WATERS ARE MORE POLLUTED THAN TESTS SAY: STANDARD TOXICITY ANALYSES COME UP SHORT

AN INTERVIEW WITH DR. SIMONE HASENBEIN

Bodies of water are "sinks", and thereby bind contaminants particularly well. If even slightly toxic concentrations in water are to be detected, the growth and swimming behaviour of small crustaceans, mini-snails and copepods should be used for ecotoxicological assessments. This was the conclusion of Dr. Simone Hasenbein, from the Technical University of Munich, who carried out a number of studies on the subject. Dr. Hasenbein recently gave an exclusive interview to IET Editor Rachael Simpson about her work in this field, the future of pesticide analysis, and what can be done to limit environmental damage as a result of the use of these chemicals.

Q: For the benefit of our readers, could you give a bit of information on your academic background, areas of interest, and tell us why you chose to carry out this study?

A: Currently I'm a post-doctoral scholar at Dr Richard Connon's lab at the Anatomy, Physiology and Cell Biology Dept. at The University of California Davis. I got my PHD degree from the Technical University of Munich (TUM) in Germany, but I conducted all my research for my previous studies at the University of California Davis, in collaboration with TUM. I have been in the US since 2010, and I've been working mainly on the effects of pesticide mixtures on the aquatic environment. My main focus currently is on the invertebrate species that all fish and higher trophic organisms need - I am convinced that if we know what is going on at the lower end of the food chain that we can help all the other organisms throughout the chain.

Before I came to the US I earned my Masters degree at TUM in Ecotoxicology, Limnology and Ecology, and I'd also already studied the effects of pesticides on invertebrate communities. Currently I'm looking at contaminants of all different kinds –insecticides, herbicides, pharmaceuticals – and what their effects are on invertebrate species, as well as algae species, in lab toxicity studies and field studies.

We recently found that contaminants are no longer found in such high concentrations out in the field so as to cause mortality, so we shifted our focus to sublethal end points. This is what made me interested in doing this study - finding out what the long term effects of lower concentrations of contaminants on aquatic communities are. Many people argue that if we're not able to detect or measure the pesticides any more then there is no effect, but of course it is not as simple as that. That's what pushed me towards doing this study, to find out what's going on in the long term, and furthermore, to see if we can prove that there is still an effect even though we're not detecting anything.

Q: What methods of analysis did you use, both in the lab and in the field?

A: The lab tests that I conducted were all based on standard US EPA protocols that are commonly used for regulatory testing or monitoring efforts, we just slightly amended them to suit our research. Usually in monitoring studies or ecological risk assessments people usually only look at mortality, whereas, as previously mentioned, we were interested in sublethal end points. We wanted to evaluate the movement and activity of the organisms at the end of the ten day lab test. For this we recorded and then analysed videos using a fairly new piece of software called EthoVision, originally from the Netherlands, a really neat bit of software that helps you analyse the movement of the organism you are studying. It has been used quite a bit for lab studies with mice and rats, but it's not that commonly used for aquatic organisms yet, and I would say this was one of the first studies using the software for invertebrates. It worked very well and really helped us to evaluate the effects of the pesticides on the movement of the organisms.

We also looked at the growth of the organisms over ten days, and for this we desiccated the organisms overnight and then weighed them. Growth is a pretty common end point already, especially for fish exposures, but not so much for invertebrates. It's not really been used before so it was nice to compare a more traditional end point (growth) to a more modern end point such as the movement of the organisms.

For the field study we used outdoor mesocosms that I installed here near the UC Davis campus. These are a fairly traditional concept I would say, and have been previously used for ecological risk assessment. As a method mesocosm are not as common anymore because they're cost intensive and a lot of work to maintain, but we shouldn't ignore the fact that they are a very helpful tool to investigate those long term effects in a more realistic setting. Because the mesocosms are outdoors, exposed to light, rain, and so on, they are more representative of the conditions that a river or a pond or lake would be exposed to as well. Also, we had naturally derived sediment in there as well as plants and of course water enabling us to present a really realistic scenario, whereas in a lab setting you usually have a beaker, very clean water, and everything is very controlled and standardised. That isn't to say that lab set ups don't have their advantages as well but in order to study the realistic effects, mesocosms are a really good tool. I used tanks out on the campus, and then filled them with sediment, and naturally derived plants and water from a nearby pond and then started sampling the organisms in there - the zooplankton (water fleas and other, smaller organisms) and also the macro-invertebrates such as mosquito larvae or (little shrimps).

We were looking at them over a total of six months, so a pretty long period compared to the ten days in the lab.

Q: How many species did you use for the invertebrate study groups?

A: Since we were using water from a nearby pond we basically took whatever was available to us. In total we had twenty of each, so twenty zooplankton and twenty macro-invertebrates, but of course some were more abundant than others, which is normal in a community like that. We had some predators at the top of the invertebrate food chain like damselflies and dragonflies, and we also had all the smaller organisms and some snails, so it was a really diverse community in terms of smaller and bigger species. Also, since we were sampling over such a long period of time, we could

see that the community was stable which was helpful for this experimental setup.

Q: The pesticides you were looking at in this study– are they pesticides that some of us might recognise?

A: When I first started picking the study pesticides I was mainly interested in the ones that are commonly applied in agricultural settings, for example Lambda Cyhalothrin which is mainly applied on rice fields or alfalfa and is a fairly common pesticide. I also looked at Permethrin which is used on dogs and cats as a tick treatment so that's something some people may have heard of. Of course, you'd usually have to read the ingredient list to see what's in the product and what you're applying, but the pesticides we looked at are definitely all pesticides used in households or on lawns. If you use them around your home you're just applying them without necessarily knowing what the impacts could be. Then the next burst of rain hits or the irrigation turns on and all of these pesticides get washed off into the nearby river or into the groundwater. Wastewater treatment plants then have trouble to remove these contaminants from the water. Since these pesticides are so hydrophobic, they don't dissolve very well in water and instead stick to all kinds of surfaces, which makes it really hard to remove them. Studies here in California have shown that these pesticides are still found in the effluent from WWTP's so unfortunately they are not being removed. That is obviously a big issue.

Q: The sublethal effects you mentioned earlier – could you give us an idea of what sublethal effects are and how they contribute to fatalities within the species you were looking at?

A: Sublethal effects reduce the ways in which an organism can respond to environmental stressors, such as change in temperature, salinity, or water quality. The sublethal effects that we were looking at in the lab, swimming behaviour and growth, are ecologically vert important. If an organism is not able to swim or to move as it wants to then it will have trouble finding food or avoiding predators. Also, if you cannot swim then you cannot find a mating partner. Organisms that are not as active or mobile as they should be are therefore more vulnerable to predation, drift and also food competition. This is especially important when looking at neurotoxic substances such as the ones we were looking at because these can affect movement. Paralysis would be the first visible symptom of acute exposure meaning that the organism is no longer able to flee or to move.

The same is true for growth. As I mentioned it's a commonly used endpoint in fish toxicity studies because it's an important ecological end point - if an organism is not able to grow properly then studies have shown that fecundity is decreased. Therefore, sublethal effects can mean ecological death because they can cause mortality in the long run which decreases a population, and that, of course, affects the whole community over a longer period of time.

Q: What did you actually find, what were the results? Were some pesticides worse?

A: For the lab exposures I looked at pyrethroids and organophosphates. The pyrethroids overall were more toxic than the organophosphates which has been known for decades, but it was interesting that Bifenthrin, one of the pyrethroids, was most toxic to Hyalella azteca, a little amphipod shrimp, causing effects mainly on their survival and growth but not as much on their swimming behaviour. Another insecticide called Cyfluthrin was most toxic to Chironomids, a mosquito larvae. Cyfluthrin, however, had the greatest effect on the motility of both organisms. What's interesting is that the different insecticides can be more toxic to one species than another. This also shows us that for ecological risk assessment it's not a case that we can use only one species for all our testing and not worry about anything else, because what we have shown here is that one insecticide was more toxic to the Hyalella whereas the other was more toxic to the Chironomids. The species had different sensitivities toward the different insecticides. Also, the sublethal effects were more sensitive than mortality alone, and overall motility was the most sensitive, and most, endpoint for both species.

For the mesocosm study, we used an environmentally relevant concentration in addition to two other treatments that were based on our prior lab exposures. Except for Lambda-Cyhalothrin, all other pesticide concentrations were lower in the environmentally relevant treatment. However, due to the different ratios of the insecticides (environmentally relevant treatment vs. lab-based treatments), the environmentally relevant treatment was in each treatment being more toxic to the community than the lab-based treatments. This was interesting because we didn't expect an effect from the lower pesticide amounts in the environmentally relevant concentrations but they still caused quite an effect, and even higher in some instances than one of the lab based treatments. This is important because the low concentrations of each compound means that they wouldn't really be that toxic individually but in combination, and over a long term, they caused toxicity to the organisms in our mesocosm tanks.

Q: What's next for you in this field? Are you planning to further your research on this topic?

A: Last year we conducted a study on herbicides, and currently we are working on mixtures of insecticides and herbicides. We are still using the mesocosm system, but in the lab we now mainly focus on Hyalella – who have turned out to be the most sensitive organism. I'm looking at long-term effects in the lab by conducting forty-two day studies using Hyalella and pretty much the same insecticides, but in combination with herbicides as both insecticides and herbicides are usually found as mixtures in water bodies. That is why I am now moving on to combining different pesticide classes to investigate their effects on the community as well as on specific organisms in lab exposures.

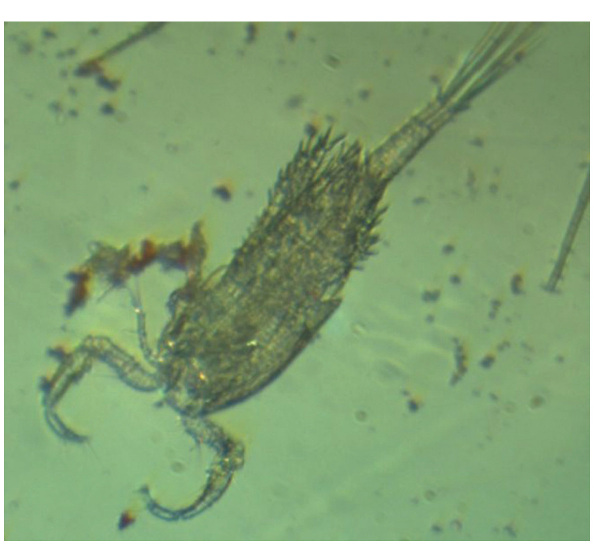
On top of that I am also looking at the molecular level, such as gene expression which is literally the first line of defense that would be affected in an organism. If you were to have an up-regulation or down-regulation of certain genes that are related to the immune system or the reproductive system, and then compared these effects to what we actually see in the whole organism, meaning swimming behaviour, reproductive effects this would give us a better idea of what is really going on within the organism, and what we can do to protect them.

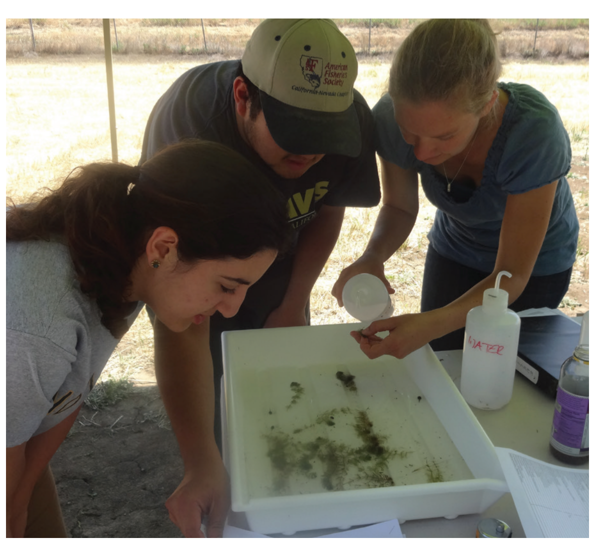
Q: Is there anything that can be done to mitigate the effects of these insecticides and herbicides, especially in waterbodies – you mentioned earlier that one of the pesticides is used for pet care and tick control. What can be done with regards to limiting the impact these things have on the environment?

A: That's a really good question, but a tough one! On the one hand, from a regulatory perspective I feel like there is a lot that can be done, but also it is really hard - there are so many contaminants and so many pesticides out there that make it very difficult to keep track of every single one and its effects. The regulatory bodies for example use safety modelling techniques to keep this under control, but I feel that there is still room for more. I was talking to some of the local regulatory agencies and they are currently not even considering sublethal effects. I am hoping that in the long run they will be convinced to include this sort of work in their modelling. That would be really helpful as a first step.

Also, educating the public is extremely important, so that they might try a more organic, natural method to repel ticks rather than a pesticide as there are so many alternatives that people could use to prevent these parasites and pests coming into their home. I feel that educating the public so they can learn about the potential effects on our water resources is a powerful tool. That's why I'm so happy that journals such as IET are interested in my research. As scientists publish in research journals the layperson usually doesn't have access to. It's nice to get the word out so more people know about the effects of the compounds we are studying – because no one really tells you otherwise!

Hasenbein S, Lawler SP, Geist J, Connon RE.: A long-term assessment of pesticide mixture effects on aquatic invertebrate communities. Environmental Toxicology and Chemistry, 13.11.2015. DOI: 10.1002/etc.3187





Simone Hasenbein, Ph.D. Postdoctoral Scholar, School of Veterinary Medicine, Dept. of Anatomy, Physiology and Cell Biology, University of California, 1089 Veterinary Medicine Drive, Davis, CA 95616, USA • Tel: +1 (530) 752-3141 • Email: shasenbein@ucdavis.edu • Twitter: @simonehasenbein • **Web: https://connonlab.wordpress.com/**