costs of more judicious approval and use of pesticides and other toxic substances. A modest beginning includes: advance notification of the public of pesticide use, increased support for farmers moving away from pesticide-intensive agriculture, buffer zones between pesticide use and water, and prohibiting the use of pesticides on school grounds and other public places.

At its core, the search for the cause or causes of frog deformities is certainly about frogs. It is unquestionably about humans as well. Frogs are predators and prey; they're vocal and covert; and they're a symbol of healthy wetlands. Deformed frogs don't live as long as healthy frogs. But grossly deformed limbs or eyes should also alert us that something has gone terribly wrong with the basic mechanisms of amphibian development. And humans have a huge stake in the fate of frogs. For the early events in the life of an embryo – cells signaling one another and arranging themselves to grow into organs and limbs – are similar in frogs and humans alike.

William Souder, the author of a forthcoming book on frog deformities, titled "A Plague of Frogs: The Race for a Solution" (Hyperion), put it this way in a recent commentary[42]

"What is certain at this point is that development is the most sensitive stage of life. Fleeting exposures to vanishingly small doses of powerful teratogens can have gross consequences for the maturing organism. We don't know if chemicals in the environment are responsible for any of the frog deformities, but the mere possibility is a chilling thought."

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