UpClose

ISSUE 11

MICHAEL LYDY

Dr. Michael Lydy, an environmental toxicologist at Southern Illinois University Carbondale, has spent decades uncovering the complex chemical and biological factors affecting pesticide toxicity. In 2014, he launched a three-year study with the U.S. Geological Survey to investigate the bioavailability of pyrethroid insecticides in urban streams and determine whether a crustacean important to environmental monitoring has developed widespread resistance to these chemicals.

How did you first become interested in insecticides?

My training as a doctoral student at Ohio State—my dissertation—was based on that class of compounds. It's actually kind of interesting. My advisor, Susan Fisher, worked with Robert Metcalf, who was at the University of Illinois. He was a famous insect toxicologist. There was a family of graduate students who studied under Dr. Metcalf and then started their own labs in other places. So, I guess I am a grandchild of that group. My whole career has been working with insecticides.

Much of your work has focused on pyrethroid insecticides. Why that class?

If you look at the history of pesticides, and insecticides in particular, you'll see that we are constantly looking for new classes of compounds to handle pests. We started with organochlorine insecticides like DDT and chlordane. DDT is probably the most famous. After using them for quite a while, we found that these have great insecticide activity. But they can also have detrimental effects. The one everyone is probably familiar with is the thinning of bald eagle eggshells, which led to a decline in the bald eagle population. Rachel Carson wrote about that in *Silent Spring,* and this helped start the environmental movement.

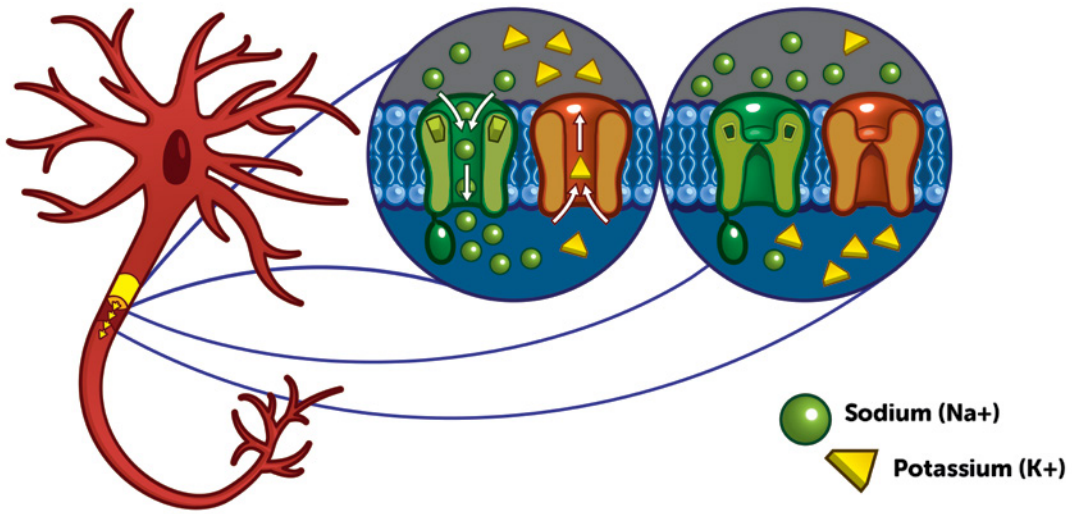
Organochlorines were phased out, and we went to a series of insecticides called organophosphates—like parathion. There were also carbamate insecticides. Carbaryl is probably the most commonly used carbamate. People use that on their flowers and in their gardens all the time. Those are problematic because they have a fair amount of innate mammalian toxicity. They have been phased out to a large extent.

Pyrethroids are the third class, and, in theory, they should be more target specific and safer. They are used as flea, tick, and lice deterrents in pet shampoos. You can also apply them to your lawn to get rid of unwanted insects in your yard. They're applied in agricultural settings too. There are a lot of positive components. The challenge with these compounds is that they are fairly long lived—not as long as organochlorines, which last for decades, but they can have half-lives over a year long.

The study of pyrethroids is quite fascinating. We have looked at the relative toxicity of these compounds to insects and crustaceans—particularly *Hyalella azteca*, which are a major food source for fish and waterfowl. What we and others have found is that pyrethroids are very toxic to *Hyalella*. The LC_{50}, which is the concentration that kills 50 percent of the population, is around 1 part per trillion for some pyrethroids. That's a lot of zeros. It's like taking a grain of salt, throwing it into an Olympic-size swimming pool, and killing most of the *Hyalella* in the pool. They're incredibly toxic. Imagine a homeowner applying fertilizer—which often have pyrethroids added to it because they kill insects—in their yard. If even a few granules of that fertilizer-pyrethroid mix enter the aquatic system, it could have devastating effects on *Hyalella* and other non-target species. And if you've ever applied fertilizer, you know it's easy to end up with granules on your driveway because you use a spreader. You may also clean out the spreader when you are done, and there are probably granules left in that.

Don Weston, my collaborator at University of California, Berkeley who is recently retired, and I, as well as the U.S. Geological Survey (USGS), have also run studies

THE SPARK OF LIFE
ACTION POTENTIALS



Neurons communicate by generating electrical signals known as action potentials or nerve impulses. A voltage-gated sodium channel embedded in the cell membrane opens, allowing sodium to rush in and alter the polarity of the membrane. Seconds later, the membrane returns to its normal state when potassium ions in the cell escape through a nearby voltage-gated potassium channel. The process then repeats like a wave down the axon.

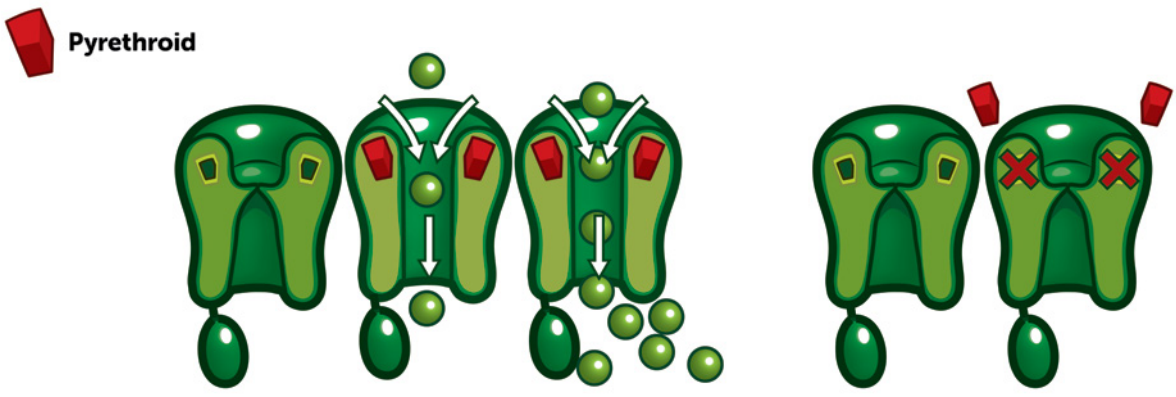
throughout the U.S. looking at pyrethroids in urban areas. We often think of pesticides as an agriculture problem, but what we've found is that pyrethroids and other insecticides are not necessarily an agriculture problem. We see more elevated concentrations and more mixtures in urban settings due in large part to commercial applicators applying a lot of these pyrethroids and homeowners wanting perfectly manicured lawns. That is especially the case in California. You have houses stacked right on top of one another, so you can imagine that a creek that runs through those areas is going to be highly impacted.

California creeks were the focus of a study you did in 2013. What was the goal of that project?

In that study, we were focused on sites where we had already found elevated concentrations of pyrethroids in sediment. We had been doing surveys throughout the state looking at concentrations in both urban and agricultural areas. We found *Hyalella* at some of the sites with elevated concentrations of pyrethroids, but we knew that *Hyalella* should not be surviving where there is a mixture of elevated pyrethroids. So our collaborators Helen Poynton and Gary Wellborn and other geneticists started looking at the sodium channel on the nerves of these *Hyalella*.

A normal nerve impulse—an action potential—is created by sodium ions flowing into a nerve cell and potassium ions flowing out. There are sites on the cell membrane known as voltage-gated sodium and potassium channels that open and close along the membrane in a wave pattern. Pyrethroids bind to the sodium channel by attaching to the receptor site that opens the gate like a lock and key and holding the gate open, which results in the constant firing of the nerve. If enough nerves are firing, the animal dies.

ATTACK AND RESPONSE
GENETIC RESISTANCE TO PYRETHROIDS



Pyrethroids kill animals by attaching to a receptor site on the voltage-gated sodium channel and holding the gate open, resulting in constant nerve firing. In some animals, including populations of *Hyalella azteca*, genetic mutations have changed the shape of the site enough that pyrethroids can't latch on.

What we found is that there is a single point mutation that changes the shape of the receptor site. When the pyrethroid tries to come in, it doesn't fit. With that single point mutation, we now have resistance to pyrethroids. We saw a 500-fold decrease in the sensitivity of mutated *Hyalella*. Animals that should be dying very rapidly in a mixture of pyrethroids are now surviving perfectly well.

What's interesting about the project is that the development of mutations in target insects is fairly common. It happens in mosquitos, bed bugs, and other insects exposed to these compounds all the time, and it's one of the reasons we had to change from organochlorines to organophosphates to pyrethroids. In fact, the same point mutations we see in *Hyalella* have been found in insects like mosquitos. That's why we specifically looked for these mutations in *Hyalella*. The concern here is that we have a terrestrially applied compound that is causing mutations in an aquatic non-target species. I don't know of any other studies that have found pyrethroid resistance in aquatic species. And it's kind of scary because we are not applying these to aquatic systems. We apply them to terrestrial systems, but they are not staying there.

Hyalella azteca is actually a species complex instead of an individual species. What does that mean?

I struggled with that a little bit also. The old definition of species was a group of individuals that could interbreed and have viable offspring. I'm not a geneticist, but I have learned from talking with them that that definition may no longer be true. It's more involved than that. It involves the genetics of the animals.

My understanding is that there is mitochondrial DNA that they use as a marker to tell the difference between clades of *Hyalella azteca*. If those DNA are different, they belong to different clades. But if I gave you *Hyalella* from two different clades, you wouldn't be able to tell them apart. You would need a well-trained taxonomist to properly identify the differences.

Hyalella azteca

But I don't know of anyone who has actually tried to breed the different clades to see if we go back to the original definition of a species—although I have an undergrad, Haleigh Sever, who will look at that question this semester. Do they have viable offspring? I have non-resistant *Hyalella* downstairs from a lab culture that has been around for over 13 years that is part of one clade. I also have a population of the wild-caught mutant population from a different clade. That one has been in the lab for 18 months and is doing perfectly well. We're going to go ahead and interbreed those to see if they have offspring, if those offspring are viable, and if they are also resistant to pyrethroids. Which one out-competes the other if they have offspring?

Do we know enough about pyrethroid resistance to predict what will happen?

It's still really early. The manufacturers of pyrethroids have done a study at the same site as ours where they took a wild-caught population from the same location and let it sit in the lab for a month or two. They say the resistance went away. They say the *Hyalella* are really resistant to start with but that it's not an inheritable trait—that is, it's one that is lost over time. We've kept a population for almost two years, and they haven't lost their resistance yet. So, our data at least suggests that the mutation stays—at least if it's not selected against.

Are resistant *Hyalella* never affected by pyrethroids, or do they just affect them differently?

Resistance in this case means pyrethroids can't affect the voltage-gated sodium channel any longer. But there are different modes of action. Pyrethroids could still affect *Hyalella* via narcosis, which is a general effect. They aren't designed to have this impact, but narcosis is something that any compound could cause. PAHs [polycyclic aromatic hydrocarbons], PCBs [polychlorinated biphenyl]—they can all have a narcosis-type effect. It's the default mechanism. Another probable mode of action is oxidative stress.

What is driving pyrethroid resistance in *Hyalella*?

Exposure. *Hyalella* are in a bath of pyrethroids that should kill them. But when there is a point mutation at the right spot, that individual lives. And if there are enough with the mutation that they can breed, the population can maintain itself.

In the case of *Hyalella*, there is actually more than one mutation site. There are a couple different locations along the genome where base substitutions would cause these point mutations. One is L925I, and the other is M918L. Some animals have both mutations. Some only have one or the other. And even though the genome for *Hyalella* has been sequenced, it's not well understood yet. There may be other factors, other parts of the genome, that are involved beyond the point mutation that could cause the differences we see in sensitivity. It's too early to know.

Also, pyrethroids are biotransformed by lots of animals. When an animal—or for that matter a person—is exposed to a contaminant, their system reacts. In terms of pyrethroids, the animal's P450 system [enzymes involved in the metabolism of toxic compounds] kicks in and tries to make the compound more polar. Or esterases [enzymes that break chemical bonds] try to break the compound into pieces, therefore making it less toxic. The problem is that a lot of times the animal gets overwhelmed—especially if they are incredibly

sensitive to the contaminant via its mode of action—to the point that their metabolism can't handle it.

You sampled one of your sites twice in a roughly three-year period. Why return to that location?

The development of resistance is most likely not something that happens overnight. Going back to the same site multiple times allows us to determine things like the abundance of *Hyalella*, whether they are still resistant, and whether there is a change in the number of alleles being affected. We don't know much about this, so the more we can learn the better. And a temporal look at the site allows us to see what's actually happening in the system and whether the genetics and relative resistance are changing.

You tested juveniles the first time and smaller adults the second. Could that have played a role in the declining sensitivity to pyrethroids?

I wouldn't think so. A juvenile will, in most cases, be more susceptible to a chemical than an older animal, but a very old individual will be as well. Age, sex—all these things come into play. There is some uncertainty associated with toxicity tests—as there is in anything. But to see a more than 500-fold difference—that is well outside the range of standard deviation.

Does one mutation create greater pyrethroid resistance than another?

No. It doesn't appear that L925I or M918L creates more resistance. And remember that not all pyrethroids are the same. It's a class of insecticides. Bifenthrin is incredibly toxic to *Hyalella* at 1 part per trillion. But permethrin, which is used more in agricultural settings, may be 100 times less toxic. There is a range of toxicity, so we can't group them all together.

How do these mutations affect *Hyalella* health?

I have a Master's student who has already addressed this to some extent. Her name is Jennifer Heim, and she's looking to see if these populations are susceptible to other contaminant classes.

Resistance could cause what's called a population bottleneck. We start with a fairly large population of *Hyalella*, but there's a selection for the resistant individuals when pyrethroids are introduced. The population could drop from 20,000 to four or five. And, in theory, the remaining population will be more susceptible to other stressors because there's less genetic diversity.

Could there also be larger food web impacts?

Yes. We have a larger study that's almost done where we are looking at the ability of these resistant populations to accumulate more pyrethroids. Pyrethroids are hydrophobic, so as long as they are not biotransformed, they will bioaccumulate in the animal. If that animal is eaten by a fish or duck, the pyrethroids would, in theory, move up the food chain.

Do we know how widespread pyrethroid resistance is?

This study was done in California, but this is not just a California story. We have also found resistance in some locations in the Midwest. And we've just started a study with USGS where we will be looking at 70 sites in the Northeast.

What impact could resistance in *Hyalella* have on toxicity research and biomonitoring programs?

There are a couple potential ramifications. One is that USGS or U.S. Environmental Protection Agency biomonitoring personnel could go to sites and think, "There's *Hyalella*, which is usually a pretty intolerant species, so the water is pretty good." But, in reality, the water quality is not very good. We've created a mutated animal that's doing well in that system, but that doesn't mean the system is clean. That could be a big problem. I think we have to figure out how prevalent the resistance is first. Right now, biomonitoring folks just need to be aware that this could be an issue. Biomonitoring is often coupled with sediment chemical analysis, and that should clue them in.

Another ramification is—as a toxicologist, I have populations that have been cultured in my lab for years. My current *Hyalella* lab population has been in culture for 13 years. And every once in awhile, toxicologists like to go out to the field to collect new animals to increase the genetic diversity of their populations. If you chose the wrong population to bring back to your lab, you could bring in a resistant population and intermingle it with your animals. My undergrad will be able to tell us whether that population will win out and leave you with a resistant population. That would impact all your future toxicity tests if it happened. We don't know if it will yet, but it could have ramifications on how we do toxicity tests.

Also, this is just *Hyalella*. We don't know if other species are having similar issues with resistance. There are midges and other animals typically used as toxicological species that we haven't looked at. Maybe this is the tip of the iceberg. We don't know. USGS will be looking at midges and mussels as part of our project together and linking it to our pyrethroid analysis. That is a good way to start the expansion into other species. I would imagine midges would have some susceptibility. They are more tolerant than *Hyalella*, but pyrethroids still have a toxic effect on them. Mussels—I am not sure. There is a lot less work done on bivalves in general, but that is one of USGS's fortes.

Could differences between *Hyalella* clades also impact toxicity research?

There may be some differences that would have ramifications long term. But, again, what is the difference between clades? Even the geneticists I've talked to can't really describe what that means. You are getting outside my realm of expertise, but the concept of a toxicity test in the bigger scope of toxicology is that you are looking at the same species test-to-test and that each lab is working with the same species. It appears that most of the commercial, federal, and university labs, including ours, received their *Hyalella* from the same original group, and therefore the clade is the same. Most of the testing is probably the same, but I don't think we have gotten far enough along to know what the potential ramifications would be if there were different clades.

But, as we talked about earlier, there are differences in toxicity when you work with a six-day-old vs. a 15-day-old vs. an adult. You have to always keep in mind what you are testing and the length of the test. There are a lot of variables going on here. I see a toxicity test as a kind of barometer of relative toxicity, but you

always include confidence intervals with those tests. Even if you are running the same test in the same lab, there is variability.

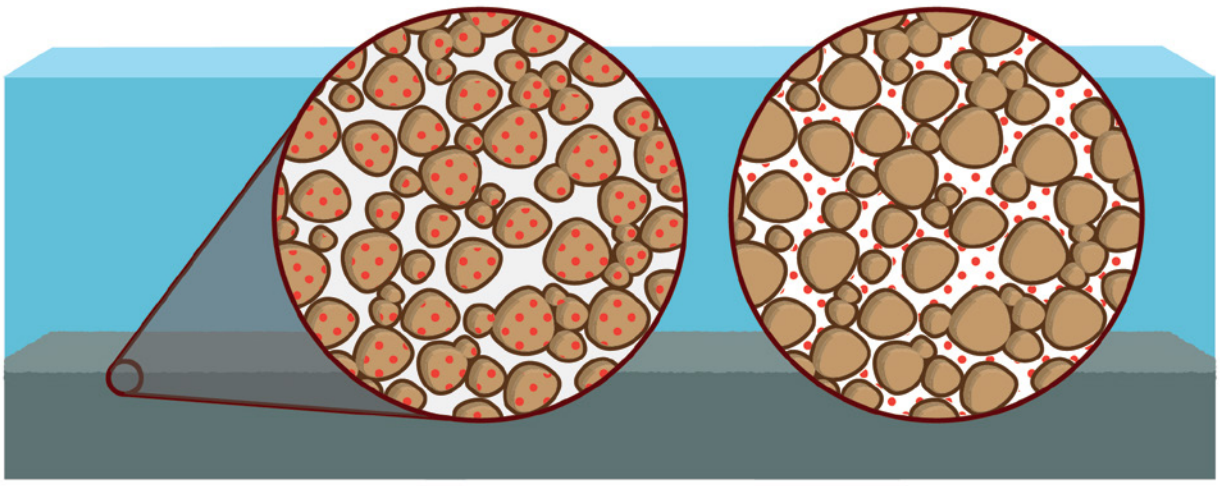
Is there a potential problem long term? Maybe. *Hyalella* might not be the best aquatic lab species in the long term because it is a species complex. But until we get a better idea of the relative differences in susceptibility among the non-resistant clades, I don't think we can answer that.

Your study with USGS is focused on determining the bioavailability of pyrethroids. What is bioavailability?

Bioavailability deals with the fraction of a compound that is available for uptake by an animal. In most cases, the compound has to be in the freely dissolved phase. Pyrethroids are hydrophobic, so they are going to bind to the organic fraction of sediment particles. They bind fairly tightly. The compound has to come off to be bioavailable—desorb off the sediment particle and into the pore water, which is the water between sediment particles. As long as the compound is bound to the sediment, it is not bioavailable. It's only when it gets into the pore water that it can be taken up by the animal and have toxic effects. *Hyalella* have gills, so they can respire and get pyrethroids into their blood stream that way. They can also go in through their cuticle [a multi-layered structure that forms an exoskeleton].

In this study, we are really trying to understand the adsorption/desorption process for how pyrethroids bind to sediment. Compounds that desorb into the pore water can also be taken up by Tenax, which is what we will be using in our study. Tenax can act as a surrogate for the animal.

PRESENCE VS. EXPOSURE BIOAVAILABILITY



Pyrethroids and other hydrophobic compounds pose little threat to aquatic wildlife as long as they remain bound to sediment particles. It's only when they desorb off the particles that the bioavailable concentrations can be taken up by animals.

What is Tenax?

It's a 2.6-diphenylene oxide resin. It basically binds freely dissolved organic contaminants. Then the compounds can be desorbed off Tenax either thermally or chemically, which is what we are doing.

The system is simple. You have a test tube filled with water, sediment, and Tenax. The tube is put on a rotator and spun so everything contacts one another, so we don't have to worry about diffusion rates. At the end of 24 hours, whatever was freely dissolved in the water is now attached to the Tenax. In theory, that is the bioavailable fraction, or the bioavailable fraction related to the tissue concentration in the animal. We—especially Federico Sinche, who works with PCBs and Tenax—have done enough studies to know that there is a good correlation between the 24-hour Tenax concentration and bioaccumulation in several aquatic invertebrate species.

The purpose of Tenax is to be used as a predictive tool. Running a bioassay can take 10-28 days. They are very long tests for accumulation or toxicity. But if I can take this Tenax, spin it for 24 hours, do a fairly simple chemical extraction of the material, run it on a gas chromatograph mass spectrometer, and get a value, I can go along the regression curve and predict the concentration. I don't even have to use animals in my test. That is the overall goal. With enough data points, species, sediment types, and toxic endpoints, we can predict and bypass the animal portion of this completely—at least at the screening level. This would be especially helpful at a Superfund site where they are doing remediation. They physically remove contaminated sediment from a site, and they want to know how much of the PCBs still present at the site are bioavailable. I can answer that in 24 hours and then a day's worth of mass spec time. If they do a bioassay, we're talking a month minimum.

This is part of a larger USGS study, correct?

Yes. Our project is coupled with USGS's National Water-Quality Assessment Program. Our study is an add-on to their larger project, but it's an important one since we are directly examining bioavailability. They are going to look at a larger number of sampling locations than the 70 sites we will study. They're doing water chemistry, biomonitoring, habitat analysis, stream characterization, and more.

The folks at the USGS lab in Columbia, Missouri are going to do toxicity tests on their own, and we will also do similar tests. Theirs will be longer-term chronic tests. Ours will be acute. Then we will do our Tenax, and they'll do chemical analysis.

Where are you in the process now?

We just started year one. The first step is to figure out what sampling sites to use on the East Coast. USGS is doing most of that, but we are helping them to make sure we're targeting the best type of site for this project.

What characteristics are you looking for?

Urban areas with rows of houses and green lawns set close together or golf courses. We are interested in the streams running through those areas.

What's the next step?

I actually have some new staff coming onto the project. Kara Huff Hartz is a chemist. She will help manage the project and is currently training on how to use the Tenax and conduct the bioassays. These first six months will be making sure that everyone is up to speed. When you are working with a federal agency, there are lots of rules and regulations on how to process sediments. Do you screen them? Do you sieve them to certain amounts? Do we do it in the field? Do we do it when they're shipped? How long can they sit before they get shipped? How long do they sit before you use them? You can imagine that when you have a lot of labs—there might be 10 labs getting sediment for different purposes—logistics are an issue. So, this first six months are logistics and personnel training.

USGS will send the collected sediments in July of 2016 probably, and that's when we'll do the Tenax. We'll also conduct chemical analysis of the sediments. We'll run organic carbon tests, which, again, is the part of the sediment where pyrethroids bind, so it's important to normalize for organic carbon when total extractable concentrations are needed. And we will conduct 10-day toxicity bioassays to see how many *Hyalella* in each sediment sample die.

When they send us sediments, we get a code number, but I have no idea where those are from. I know it is from the East Coast, but I have no idea where the sites are. It's better that way because there's no potential bias because it is just a number to us. We run the bioassays completely blind. And obviously there is a lot of quality assurance. We'll run lots of blanks [samples used to confirm equipment is free of contamination] and matrix spikes [samples with known pyrethroid concentrations used to determine the bias of a testing method] for each of these tests. It's a lot of work.

How will you know it was the pyrethroids that killed the *Hyalella*?

That's another component of this study. There are lots of compounds in urban sites that could kill *Hyalella*: pyrethroids, PAHs, organochlorines, maybe even ammonia. What we do is run focused TIEs—toxic identification evaluations. These are tests that let us exclude other classes. Don Weston and I developed the focused TIEs for pyrethroids.

Pyrethroids have a negative correlation with temperature, which is quite unique. In most cases, the toxicity of a compound increases if you increase the temperature because you get increased respiration, which means there is more chemical uptake. The animal dies because they get more of the compound in them—along with a few other reasons. Because pyrethroids have a negative relationship with temperature, they become more toxic as you decrease the temperature. This happens for several reasons, but it has to do with the sensitivity of the nerve. So, if you simply do a sediment test where you decrease the temperature, you should see an increase in toxicity if pyrethroids are the main cause of the toxicity in that system.

There is also another focused TIE parameter that we can use: piperonyl butoxide. It's a P450 inhibitor. As we discussed earlier, P450s can break down

pyrethroids. But if I am blocking the biotransformation, I should see increased toxicity because the compound is not being broken down to the less toxic metabolites.

So, there is a series of these tests that will also be done at the end of year one and in year two to determine whether there's enough evidence from the focused TIEs to say that it's probably pyrethroids killing the *Hyalella*. Other compounds are most likely present in the sediments. The focused TIE procedure will tell us that pyrethroids are there in high enough concentrations that it appears they are the ones causing the toxicity.

Is toxicity measured with Tenax as well?

Tenax measures the bioavailable fraction of pyrethroids in a system, and this study is looking at the relationship between freely dissolved pyrethroids and their acute and chronic toxicity. If a good relationship is found between the Tenax concentrations and toxicity, Tenax could be used to predict toxicity in the future.

I always liken *Hyalella* to a canary in a coal mine. If you bring the canary down and it dies, you better get out of the mine shaft. It's the same concept here. The animals are dying, and we're trying to figure out why. The Tenax measurement tells us whether there is enough pyrethroids available in the system to kill them, and the focused TIEs tell us it is probably the pyrethroids that are causing that, not the other chemical classes.

The other thing in year two is to go back to locations where USGS biomonitoring indicated there were *Hyalella* and our sediment bioavailability tests indicate via the Tenax that there are bioavailable pyrethroids at the site. We know pyrethroids are very toxic to *Hyalella*. If USGS finds them at a site and we find pyrethroids there, that population may be resistant. We will send people to those sites, collect *Hyalella*, and test them at elevated pyrethroid concentrations. If they survive, we will do some genetic testing to determine if there is a mutation and what the mutation is.

What about this study is most exciting to you?

I've worked with pyrethroids with Don Weston and others for about 15 years, but finding resistance has really spurred on some interesting thoughts. Resistance is very troubling to me as an environmentally concerned person. But as a scientist, it's a fascinating environmental problem. I like to learn. If you don't like to learn, I don't understand why you would be in academia or in science in general.

I worked with USGS as a hydrologist back in the early 1990s. Many of the folks involved in this are people I knew a long time ago. It's fun to get back working with the folks I worked with then.

Of the projects we have had in the last five or six years, this is probably one of the ones I'm most excited about.

Who in your lab is working on this?

There will be two undergrads, Haleigh Sever and Andrew Derby, working on the project. Federico Sinche is a PhD student from Ecuador who will be the main graduate student working on this. He works with Tenax on PCBs in Superfund sites. And Kara Huff Hartz is an associate scientist, and she will be leading the mainstay portion of this in the lab.

I have been in academia for a long time. The life of the lab is grad and undergraduate students. I have even had high school students from Carbondale High School work in the lab. Without them, I don't think we would function. They are the hands and the engine by which the lab runs.

I think that different students—different levels of students—get different things out of the project. Someone like Federico—this is a deviation from his project with PCBs. This will help him learn how to handle a different chemical class. Pyrethroids have functional groups that PCBs don't have, so they are much more challenging to work with. And his study is almost exclusively looking at bioaccumulation, whereas these are toxicity tests. They're totally different types of studies. His background is in nanoparticle work, so he is coming in from a totally different field. I think this will be a great learning experience. I also have a hierarchy in the lab where graduate students help train undergrads. He wants to be a professor, and he will be able to train some of the undergrads as part of this.

Was it important to you to bring in undergraduate students?

I think undergrads get something different from this. They come in wide-eyed and bushy-tailed with questions: What is a hypothesis? What is science? What do I want to do when I grow up? I have two sophomores working in my lab right now who are really talented, but they're sophomores. It's really challenging for them to see the big picture because they haven't been here long enough.

My goal for the students is to put a fire under them and make them toxicologists. People don't come in as sophomores and say, "I want to be a toxicologist." However, if I cannot convert them to be lifelong toxicologists, at a minimum they will be well trained to do science and think like scientists.

I always take applications this time of year for high school students and undergrads to come and work in my lab. I will get 10-15 really high-quality applications, some from high school students. I try to promote that as much as possible. I promote science. That is why I am here. I am here as an educator. I see that as my most important role.

COVER PHOTO
U.S. Geological Survey

Illinois Indiana Sea Grant
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IISG-16-008