

Insecticides can affect fish behaviour

August 24, 2023 | Annette Ryser Topics: Ecosystems | Pollutants | Society

The safety of insecticides is predominantly evaluated based on lethal effects on other animals. However, new findings from Eawag support the notion that also lower concentrations can be problematic, because they affect the nervous systems of fish and cause changes in their behaviour. This can indirectly affect the survival of fish populations and may be one of the reasons for the fish decline that we are observing in Switzerland. Researchers are uncovering the mechanisms of action and suggesting how these effects could be taken into account in the assessment and evaluation processes of insecticides.

Insecticides have a bad reputation. Most of them are designed to target the nervous system of fruit and crop pests (such as the aphid), but they often affect the nervous systems of other organisms as well. These include bees, aquatic insects – and vertebrates, such as fish or even humans. In some cases, this already occurs in very low concentrations. "Many fundamental aspects of the nervous system have changed very little over the course of evolution", explains Ecotoxicologist Sarah Könemann, who researched the effects of insecticides in her doctoral thesis at the aquatic research institute Eawag and EPFL. For example, some insecticides target certain molecules in the nervous system of insects, but we have almost exactly the same molecules in our nervous system. This is why insecticides can have an effect on humans, too.

In most cases, the concentrations in which insecticides are found in the environment are not high enough to be acutely toxic, i.e. lethal, for vertebrates. "However, there is a very wide range between a lethal effect and no effect", explains Könemann. "This is where I wanted to take a closer look."

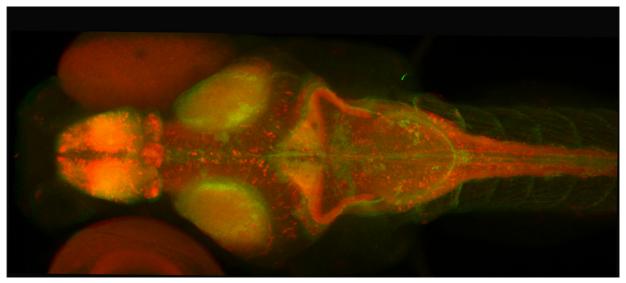
Fish can smell certain insecticides

For this reason, the researcher examined how six common insecticides affect the movement patterns



and neuronal activity in the brains of zebrafish larvae. She discovered that these fish perceived the insecticides imidacloprid and diazinon as stress when they were exposed to a high concentration of them in a short time. And: Könemann was able to demonstrate that the fish larvae smelled these insecticides and subsequently avoided the substances. "At first glance, this seems like a sensible reaction", says the researcher. "They flee from the insecticide and thus prevent chronic effects."

Yet, such a change in behaviour can have negative consequences as well, e.g., if the fish avoid certain habitats as a result. This could lead to them no longer finding sufficient sexual partners or abandoning areas with a particularly rich food supply. "Such effects may therefore be another factor negatively impacting fish populations, which are already challenged by other stressors."



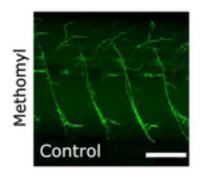
Confocal microscopy image of a zebrafish brain stained with fluorescent dyes visualizing the active (red) and inactive (green) neuronal cells (Photo: Eawag, Sarah Könemann).

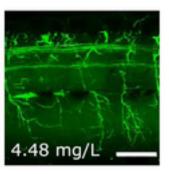
Behavioural change at the push of a button

In her doctoral thesis, Sarah Könemann also looked at the developing nervous system of zebrafish embryos. "We anticipated that certain insecticides would have a particularly strong influence during the sensitive developmental phase, when the nervous system is forming", explains the researcher. And indeed, for three quarters of the substances studied, she was able to demonstrate an effect on the behaviour of the fish: Larvae moved less than the control larvae.

Könemann was also able to identify structural changes in the muscle fibres and the peripheral nervous system that could explain the altered movement patterns. But there were also some surprises: Most of these effects (with the exception of those caused by pirimicarb) were reversible when the larvae were no longer exposed to the insecticides. In other words: "We were able to reverse most of the behavioural effects as well as the structural changes within 72 hours."







The insecticide methomyl, for example, led to structural changes in nerve cells of the peripheral nervous system of zebrafish larvae: Compared to control (left), more branches were formed in the nerve tracts on the right. This effect disappeared when the larvae were no longer exposed to the insecticide (Photo: Environ. Sci. Technol. 2022 56 12 8449 8462).

Significance for the approval of insecticides

Könemanns investigations also revealed that a group of newer insecticides, which were specifically developed to act on a certain organ that only insects have, did not have farreaching influence on the behaviour of the fish studied. This is the case for pymetrozine and flonicamid. "This shows us that the impact on vertebrates in the environment can be reduced if the substances are developed in such a way that they more specifically act on the target-organisms", says Könemann.

She advocates that when evaluating and approving insecticides, one should not only consider whether they will eventually lead to the death of the test animals, but also consider whether their use (even at much lower concentrations) results in behavioural changes. "There is a lot of research going on in the field of ecotoxicology at the moment, especially in the development of tests that can determine the effects of insecticides or other chemicals on the developing nervous system of vertebrates", says Könemann.

However: "Simply investigating whether a substance affects the behaviour of animals is not enough, in my opinion, to be able to classify the significance of such an effect. I recommend also looking closely at the mechanisms that lead to the behavioural changes and the potential of the organism to recover."

Award-winning research

It is worth thinking outside the box to gain new insights. In her doctoral thesis, Sarah Könemann applied methods from the neurosciences to an ecotoxicological context and was awarded the Rifcon Early Career Scientist Award from the globally active Society for Environmental Toxicology and Chemistry (SETAC) in 2022 for her detailed and interdisciplinary work.





Sarah Könemann (left) and former Eawag Director Janet Hering at the 2022 SETAC Europe meeting in Copenhagen

(Photo: Eawag, Barbara Jozef).

Why are problematic insecticides found in water bodies?

In Switzerland, insecticides are approved primarily to be used as plant protection products and biocides. They are mainly used in agriculture, but also in urban areas, the food and feed industry, as well as forestry. They generally enter water bodies through the air and via run-off during rainfall. Although the standards for approving insecticides have been made more stringent in recent years and many substances have been banned, they can still be found in the environment. This is surprising insofar as the most current insecticides degrade quickly in nature. «The fact that we can detect them in our environment in Switzerland (albeit in extremely small quantities of a few pico to nanograms per litre) nevertheless means that they are still being released, despite the bans», says Ecotoxicologist Sarah Könemann. This is made possible by emergency approvals or their use as biocides in stables, which is not explicitly prohibited, unlike the use of the same substance in a field.

Cover picture: Insecticides are designed to target the nervous system of fruit and crop pests (such as the Colorado potato beetle), however they often affect the nervous system of other organisms as well. (Photo: Shutterstock, Sidorov Ruslan)

Original publications

Könemann, S.; Meyer, S.; Betz, A.; Župani?, A.; vom Berg, C. (2021) Sub-lethal peak exposure to insecticides triggers olfaction-mediated avoidance in zebrafish larvae, *Environmental Science and Technology*, 55(17), 11835-11847, doi:10.1021/acs.est.1c01792, Institutional Repository

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von Wyl, M.; Könemann, S.; vom Berg, C. (2023) Different developmental insecticide exposure windows trigger distinct locomotor phenotypes in the early life stages of zebrafish, *Chemosphere*, 317, 137874 (10 pp.), doi:10.1016/j.chemosphere.2023.137874, Institutional Repository



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