Environmental pollutants make worms susceptible to cold

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Enchytraeus albidus. Credit: Aarhus University/SDU

Some pollutants are more harmful in a cold climate than in a hot, because they affect the temperature sensitivity of certain organisms. Now researchers from Danish universities have demonstrated how this happens, and it can help us better predict contamination risks, especially in the Arctic.

Imagine you are a species which over thousands of years has adapted to

the arctic cold, and then you get exposed to a substance that makes the cold dangerous for you.

This is happening to the small white worm *Enchytraeus albidus*, and the cold provoking substance, called nonylphenol, comes from the use of certain detergents, pesticides and cosmetics.

Nonylphenol is suspected of being a endocrine disruptor, but when entering the worm it has another dangerous effect: It inhibits the worm's ability to protect the cells in its body from cold damage.

Enchytraeus albidus is a species of earthworms, which can be found from the Arctic to temperate regions of Europe. It is a so called ectotherm; an animal whose body temperature closely follows the temperature of the environment in which it lives. It is grown as feed for some aquarium fish.

Liquid Membranes and Artificial

Cell membranes are mainly composed of certain lipids – phospholipids. When ectothermal animals are exposed to cold, their cell membranes become stiff, just as butter does when you put it in the refrigerator. This is not good, because cell membrane must have some fluidity, so that for example transport proteins can pump molecules and ions into and out of the cells in the right way. Conversely, heat can also be a problem, because it makes cell membrances too fluid.

Fortunately, *Enchytraeus albidus* - and many other ectothermal animals - can adjust the fluidity of membranes by altering the lipid composition of their cell membranes.

Now it turns out that nonylphenol makes the worm's cell membranes more stiff, so it is harder for the worm to tolerate the cold. The

researchers from Aarhus University, University of Southern Denmark and Roskilde University have demonstrated this by experiments with artificial cell membranes.

Realistic Cocktail

The researchers' work can pave the way for more accurate assessments of how pollutants affect ecosystems – a field called ecotoxicology.

"Usually, when you make ecotoxicological risk assessments for hazardous substances, you make experiments with different organisms at room temperature, or at the optimal temperature for the organisms. But in nature, the organisms rarely have optimal conditions. They are stressed by high or low temperatures, drought or disease. This gives a risk of underestimating the real effect of substances in nature", explains research leader of the project, professor Martin Holmstrup from Aarhus University.

His research team has tested many different hazardous substances at low and high temperature or drought, and the researchers have seen synergies between the effects of such substances and for example low temperature.

"We found many instances where the two effects reinforce each other, and we set out to uncover the mechanism behind this cocktail effect. Now we have uncovered part of it, "says Martin Holmstrup.

Several Animals, Chemicals and Effects

So far, the scientists have revealed the mechanism of two pollutants, both lipophilic (meaning that they can be dissolved in fat) - namely nonylphenol and the tar substance phenanthrene.

As nonylphenol makes the membranes more stiff, the researchers see the opposite effect from phenanthrene. This substance makes the cell membranes more fluid and thus the worms more resistant to cold when occurring in the right concentration.

The same effects were observed in another organism, the tiny springtail (Folsomia candida; a kind of primitive insect). As opposed to the small earthworm this animal does not live in the Arctic.

How to Make a Cell Membrane

The scientists analyzed the two substances' effect on artificial cell membranes using methods developed at the Center for Biomembrane Physics at the University of Southern Denmark.

"We pulled all lipids out of the organism and created a mean membrane whose physical properties we could study. We chose to characterize the membranes elastic flexibility and express it in a single quantity, namely the bending rigidity. And we found that phenanthrene makes the mean membrane much more flexible both for the worm and the springtail, while nonylphenol makes the membranes more stiff", says John H. Ipsen, associate professor at the University of Southern Denmark.

Additionally, calorimetric measurements at Roskilde University have confirmed that the temperatures where a phase transition of the artificial membranes happens (i.e. solidification or melting), are affected by the two substances.

Close to Reality

In parallel, the researchers tested the two substances on worms and springtails by polluting the soil that the animals inhabitated in the

laboratory, They measured both how their cold tolerance was affected under changing temperatures and the amount of the substances they had accumulated.

Martin Holmstrup from Aarhus University emphasizes that the experiments and measurements were carried out under circumstances are very close to reality, except for one point:

"We have used chemicals in concentrations higher than found in nature. We need further tests and investigations to find the influence of lower concentrations in nature. But now we know the mechanism", he concludes.

Provided by University of Southern Denmark