

Order of Exposure Can Make the Poison

(*Beyond Pesticides*, July 5, 2007) A new study showing that the order of exposure to multiple pesticides may be just as important as the dose, timing and length of exposure adds another dimension to the complex task of risk assessments. Using **carbaryl** and **chlorpyrifos**, University of York scientists have observed significant differences in mortality rates of freshwater invertebrates depending on the order of exposure to these frequently used agricultural chemicals.

The study, *Modeling Combined Effects of Pulsed Exposure to Carbaryl and Chlorpyrifos on Gammarus Pulex*, suggests the sequence of pesticide exposure may be just as important of a variable as the dose, the timing of the dose and the length of exposure when factoring environmental and health endpoints.

The researchers conducted the study by exposing the freshwater invertebrate *Gammarus pulex* – a tiny shrimp – to pulses of the two insecticides (both of which affect the nervous system through acetylcholinesterase inhibition) mimicking exposure to chemical mixtures in the environment – for example, farmers may apply several different pesticides over the growing season that run off into the aquatic environment. After receiving a pulse of one pesticide, the shrimp were given 14 days, a time period chosen based on previous experiments, to recover and expel the chemical from their systems before exposure to the second pesticide.

When first exposed to carbaryl and then chlorpyrifos two weeks later, mortality rates were observed to be 31% for carbaryl and 21% for chlorpyrifos. When reversed, with chlorpyrifos exposure occurring first, the mortality rates were 12% for chlorpyrifos and 55% for carbaryl. The significant difference in the mortality rates from the carbaryl pulses have led the authors to hypothesize that the shrimp were not able to recover completely from the chlorpyrifos exposure and therefore require greater “damage recovery” times.

As reported by *Environmental Science & Technology*, Jim Lazorchak, an EPA ecotoxicologist, 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, he says, “but that’s the direction [eco]toxicology needs to go.” He continues, “The order in which you are exposed is just as important as the concentration and duration you were exposed.” The question now becomes “why is the order important?”

The study renews a central discussion over real world scenarios where mixtures and synergistic effects are common. This is a situation that not only impacts our environment but also our health. For example, several **studies** conducted by a team of Duke University researchers lead by pharmacologist Mohammed Abou-Donia suggest that DEET in conjunction with permethrin-impregnated clothing may be linked to Gulf War Syndrome. Exposing animals to the same doses of DEET and permethrin have been shown to result in similar effects

Synergistic effects between multiple pesticides and/or other chemicals represent one of the greatest gaps in EPA's ability to protect the public from the adverse health effects associated with pesticide use and exposure. This current study sheds further light on just how little is understood about exposure to pesticide mixtures and the many variables that can occur.

Source: *Environmental Science & Technology*

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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](https://doi.org/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](#) 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 [$\mu\text{g/L}$]), the tiny shrimp seemed to handle a pulse of chlorpyrifos (about 0.5 $\mu\text{g/L}$) 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

Modeling Combined Effects of Pulsed Exposure to Carbaryl and Chlorpyrifos on *Gammarus Pulex*

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Abstract:

Aquatic risk assessment can be improved if we are able to quantitatively predict the effects resulting from sequential pulsed exposure to multiple compounds. We evaluate two modeling approaches, both extended to suit multiple compounds, the semi-mechanistic threshold damage model (TDM), and a model based on time-weighted averages (TWA). The TDM predicts that recovery of damage to *Gammarus pulex* from exposure to chlorpyrifos takes longer than that from exposure to carbaryl and consequently that the sequence of exposure matters. We measured survival of the freshwater invertebrate *Gammarus pulex* after sequential pulsed exposure to carbaryl and chlorpyrifos. Two groups of organisms were exposed to a first pulse of either carbaryl or chlorpyrifos for 1 day and then, after a recovery period of two weeks, to a second pulse with the other compound. The comparison of mortalities caused by each pulse, as well as combined mortalities in both treatments, show that the sequence of exposure to pulses of contaminants does indeed matter. Previous exposure to chlorpyrifos leads to significantly increased mortality from subsequent pulses of carbaryl, but not the other way round. The TDM facilitates a process-based ecotoxicological explanation by simulating the recovery dynamics and outperforms the TWA model.

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