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Order matters in pesticide exposures

Risk assessments should consider order of exposure when it comes to mixtures.

The sequence in which organisms are exposed to chemicals could matter just as much as dose and length of exposure, according to new research published in *ES&T* (DOI: 10.1021/es070283w). Most risk assessments consider only one chemical at a time when looking at toxicity or other deleterious effects. But mixtures are important, especially in agriculture, where farmers may apply different pesticides throughout the growing season that run off into streams in pulses.

Roman Ashauer

Tiny freshwater shrimp from this U.K. stream ended up in one of two scenarios (upper right): exposed sequentially to carbaryl and then chlorpyrifos, or vice versa, with higher mortality rates depending on the order of the pesticide pulses.

Roman Ashauer and colleagues at the Central Science Laboratory and the University of York (U.K.) studied exposure to the pesticides carbaryl and chlorpyrifos. Both act on the nervous system by targeting the enzyme acetylcholinesterase (better known as AChE). Tiny freshwater shrimp, *Gammarus pulex*, evacuate the two pesticides in so-called depuration times that differ by only a few days.

Ashauer collected *G. pulex* from a small stream, Bishop Wilton Beck, along with water samples for testing. The team then exposed the approximately 11-millimeter crustaceans to a pulse of one pesticide, followed by a 14-day period of depuration, and then a pulse of the second. They selected this timing to ensure that the first pesticide was out of the shrimps' systems.

Two weeks after a hit of carbaryl (about 27 micrograms per liter [µg/L]), the tiny shrimp seemed to handle a pulse of chlorpyrifos (about 0.5 µg/L) fairly well, with mortality rates of 31% for carbaryl and 21% for

chlorpyrifos. But when the order was reversed, *G. pulex* suffered more damage, with mortality rates of 12% for chlorpyrifos and 55% for carbaryl. Although the death rates for chlorpyrifos were relatively similar no matter the order (21% and 12%), "the mortalities from the two carbaryl pulses are significantly different from each other," Ashauer says.

The shrimp seemed not to have recovered completely from the first chlorpyrifos pulse, even though they should have according to depuration data from previous experiments. The authors hypothesize that the lingering damage from the chlorpyrifos primed the shrimp for even greater mortality with the second, carbaryl, pulse, but exactly how remains to be determined. They suggest "damage recovery" times of about 15 days for carbaryl and 25 for chlorpyrifos.

"If you just do any kind of sequence experiment, you might not find these effects," says Ashauer. In this case, the timing had to be just right: had the time between pulses been much shorter (so the shrimp could not recover in time) or much longer (so the shrimp recovered completely from both), the effect of order may not have been evident. Ashauer notes that "one of the most interesting experiments in the future would be two compounds that have different target sites."

The experiment was "exactly what we want to see" in ecotoxicity and mixtures modeling, says Andreas Kortenkamp, head of the Centre for Toxicology at the University of London's School of Pharmacy. The paper's strength lies not only in pointing out that "order does matter if there are differences in recovery," Kortenkamp says, but also in indicating that modeling approaches can work in forecasting such sensitivities.

Jim Lazorchak, an ecotoxicologist at the U.S. EPA in Cincinnati, calls the experiment "groundbreaking". The team is "trying to explore modeling to predict realistic exposures," he says, particularly for exposures to nonpoint sources of pesticides. Typical assessment methods don't incorporate timing and order, which are critical in assessing real-world situations, where even more stressors occur, he emphasizes, from changes in water availability and climate to lack of food and habitat loss. "As far as assessing different exposure regimes, few people are getting involved" in such complex scenarios, like Ashauer and colleagues, he says, "but that's the direction [eco]toxicology needs to go."

"The order in which you are exposed is just as important as the concentration and duration you were exposed," Lazorchak says. The question now becomes "why is the order important?" —NAOMI LUBICK

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