UpClose

with Melody Bernot

Dr. Melody Bernot, an ecologist at Ball State University, is probing deeper into some of the biggest questions in pharmaceutical and personal care product (PPCP) research. Her work over the last five years has taken her across Indiana to learn more about how pharmaceutical compounds are entering waterways and what happens to them once they are there. Back in the lab, she is working to understand how moderate concentrations of these chemicals affect individual aquatic species and food webs as a whole. Dr. Bernot was one of the first to measure pharmaceuticals in the nearshore waters of Lake Michigan. That project, funded by Illinois-Indiana Sea Grant, uncovered important relationships between PPCP concentrations and factors like location, season, and nutrient levels in water.

IISG sat down with Dr. Bernot earlier this summer to talk in detail about the results of her Lake Michigan study, how they measure up with past findings, and what these discoveries mean for future PPCP research.

How did you become interested in pharmaceutical pollution?

I have always been interested in water contaminants. My background is in nitrogen and agricultural pollution. I got my graduate degree at Kansas State University, so we were really interested in how agriculture influences freshwater ecosystems. I really didn't get into pharmaceuticals until I came to Ball State. I opened it up with my first graduate student, Aubrey Bunch, who is now at the U.S. Geological Survey Water Science Center in Indianapolis. I said, "I am really interested in many aspects of non-point pollution. What do you want to do?" I laid out a bunch of things like herbicides, nutrients, and pharmaceuticals, and she jumped on pharmaceuticals. That was my origin. It was very much driven by my first graduate student.

Then when we started working on it, every hypothesis we had was wrong. I just immediately became captivated. For Aubrey's first descriptive study, we were looking at spatial dynamics, and we thought urban areas were going to have the highest pharmaceutical concentrations. Low and behold, they didn't. And study after study, we consistently see higher concentrations in rural areas than in urban areas. And concentrations are higher in winter relative to summer. We were predicting different patterns. It really captivated me in that respect and has really taken hold of me. This is something I am very excited about and see myself pursuing well into the future.

What connections do you see between pharmaceuticals and other contaminants you have studied?

I think they are very connected. It is all about chemical movement in waterways. Essentially half of my lab is working on basic freshwater ecology. They are looking at algal assimilation, algal degradation, bacterial metabolism, and things like that. The other half is looking

at specific contaminants—mainly pharmaceuticals, though I do have a doctoral student, Daniel Elias, working on herbicides as well. Understanding the basic mechanisms of a system and how a contaminant moves in that system feed off each other. I think some people might see it as disjointed. But I really feel like studying contaminants is a natural growth from what we have been doing with freshwater dynamics.

In your Lake Michigan study, you looked at individual pharmaceutical concentrations and total concentrations. Why both, and what can the total tell you that individual compounds can't?

Using total concentrations is really tricky because it is dependent on what you measure. There are hundreds of chemicals out there that we didn't measure. The total is very much a qualitative measure, but I think it is a better one. Looking at the total concentration of the compounds we did measure might give us a better overall indication of what is coming into the water and even potentially where the source is. For example, labile compounds break down quickly, and you don't always see a signal from them. Looking at total concentrations of pharmaceuticals means that you might be able to understand overall patterns a little better even if you can't measure the specifics.

I find total concentrations really useful when trying to understand dozens of compounds. I am an ecosystem ecologist. I am not a toxicologist. I am not a microbiologist. I was trained in ecosystem ecology with a terrestrial background, so I don't really care about the specifics as much as I do about the whole in terms of what dynamics are changing. Looking at total concentrations allowed me to focus my efforts a little bit better. Total concentrations help us figure out which compound is the largest component of the total in any given sample and to compare across samples which compound is not influencing the total here but is influencing the total there. I think it has given us a lot of insight in terms of what to do next and where to look. I can see over the last six years how much more we know now than we did when my first graduate student said, "I want to study pharmaceuticals." I think this is the first time in my career that I have really seen the questions grow.

What is a labile compound?

Labile compounds have a shorter half-life and are easily degraded. They may not stick around in an ecosystem very long. They may be there for a second or a day. Labile compounds usually have a carbon that is easily accessible, so they can be used as a carbon, and sometimes a nitrogen, source. Recalcitrant compounds are ones that have very long half-lives. They are not easily degraded. They stick around for a very, very long time. We have a mixture in pharmaceuticals. We have lots that are recalcitrant and lots that are labile, and their toxicities are different. Going back to why we study PPCPs—the complexity of it is enormous. I find it really fun to try to untie some of these things and figure out which ones matter and which ones don't.

How did you decide which chemicals to measure?

Initially, we measured everything we could. But now that we have the whole suite of compounds, we've figured out which ones we think we should target based on their concentrations, detection frequency, and toxicity. We will continue to measure the whole suite because I do think there is a need to continue surveillance. We can't get rid of that altogether. But we are starting to get a better grip on some of these chemicals—when we can expect them and what concentrations they are going to be at. We need to continue surveying, but we want to ask more targeted questions about specific compounds now.

Our work, especially in the Upper White River, has really highlighted carbamazepine, an anti-epileptic drug. It is high in this area for some reason. I am not quite sure why. It is exceeding 200 ng/L, and I think the nationwide average is closer to 30 ng/L, an order of magnitude lower. We consistently find it in the Upper White, and we found it in Lake Michigan as well. It is very recalcitrant. That is one that we are doing a lot of work on, as well as caffeine, triclosan, DEET, and EE2 [a synthetic estrogen steroid used in birth control]. Those are the primary compounds we are focusing on.

But we aren't losing the whole suite. For example, we are also looking at acetaminophen. It is probably not toxic itself, but it could cause toxic responses when combined with other things. Anytime we do anything, we measure the whole suite. Because, really, who knows which one is most important?

You did grab sampling for this study. What are your thoughts on that method vs. Polar Organic Chemical Integrative Samplers (POCIS), which takes continuous samples over longer periods of time?

Our numbers compare very well with POCIS sampling. We have only been able to compare them in the Lake Michigan study. That was just four sampling events, so you have to take this with a grain of salt. But the numbers we got in Lake Michigan with grab sampling were very comparable—within the same range, not orders of magnitudes different—with POCIS samples in Lake Michigan. I don't feel grab sampling is doing a disservice to understanding the patterns or understanding the load. We get a good representation.

I haven't seen a side-by-side study of grab and POCIS sampling, but I think that would be useful. We have proposed one a couple of times, and hopefully we will do it eventually. I work with the U.S. Geological Survey a lot on the Upper White River, and they use POCIS. So in some respects, we have been able to compare the methods. They weren't field replicates per say, but we feel pretty confident that POCIS is giving comparable numbers. And that is almost concerning. POCIS samples are sitting out there for 30 days. Shouldn't they have higher numbers? Not having ever used POCIS myself, I am not sure what the limitations are.

The grab sampling we have done has been an eye-opener because we were measuring throughout the year as opposed to just the spring and summer. We couldn't get out on the lake as early as we wanted to, but we sampled through ice in the Upper White—cracked a hole and sampled through ice. I think our grab samples gave us at least an equally good assessment of what is there relative to the POCIS samplers.

Is there something that grab sampling gives you that POCIS doesn't?

Not to my knowledge. POCIS gives you the cumulative amount. But then the amounts we got with grab sampling—the ones we have been able to compare, which is limited—haven't been that different. We really need to have a POCIS vs. grab sampling assessment.

You found lower concentrations of pharmaceuticals in the lake than connecting rivers. Why is that, and was it surprising?

Volume of water. It is just the result of dilution as the pharmaceuticals move out.

I was surprised there was no effect of depth. We designed the sampling scheme the way we did because we thought there would be a difference in concentrations because of the hydrology of the lake. You would expect there would be a difference in shallow water vs. deep water.

Are these concentrations a reflection of usage or how long compounds take to break down?

I am not sure. I don't think we will be able to definitively say one way or the other until we start doing in situ stuff, which we may never do. I think that caffeine is a really great example of this problem. In inland systems, caffeine has significantly higher concentrations in winter. I am not sure if we just use caffeine more—I suspect that we do, and there are some social science papers out there that suggest higher consumption of caffeine in winter—or whether there is lower degradation because of lower radiance and temperatures. It is probably a combination of the two. Caffeine, I think, is the one that speaks to this problem more than anything.

There are a lot of drugs that increase in the winter, like acetaminophen. But getting at something like acetaminophen use is really hard. I have tried. I have worked with the Indiana State Department of Health a little bit on this to try to understand use patterns so we can get at those kinds of questions. We haven't gotten very far yet. At least I haven't. It is very hard to tease out how much is being used at any given time. I mean, people have aspirin bottles that have been around for 10 years, so who knows if sales are linked to actual usage. I don't think so. It is very hard to get at usage questions.

You found that pharmaceutical concentrations were most closely correlated with saturated oxygen and carbon levels. Was that surprising?

It wasn't super surprising. A positive correlation with total carbon suggests the source is wastewater input as opposed to something else. Negative correlations with oxygen suggest that lower oxygen concentrations facilitate the degradation of pharmaceuticals. So, not too surprising. But supportive. However, some of the spatial and temporal patterns were surprising. We haven't found the mechanisms yet. In the Chicago River, the flow seems to explain a lot. At the other sites, we are still unsure of the mechanisms.

I think that we have been surprised by some of our results over the last six years. But the more cumulative knowledge we have, and the more consistently we see that oxygen or other things are related to pharmaceutical concentrations or individual compounds, the more we can be sure.

It's like discharge. A lot of work suggests that wastewater discharge is positively correlated with pharmaceutical concentrations. But we never find that. Treated discharge from wastewater treatment plants can be negatively correlated, or discharge could be coming from combined sewer overflow (CSO) input and be positively correlated. I think those two mechanisms blur how discharge may influence pharmaceutical concentrations. Early on, we thought it was all coming off high wastewater discharge. But as we accumulate knowledge, we see—"well…."

Sulfamethoxazole was driving some of the temporal and spatial patterns you found, right? How does a single chemical drive the behavior of the total?

It is just driving the patterns of the 24 compounds that we measured. We have to qualify that. But it is sheer concentration driving those patterns. Sulfamethoxazole was almost an order of magnitude higher than any other compound we measured. I am not sure what is going on there. It is an antibiotic, so maybe there is a significant source that brought it in during that period of time.

You also measured DEET and triclosan but weren't able to report those in your study. Why not?

We did measure DEET and triclosan, but every single one of our field blanks were contaminated. We do not have that problem in lotic ecosystems [rivers and streams]. I had never had a field blank contaminated in my life. We have done hundreds of pharmaceutical measurements and never had any contamination. But during every single trip to Lake Michigan, our DEET and triclosan field blanks were contaminated. I think it is in the wind. I think that is the only explanation. After the first sampling trip in August, we washed everything, and we consistently went to great lengths month after month to try to prevent this because we had never had a field blank contaminated in our Upper White studies. But they were in Lake Michigan. And I think it has to be in the wind. I mean, you really just can't avoid it out there on Lake Michigan. There are particulates coming in. So my guess is it was direct contamination from the wind or there were particulates on our equipment.

What is really interesting is that there were higher concentrations of DEET and triclosan than any other compound, but we couldn't talk about them because our field blanks were contaminated. They were in the hundreds of nanograms. So, it doesn't come out in this paper, but DEET and triclosan are really high up there on our list of things that are potential concerns in Lake Michigan.

How do you decide where to go from here given all the new questions your Lake Michigan study raised?

This was our first study of PPCPs in Lake Michigan. Basically, our intent was to go out there and see what the patterns were. It was a very descriptive study. We were able to identify what was there and the variations between different seasons and watersheds. So we now know we have different mechanisms. This first study in Lake Michigan really gave us the jumping off point. Now we are starting to think about source, specific compounds, bioaccumulation, and ecosystem effects. The study did highlight some compounds, ones that we consistently found. We picked five to focus on out of the 24 we found. Whether or not we will continue to report on the others, I don't know.

I really think there is a lack of data at environmentally-relevant concentrations in organismal work. Even if you look at ones that say they are environmentally relevant, they are still at maximum concentrations. You really don't have to move out to those high concentrations, the maximum ever observed, to call that environmentally relevant. We stick to the mean concentration range we have measured and try to stay away from the maximum concentration when testing for effects, although we normally do have an end point at the maximum concentration so we can better understand what is happening. But we see compounds having effects at mean concentrations. For example, Amanda Jarvis, one of my master's students who is co-advised with Randy Bernot, is doing a study on carbamazepine's effects on macroinvertebrates. Her Ephemeroptera [mayflies] took longer to develop than they normally do. When they molt, they are very light in color, and then they quickly turn to a dark color. But hers weren't turning dark. It took them 6-8 days to turn dark instead of one day. Amanda also found changes in growth, reproduction, sex ratios, and other minor things. Whether these translate to ecosystem effects is also something we are looking into.

Amanda also has a mesocosm experiment [a method used to look at a part of an ecosystem under controlled conditions] that has been populated with algae, invertebrates, snails, and fish. She is incubating it over time and looking at how communities change. Then I have a doctoral student, Jee

Hwan Lee, who is looking at pharmaceutical bioaccumulation in yellow perch in a natural food web vs. one with an invasive species, the quagga mussel. Invasives might cause faster bioaccumulation in the food web. The dynamics might be very different. That study is in collaboration with Tom Lauer and Randy Bernot here at Ball State as well as Daniel King at Taylor University. We are getting our organisms for that study from Lake Michigan.

What is the timeline for these projects?

We are in the middle. Some of our experiments will finish this summer and be submitted in the next year or so, but those aren't Lake Michigan studies. We have Upper White studies that will be submitted by the end of the summer and others that are continuing. There we have taken a look at EE2 and carbamazepine more specifically. It is still descriptive work, but we are coupling it with more organismal work, looking at impacts on trophic interactions and direct toxic effects on reproduction and growth. There is a lot to work on, but those are the things we are interested in now.

You have also done several studies in rural streams. How do the Lake Michigan findings compare with those results?

They were consistent, which is what I like. As I said, our hypotheses have never been aligned with our findings. They are starting to get there, but earlier on they weren't. For instance, spatial patterns show high concentrations of pharmaceuticals in rural areas. In Sugar Creek [an Indiana stream], we consistently see high concentrations of human pharmaceuticals in the watershed even though it is a low-population area. And they are frequently higher than the veterinary pharmaceuticals even though there are a lot of animal feeding operations in the area. We have a group of farmers that tell us how much lincomycin and sulfamethazine they are using. We know that they are abundant in the watershed, but what we find in the water is a little bit of veterinary pharmaceuticals and a lot of human pharmaceuticals. That is consistent with our previous studies. Concentration ranges are consistent with our previous studies. And temporal patterns are consistent with our previous studies. That is good. We always like it when it is consistent. It has made me feel a lot better that we are consistently seeing these patterns. Now we can start getting away from asking, "Is it out there?" and "When is it out there?" and move towards understanding the sources better.

I think we need to take a much closer look at things like septic flow. High concentrations in rural areas could be because of failing, ineffective, or leaky septic tanks. I am not sure. But I do think that the rural septic sources need to be evaluated so that we can better understand the source, the mechanisms, and the potential for contamination. Those are the next steps.

Is it surprising that septic tanks are contributing as much as they are?

Well, we didn't measure septic tanks, we measured the water. The hypothesis is that it is coming from septic tanks. Where else would it be coming from?

We did a broad sweep of the Upper White in one study, and then we did a comparison of a CSO-fed system vs. a rural system. We saw no difference between the two even though we had several overflow events. We also had two papers before we started the Sugar Creek study showing that rural areas consistently have at least equal human pharmaceutical concentrations as urban areas. This doesn't surprise me now, but if you had asked me five years ago, I would have been very surprised. I still find it pretty fascinating. There is no doubt there is a lower contributing population in rural areas, but there is something about them that is letting

more chemicals into waterways. There are a lot of questions still remaining. Hopefully we can tackle those sooner rather than later.

What other projects have come out of your Sugar Creek study?

We have a lot of current projects, mostly looking at abundance, organismal influence, trophic interactions, and things like that. We have a lot of things pending on veterinary pharmaceuticals specifically. Pharmaceuticals are expensive to measure. We don't measure ours in house, which I am actually quite thankful for. We contract out our samples, primarily to the Indiana State Department of Health, and I really like that. The students can go and help perform the analysis so that they get that experience. But at the same time, we don't have to maintain significant mass spectrometry [a technique used to determine the elemental composition and molecular structures of a sample] in order to get strong numbers that you know are robust. I really like contracting the samples out. But it is costly. We would be doing a lot more pharmaceutical studies if it weren't so expensive. But, unfortunately, that is the nature of the game to some degree. We go where we can and sample as much as we can.

How do you decide where to sample?

It depends on the study. Sometimes we focus on a specific watershed. In our broader studies, we try to cover a wider range and represent the diversity that is out there.

Locally, we try to cover most of the Upper White and span sites across rural, agricultural, and urban land use. In Lake Michigan, it was about accessibility and contributing population. And the sites also overlaped very well with a lot of Tom Lauer's work. He has been working on Lake Michigan for decades to understand perch populations. Our sampling locations overlapped very well with perch measurements they were doing at the time. It was a function of convenience as well as getting a good contributing population.

Do you have a set sampling schedule or do you try to take samples after events that might impact concentrations, like rainstorms?

Both. It depends on the study. For both of our farm papers that came out earlier this year, we knew we were going to go out seasonally, or we knew we were going to go out every two weeks. That was a set schedule. But in the CAFO [Concentrated Animal Feeding Operation] paper, we targeted extended sampling prior to manure release and following manure application to the fields. We also did targeted sampling following spring runoff. So, we had a standard frequency and then we augmented it with targeted measurements.

Did you see higher concentrations after manure applications or storms?

The post-manure storm is an interesting story. There was this huge rain event, and we were out there 12 hours after. I am not sure if we missed the chemical runoff or if it never came off. I thought we timed it perfectly to catch all the runoff. I thought for sure we had it. But nothing.

No difference in concentrations?

Yeah. I honestly don't know if it got degraded before it hit the tile [a drainage system that carries water from agricultural fields to nearby streams]. I think if it got in the tile, we would have seen it. We expected to see a big flush after the manure application, but we didn't. I think we didn't catch it.

Spatially, we see higher veterinary pharmaceuticals next to the drainage outflows, but they are very quickly degraded. You find higher concentrations right next to the CAFO, but by the time water flows to the mouth of the stream, they are gone.

Do veterinary pharmaceuticals degrade faster than human pharmaceuticals?

It depends on the compound. They don't degrade faster than caffeine or something like that. It is all relative. But we expected to see them. We did see them, but we didn't get a manure pulse. We knew that there was lincomycin and sulfamethazine in that manure and that it had just been applied a day or two before. Then we had this really nice, significant rain event. But we really just did not pick up an increase in compounds from the manure when we tested the water. That was really surprising, and I am not sure if we were too late. I couldn't have asked for better timing. I was thinking, "Oh, we nailed this." You never get timing like the way we felt we had it. So, we will keep investigating that for sure.

We have talked about a lot of projects. Are you particularly excited about any one specifically?

I have several right now that I am really excited about. And Randy Bernot also has a triclosan study on mussels that should be coming out by the end of the year that is pretty exciting.

We are doing a very targeted carbamazepine study to see what effects it has on organisms. We didn't find much carbamazepine in Lake Michigan, but we find it in unprecedented amounts in inland waters. For our study, we exposed mayflies to low concentrations of carbamazepine, and we just saw crazy mayflies. They were running around in circles. We found measurable effects in growth and reproduction as well, but their behavior was crazy. Amanda Jarvis used qualitative bird behavior models to try to quantify some of the effects. It actually didn't end up being statistically significant, but what we observed were crazy mayflies.

It has been really exciting to test at these low concentrations. We have done ecotox work before on microbial respiration, snail growth, and things like that, but it has always felt very toxicology to me and less ecology. I think that might have been a shotgun approach. You start somewhere and then see where you go. We are merging our studies with real-world systems better now and trying to understand trophic dynamics and whole system impacts. I am really excited about that, especially with the carbamazepine work.

Then we are doing a comparison of EE2 and carbamazepine impacts in food webs with native and non-native species. EE2 and carbamazepine have very different modes of action. We are trying to understand how the mode of action of a pharmaceutical may influence how an organism responds. The study has three levels. First we take individual trophic levels and look at responses individually, then we combine the trophic levels and look at responses across levels, and then we look at accumulation in organism tissues as you move up the food web. I have never done any bioaccumulation work, so I am kind of excited about that.

And Jee Hwan Lee is putting cannulas in yellow perch, which lets you measure drug content in the blood while they are alive. The idea is that fish probably metabolize these drugs differently than humans. But without looking at loss of concentration in the blood over time, like classic human metabolism studies, we really don't know how they are metabolizing it or what organs and tissues the chemicals are going into. We are trying to understand the pharmacokinetics of it. I think that will help us understand potential effects a lot more. We are moving past just what we can observe and trying to understand how the organism is processing it. And looking at it from two different modes of action will help. We know all this for humans, but it is likely different for fish.

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