UpClose

with Rebecca Klaper

Dr. Rebecca Klaper, an ecologist at the University of Wisconsin-Milwaukee (UWM), is searching for practical solutions for the design, use, and management of pharmaceuticals and other emerging contaminants found in the environment. As the director of the UWM Great Lakes Genomic Center, much of her work investigates the impact these chemicals have on gene expression in fish and other aquatic species. This approach, along with traditional toxicology techniques, shines a light on the different ways emerging contaminants impact species growth, development, and reproduction. Together with her research team, Dr. Klaper has also conducted studies to uncover location and temporal patterns of pharmaceutical pollution and to better understand the combined toxicity of chemical mixtures found in Lake Michigan.

IISG sat down with Dr. Klaper to talk in detail about what happens to pharmaceuticals when they enter waterways and the risks they may pose individually and in combination.

How did you become interested in pharmaceutical pollution?

When I was in the last years of my graduate work, I saw an article by the US Geological Survey. They had done a survey looking for pharmaceuticals and emerging contaminants in streams across the United States. It was something I had never even thought about. It was fascinating, and it kind of sat in the back of my mind as I was going through the rest of my training.

I was trained as an environmental ecologist and started out looking at the impacts of natural plant products on invertebrates and insects—how those products influenced the life cycles of these organisms. I went from there to a postdoc where I studied similar things as well as the impacts of those products on the genetics of organism populations. And from there I went to the Environmental Protection Agency (EPA) and did a fellowship with the risk assessment group. It was an American Association for the Advancement of Science fellowship. So, before I was doing very theoretical ecology, but I started thinking about ways to transfer that into something that was more applied, something that could help solve real-world problems. It was kind of a natural fit to go from natural products and their effects on organisms to man-made products and their effects on organisms. When I joined the faculty at the University of Wisconsin-Milwaukee, I ended up shifting my focus and decided to tackle emerging contaminants as a group.

Are effects on population genetics and life cycles still the focus of your work?

That is definitely a focal point. I am interested in the effects of different emerging contaminants, ranging from pharmaceuticals and personal care products to nanomaterials, which started to become a big focus while I was at the EPA. I look at how those impact survival and reproduction. I have also implemented behavior studies to see how they impact the behavior of freshwater organisms. One of the techniques that I use is gene expression. The gene expression of an organism can tell you what is going on in an organism in response to a potential toxin.

How does gene expression help us understand toxic effects?

All of our cells have our genetic code represented. Our genes are in every single sell. But not all of our genes are expressed at any one time. There are some that sit there just kind of waiting for something to happen. Basically, certain genes get "turned on" when your body and your cells need to do certain things. And your liver has different things that turn on than your lungs because they are doing different processes. So, that pattern of gene expression tells you something about the function of what is going on in those cells and in that tissue.

When you come in contact with a toxin, your gene expression pattern changes because your body is trying to deal with that toxin in some way. The toxin might turn on genes that code for proteins that either process the toxin, bind it up, or break it down to get rid of it. By that gene expression pattern, we can tell what is going on in the organism. It also gives an indication of whether the organism is getting sick or reproduction is going down. And, if you are looking at genes associated with neurobiology, it gives you an indication of whether there might be some behavioral changes down the road.

Will an individual pharmaceutical turn on the same genes in different species?

The great thing about this is that some of the ways organisms deal with toxins are very similar across all vertebrates, for instance. So if you are talking about a fish or a human—some of those pathways are very similar. The code might be just a little bit different, so you need to figure out what that code is. But the overall pathway is very similar. So, actually, fish can tell us a lot about how humans might be impacted by the same chemical. They can kind of be a sentinel for human health in that way.

Could that make it easier to manage these contaminants to protect human health?

It does help. The National Institutes of Health and the EPA regularly use these other organisms as sentinels for what might happen in humans. People are probably more familiar with rat or mice studies, but we are also using fish to look at the effects of toxins on development. There are a bunch of pathways that are really similar between fish and humans, believe it or not, when we are developing. If something is disrupting fish development, it is probably also disrupting human embryo development.

There are more and more of these species being listed as models for different pathways. We use fruit flies, which seems like something so incredibly different from us. There are some genomic pathways and genetic defects in fruit flies that tell us something about our own biology. It is really exciting that we can learn so much from other organisms.

How close are we to being able to use insights from other organisms to manage specific pharmaceuticals in the environment?

There are still a lot of holes in our knowledge. There are some chemicals that act similarly in fish and humans, let's say. And some alarm bells are going off with things like plastics and some of the endocrine disrupting chemicals that bind estrogen receptors pretty similarly across organisms. We have an indication that at very low levels in the environment those are something we need to be concerned about. That is why you see more monitoring programs and science going on in that area. And it was partially a siren call by some of the scientists in that area that pushed that.

There are lots of different pathways that could be disrupted by a chemical. We have much less information about chemical influences on human development. A mom who is exposed to a particular chemical that ends up going to the fetus—how much does that really influence the developing baby? Our immune systems are becoming a bigger topic now too. We

know that it is more than just being allergic to something. Your immune system can be very responsive to chemical exposure, but it is much less studied than some of the other pathways. So, we still have lots of big gaps, but there are some chemicals where alarm bells are going off, and we know we want to do something.

What are some of the other pathways pharmaceuticals can disrupt?

There are developmental pathways, which deal with how an organism develops over time. Chemicals can affect the neurobiology of an organism. Some chemicals can also be immunotoxic, meaning that they can shut down the immune system or overstimulate it to the point where your body is not performing like it should—it is not recognizing pathogens, or it is recognizing your body as a potential harm and starts attacking tissues. Things can also be cancer causing, which people generally think of when they think of chemicals.

It is really hard to measure all those different things for all those chemicals. So one of the efforts right now is focused on developing a set of screening tests to determine which chemicals are most dangerous.

What groups are behind that effort?

There is an EPA project called the Computational Toxicology Initiative. What they are doing is using different cell lines and some other very basic tests to try to screen large amounts of chemicals that have been in the marketplace for a while but we just don't have enough information on. What they do is called a tiered testing approach. They start with the cell lines, and if they hear some alarm bells going off, they will move into a bigger test, like an organism test or a lifecycle test, which are more expensive and harder to do and actually use animals. They are trying to get away from animal testing and just do some simplified assays.

One of the problems with that, though, is that you can miss some of the impacts. Those tests tend to be very short-term assays and on a cell that has been taken away from the organism. Cells act very differently when they are inside the organism. A lot of these chemicals don't have an acute toxicity. They don't necessarily cause an effect in the short-term. Where you see an effect is over the life cycle of an organism, over a long period of time. One of the things we are finding, which is a little frightening, is that a person can be exposed to a chemical but you see the effects not necessarily in their generation but in their children's generation or their grandchildren's generation. That makes it very hard to monitor what exactly caused that change. But that is the kind of research that is going on in laboratories around the world right now—how to test for what we call epigenetic effects. What changes in the offspring's genome might happen due to what happened to the parents?

Some of the work you have done focuses on how pharmaceutical concentrations in Lake Michigan vary by location and time. Why is it important to understand those patterns?

A lot of the data so far from sewage treatment plants indicates that there is a lot of variation that happens, even in a 24-hour period, within a sewage treatment plant. You can image why that might happen. People wake up, they take their medications, and then, as you go through the day, your body evacuates that medication. Concentrations also spike in the morning when everyone wakes up and goes to the bathroom. So, the concentration of these chemicals goes up and down through time. And then we also see changes over seasons. People tend to use more antibiotics in the spring or winter when they are catching colds and are inside all the time. You see a lot of antihistamines then too. There can be a seasonal or even hourly

aspect to what is in the effluent. We did a study over several different time points to capture some of this variability.

And, actually, one of the things that we also know about sewage treatment plants is that their operation is different depending on the water flow. In the Milwaukee area, we have a combined sewage system, so the rainwater goes into our storm sewers but ends up in our sewage treatment plant. There are lots of things that get washed off the street—like bacteria and chemicals—that the treatment plant can get rid of before the water hits the lake. Overall this is a good thing, but it changes the flow of the wastewater treatment plant. You get an increase in flow. There is also a lot of leakage. We have really old infrastructure all over the country. Wastewater treatment pipes end up getting a lot of rain water infiltrating, which ends up changing the flow regime of the treatment plant. In some cases this is actually overwhelming our treatment plants. And this increased flow can change the chemical breakdown process within the sewage treatment plant.

As for location patterns, one of the reasons we wanted to study different points within the lake is because, of course, water comes out of a pipe, but then it moves. Measuring just right near the wastewater outfall doesn't provide a good indication of what happens to those chemicals once they hit the lake. They could end up binding to little particles and settling out. They could breakdown because now they are coming into contact with UV light and microorganism within the water. The thought in the past has been that things will breakdown really quickly and just go away. And I should say that we did a companion study to this that was a sewage treatment plant study. We measured the chemicals in the sewage treatment plant 24 hours before we took samples from the lake, so we had kind of an idea of what had been evacuated during the previous 24 hours and an estimate of what we might find coming out of the sewage treatment plant for each date. Then we sampled in Lake Michigan starting at the outfall and moving out. We measured a certain distance out into the lake and used the prevailing wind and water patterns to decide where we were going to sample. We basically picked as many sites as we could given the funding that we had received from the Milwaukee Metropolitan Sewage District. They really wanted to know what they were contributing to the lake, which is really forward thinking.

So, we had this spatial pattern along with the time pattern to see what kind of variability there was. Now, of course, we would like to do more. We would like to go out into the middle of the lake and see what we find. You know, we really weren't planning on detecting anything 3 kilometers away from the shore, especially when that wasn't the prevailing way that the water was traveling. The question is, why are we finding things way out there?

Do you have a hypothesis?

Not a specific one other than the fact that these things are probably not breaking down as fast as we thought they were. Even the ones that we thought would bind with little sediment particles and fall out are probably not doing that. They are moving around to some extent, either because the particles are moving around or the water is carrying these chemicals farther out.

Does that mean that dilution does less than we thought to mitigate these pollutants?

Definitely in the nearshore areas. Like I said, we would like to do a study where we go even farther out into the lake and farther away from any of the outfalls, in areas where there isn't a lot of input, to see if we are still seeing measurable concentrations.

What constitutes the nearshore areas?

Nearshore is just what it sounds like. It is where the land meets the water. Some people define nearshore differently. We were measuring several kilometers out. That is still fairly nearshore, but it is far enough away that we wouldn't have expected to see the concentrations that we did.

In your study, you tested for pharmaceuticals in both water and sediment. Why both?

Chemicals differ on their water solubility. Part of the thought is that some of these chemicals will bind to sediment particles because they are not very water soluble, so they naturally go to something that we call lipophilic. They want to go to something that is more fatty or oily or has a different chemical property than water. So they preferentially bind to some of the sediment particles. The thought is then that they would fall out and sink into the sediment at the bottom of the lake.

There are some chemicals that we knew from a preliminary study we had done that go out of the outfall and just disappear. The question is, where does it go? Does it completely breakdown and that is why we are not seeing it? Or is it sitting in the sediment at the bottom of the lake, which could still have an impact? Doing a water study is really important to find out what is in the water where fish are swimming around, but there are all sorts of critters that are living in the sediments that are really important for the lake ecosystem and, in fact, the fish eat them. The chance of fish coming in contact with these chemicals is not only from the water column but also from the sediment at the bottom of the lake. So we wanted to test both.

How did you decide which chemicals to test for?

They were on a list the EPA developed. It is hard to do measurements on these things at the concentrations that they are found at in the environment. The government and scientific organizations have tried to come up with standardized methods so we know the results from one lab are comparable to another. So, those particular chemicals were on a list of appropriate chemicals for the methods that we were able to use to take our measurements. Rather than just pulling things from the literature, we were trying to go with a standardized assay that other people were using.

You found carbamazepine in the effluent but not in the soil or lake water even though carbamazepine does not breakdown easily. Was that surprising?

Yeah. We don't know where that went. It is a really good question. We know that it goes through the sewage plant, and other people have noted that too. We know that if it goes through drinking water treatment, it doesn't breakdown either. We don't know where it is going once it hits the lake. Maybe it is getting absorbed by organisms or—yeah, we have no idea. It is a really big question mark. I have no concrete theories for why we aren't measuring that.

Are there other chemicals that seem to just disappear in water?

Yeah. The problem with our detection techniques is that we don't know what some of the bi-products are. There are some chemicals that we know are altered when they hit the environment, but we don't know what their bi-products are. We also don't know what the potential bi-products are from the disinfection process. It could be that carbamazepine hits the lake and then maybe some bacteria changes it into something just slightly different so that we aren't measuring it using the chemical technologies we have right now.

And the impact of those bi-products is still a huge question, partially because we don't know what they are. That is a tough field. I am not an organic chemist, so I can only imagine what they are going through trying to figure out what all these bi-products are.

You also found metformin more consistently and at higher concentrations than past studies. Why is that?

Metformin is a type 2 diabetes drug. Among the population of many urban centers, definitely in Milwaukee, the rate of type 2 diabetes is pretty high. The prescription level is probably higher here than it is in some other locations, so you see lots of it going through the sewage treatment plant. And we also see it going out into the lake.

There were a few studies in Europe before ours that measured metformin in the water, but it is not commonly tested for. It is not all over the literature yet. I would think that, after what we found, it will be something that is more commonly tested for. It was a surprise for us because it wasn't something we had really thought about originally. People are very focused on endocrine disruption, not necessarily on other types of functions.

Even though you found it in high concentrations, metformin had a pretty low risk level, correct?

Yeah. One of the things my student Ben Blair did was go through and look at the literature and the databases that the EPA keeps about toxicity quotients for these chemicals. This tells us what other scientists have determined to be the lowest concentration where you would find any kind of environmental concern. He based his analysis of how dangerous the chemicals we measured are on the literature of what people had found for each of those chemicals.

Some of the chemicals we tested for aren't listed in our risk analysis because we just don't have a lot of information about them, and metformin is one of these. We were very conservative about including things in our study if there was just not enough information. There are not a lot of environmental studies looking at the impacts of metformin. Actually, that is one of the things we are doing in our lab right now, looking at the impacts of that particular chemical on fish populations and what kinds of disruptions it could cause. It is an anti-diabetic drug, so is it having any impact on the metabolism of organisms? And we are also looking at standard things like mortality, reproduction, growth, and development.

You identified 17 risky chemicals in the effluent but only 13 in the lake. Why the difference?

Their concentrations dropped once they were in the lake. Basically, we didn't find them at the concentrations that reach the critical level of potential effect. But the reason that they dropped could be from dilution, from breakdown, or it could be that it is getting bound to some particle farther out. Some process is happening that is making it so we aren't measuring them in the lake at levels that cause concern.

How do researchers determine the lowest concentration level that causes environmental impacts?

The government tries to determine a concentration level that causes no effect, and they do that for different organisms. They have a testing regime that chemical companies and the government have to go through for different chemicals. If the chemical will go into an aquatic ecosystem, they test things like algae, bacteria, and daphnia. If it is something that they think humans will come in contact with, they'll test mammal species—mice and rats. These are surrogates for all of the organisms in the environment.

What they want to do is base the risk level of a chemical on those numbers. What is the lowest level of that chemical that we think will cause no effect? They try to set a number, or they might build in a conservative factor—multiplying the number by 10, 100, or 1000—just to make sure they are covering all the developmental stages of an organism or anything that might be a little more sensitive than standard test organisms. That is how they set those levels.

And when you did the risk analysis for the chemicals you measured you were just testing whether concentrations were above or below that pre-determined level?

Basically, yes. And that level could be based on any organism. It could be that there is nothing in the literature about fish but that particular chemical is really toxic for algae. For instance, triclosan and triclocarban have been shown to be toxic to algae, so the concentrations that disrupt photosynthesis in algae and other plants could be where the "no effect" level is set for those chemicals.

Are any of the risky chemicals you found detrimental to humans?

We did measure some hormones that are used for agricultural or human medical purposes, so we know they can potentially have an impact on human health. But we weren't specifically targeting drinking water. We were really looking for environmental impacts and the potential for some kind of ecosystem impact when we were determining those risk quotients.

Is there a connection between the measurable concentrations of a chemical and its riskiness?

It depends on the chemical. For instance, some of the estrogenic compounds can be difficult to measure at our detection limits. And we know from the literature that the detection limits using most techniques are higher than the concentrations that would have measurable impacts on organisms. But, caffeine, for instance, has an impact on certain organisms but only at really, really high concentrations. So even though the levels we are finding them at are "high," they are not to the point where they would necessarily cause an impact.

Caffeine was high on the list in your risk analysis, higher than chemicals we think of as being risky, like triclosan. Why is that?

This goes back to the question of its risk to certain organisms. So, it is risky to some organisms but not to others. For us, obviously, at the levels it is found, it is not all that risky. But for other organisms, it might be worse. And caffeine is so prevalent because we all use it in our beverages in the morning, afternoon, and evening. Sewage treatment plants do a really good job of removing a lot of it, but because there is so much going in, it is not able to remove all of it. Caffeine ends up being in the ecosystem in large quantities. We actually often use caffeine as a tracer for sewage treatment effluent to figure out where effluent might be leaking.

Which organisms are negatively impacted by caffeine?

I would have to go look at the literature for caffeine to see what triggered that higher risk level because we based that on a database with a lot of information. It is at that concern level because there is some organism in the database for which caffeine is toxic at the concentrations we found.

Have we been able to test the accuracy of these estimates, particularly for those pharmaceuticals that might have multi-generational effects?

For a lot of these chemicals, the answer is no. There are some chemicals for which we have evidence that there are cross-generational effects. For things like plasticizers and fire retardants, we have some indication in the literature that it probably has effects over multiple generations.

Actually, if you look at regulations, sometimes allowed levels will drop down overtime because they have determined that the risk is actually worse than they originally thought. And sometimes it will go the other way.

Are there some organisms that we should be more concerned about than others when we are considering whether and how to regulate a chemical?

As an ecologist, I know that all things are connected to each other. If you are really disturbing one pocket of the ecosystem, you are probably disturbing other parts of it as well. I think one of the big questions is how much do we value ecosystems as a resource vs. our own human health. These chemicals are accumulating in fish, and we eat the fish. It is obviously having an effect on us too. If an ecosystem dies, even just from a recreational point of view, you are not going to have fish to fish for fun. There are lots of different impacts that I think people don't consider when they think, "Well, it is just effecting that algae."

What projects have come out of this Lake Michigan study?

We are looking at the impacts of some of the chemicals that we measured on different organisms, both individually and in mixtures. In the databases, we often have information about individual chemicals, but we really have very little information about what happens when you mix them all together. For instance, there was another study we did that looked at the mixture of a plasticizer and a pesticide. They are both known to be endocrine disruptors at very high concentrations, but the question is what happens when you drop down the concentrations to what we find in Lake Michigan, let's say. What my student Jordan Crago found is that each of those chemicals individually really wasn't a concern at environmentally-relevant concentrations. They were below the level of concern. When you mixed the two together, though, it dropped testosterone levels in fish.

The issue of mixtures is a huge one, and one that we are trying to tackle. How to do those studies is a really big question. We are talking about a soup of hundreds of thousands of chemicals. Which ones do you pick? We are trying to pick the ones that had environmental impacts individually but that also have an impact on some similar pathway in the organism. Do they collectively have the same impact on that pathway? Some of the chemicals may turn that pathway up, and some may turn it down. If you put them together, do they just cancel each other out? Is there no effect? Or does it increase the effect? Does it cause something else to happen that we don't know? So, we are starting with some of the ones we found out in the lake that were on our list of concern. We are trying to do some individual studies and mixture studies to see what kind of impacts they might have together as a soup.

We also have studies looking at the impacts of some of the neurochemicals in particular. Like hormones, these are active at very low concentrations. Hopefully, there should be a couple of studies coming out soon where we looked at environmentally-relevant concentrations and the effect those neurochemicals have on things like fish behavior. We found that you see impacts on fish reproductive behavior towards the higher end of environmentally-relevant concentrations. One of the things we would like to do is use that as a model for what might happen to humans or other vertebrates that might come into contact with low concentrations of these chemicals. There is a totally different behavioral change depending on the concentration an individual is exposed to.

How do you determine the risk levels for mixtures?

Generally for the mixtures, we are analyzing it as an individual study in our lab as opposed to the database study that we were talking about. So, we are analyzing risk a little differently. We are testing for the lowest level of concern, which would then go into a database like the ones we were talking about. We are determining the lowest level of concern for this specific

assay. If the environment has a higher concentration than that, we should really be concerned. Same thing with the hormones I was talking about or the nervous system chemistry of the brain. If it ends up having an impact at a much lower concentration because of the mixture, we should probably be concerned if all three of those things end up in the water together.

What we are trying to do is fill in the holes. There are a lot of holes in the databases. We are trying to pick at least a few chemicals we just don't have enough information for and fill in the database. We are starting with ones that we determined were risky and that have some mechanism of action that may be really important for things like fish, algae, or bacteria.

Is it possible that the impacts of different pharmaceuticals could just cancel each other out?

It is theoretically possible. We haven't necessarily found that in our mixture studies. But it is theoretically possible.

Does that mean the risk levels of individual compounds might not tell us much about the real danger to organisms?

That is a good question. There are some studies, and we are doing this in our lab too, that look at what we call whole effluent toxicity. So, you have the stuff that is coming out of the plant at any given time. There is a really great study, which I think was done partially in Illinois with some folks from Minnesota, that looked at whole effluent toxicity. It is a way of getting at this soup question without identifying all the different chemicals that are in there. It just looks at whether exposing a fish to the stuff coming out of the sewage treatment plant causes an effect.

So, there are two approaches to this question. There are some people doing the individual chemical and mixture approach and others approaching it from the whole soup question. The whole soup question is probably providing just as much information as the individual chemical approach. We are getting some indication that there are some particular effects on fish, let's say. But that approach also leaves a lot of questions because there is that variability in sewage effluent over time—over 24 hours, over two weeks, over different rainfall events. Even then, we are probably not capturing the information fully. But it is providing us with another piece of the puzzle.

Is there anything else you want to say to people interested in the risks associated with pharmaceutical pollution?

I think that it is important to realize that this research shouldn't create a big freak-out. Often people freak out when they read some of these reports. You have to put things in the context of what is most dangerous. Really, that is what our science is trying to do. Should we really be more worried about emerging contaminants or just focus on the regular, old, nasty contaminants that we have in sediment and know are bad? Should we be focusing on those? The science should help us figure out ways of dealing with different types of exposures that we run into rather than causing an alarmist reaction. We try to be conservative in what we are saying so that we are really providing the best information, the best science, to back up any changes that may need to happen.

Sea Grant
Illinois-Indiana

IISG-13-87

www.unwantedmeds.org