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TEXT Willem Koert

Tinka Murk does research on the insidious influence of hormone disrupting substances on the environment and humans. These endocrine disruptors affect the hormonal balances of fish in very intricate and complex ways. The Wageningen professor suspects that they also play a role in the increase of hormone-related cancer in humans. "I'm worried about what we are doing to our surroundings and ourselves."

# TAKING A CLOSER LOOK AT ENDOCRINE DISRUPTORS

One of Professor Tinka Murk's hobbies is diving. Underwater she can easily spend half an hour watching an octopus hunt over the seafloor. "Or a fish that gets cleaned by small fish after starting to swim in a vertical position. If it swims horizontally the little fish ignore it, but as soon as it turns vertical they flock to it. The complexity of nature has always fascinated me. As a child I could spend ages watching all those creatures you find if you turn a stone over."

Murk has worked at the sub-department of Toxicology at Wageningen University since 1989, and was recently appointed personal professor of Environmental Toxicology. She intends to focus on the insidious influence of man-made hormone disrupting substances on nature. "Their technical name is endocrine disruptors," says Murk. "They are scientifically interesting because of the subtle ways in which they work. Unravelling how endocrine disruptors mess about with the functioning of organisms makes

you realise how ingeniously nature works. But, I'm also worried about what we are doing to our environment and ourselves."

Tinka Murk studied environmental biology in Leiden in the eighties. After graduating she worked for the Health Council of the Netherlands, exploring the research field of eco-toxicology. During that period she discovered that Dutch companies exported dangerous waste to the Third World. A memorandum that she wrote resulted in recommendations from the Health Council, which placed the practice firmly on the political agenda. "I interviewed about thirty eco-toxicologists for that report," recalls Murk. "One of them was Jan Koeman, who was chair in Wageningen at the time. In 1989 Koeman invited me to apply for a job here."

## Environmental toxicology

Fifteen years later, Murk is now chair herself. "Toxicology in Wageningen rests on two pillars," she explains. "Nutrition and environment. The environment side has had a pretty low profile in recent years, but I hope that my appointment will help to put environmental toxicology back on the map." What may also help is Murk's relationship with the marine research institute Wageningen Imares, where she has been working one and half days a week since the end of 2006. Wageningen Imares has facilities that her colleagues can only dream of. On the other hand, however, Murk's toxicology expertise and advanced methods may also give

photo: Guy Ackermans

research at Imares a new impulse.

“A PhD researcher at Imares wanted to do experiments with endocrine disruptors on young sole,” continues Murk. “But a problem with endocrine disruptors is that their harmful influence is particularly noticeable in very young organisms whose tissues and hormone systems are not yet fully developed. Young sole measuring ten centimetres are already past their most sensitive period. Fortunately at Imares they managed to get sole to reproduce and were able to raise larvae. This meant that for the first time we were able to measure the effect of endocrine disruptors on very young sole, from the moment they hatch out of the egg.”

The results of these experiments, in which young sole were exposed to endocrine disruptors for four days, are disturbing. The concentrations of endocrine disruptors used are deemed not dangerous according to current OECD test methods (carried out with older organisms), but nevertheless these levels led to death in the long run. The concentration of endocrine disruptors in the larvae was at levels found in fish from severely polluted water.

### Herring

The experiment results perhaps explain the remarkable discoveries of North European fisheries biologists who monitor the herring stocks. The biologists have found sufficient herring spawn, but notice that only a small proportion of this matures into adult herring. Murk suspects that endocrine disruptors are to blame. “It’s a theory that doesn’t get much publicity. It doesn’t fit in the current paradigm, which says that emissions of contaminants have been reduced so much that they are no longer an environmental hazard. I’m not saying that the paradigm is wrong. I am saying that we do not yet have proof, and the current methods of testing are not capable of measuring the risks of substances such as endocrine disruptors which work so insidiously.”

Endocrine disruptors is the general name given to a large group of compounds of which dioxins and PCBs are the most well known. PCBs were used for a long time in transformers, condensers and pumps. PCBs from discarded equipment leaked in large quantities into the environment, and dioxins were released from unfiltered waste incinerators.

Governments introduced drastic measures and managed to reduce the emissions of these toxins, but it was not long before worried scientists sounded the alarm about another group of compounds: brominated flame retardants, which manufacturers add to electronic equipment and plastics to stop them catching fire, are now being released into the environment. And as politicians apply themselves to formulating new measures to deal with these, publications are starting to emerge on yet another

group of suspicious substances: perfluoro compounds, found in protective coatings and food packaging.

“I’m definitely concerned about perfluoro compounds,” says Murk. “They behave differently in the body than dioxins, PCBs and brominated flame retardants. All accumulate in fatty tissues and are difficult to get rid of because they do not break down easily. But perfluoro compounds differ because they are soluble in water. They attach themselves to proteins in the blood and take over the place of fatty acids in the cell wall.

From studies we know that baby rats that had absorbed relatively small quantities of perfluoro compounds from the mother animal died shortly after birth or showed retarded development. That’s why we are doing research at Imares on the effect of perfluoro compounds on sole larvae and on the sea potato (*Echinocardium cordata*) is a kind of hairy ball that lives in sediment. Imares has been breeding them as an experimental species because their hormone system is remarkably similar to that of vertebrates such as fish and humans.

### Complex puzzle

The difficult thing about endocrine disruptors is that they work through different mechanisms and can influence each other’s effect. Hormonal disturbances have turned out to be an increasingly complex puzzle. “Take disturbances in thyroxin,” says Murk. “Fish, frogs and sea potatoes need this hormone to be able to change from larva into an adult organism. Humans and other vertebrates need the hormone for the development of the nervous system and energy regulation.

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**‘THE RISE OF HORMONE-RELATED CANCERS MAY INDICATE THAT HUMANS ARE ALSO AFFECTED BY ENVIRONMENTAL CONTAMINANTS’**

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Endocrine disruptors that leak into the environment from electronic equipment probably affect the hormonal balances of fish.

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Some endocrine disruptors can force thyroxin off its receptor, but they can also do much more. Thyroxin is transported through the body by protein compounds such as thyroxin-binding-globulin and transthyretin. The substances we are studying can force thyroxin from its position on the protein and therefore reduce the availability of thyroxin. But vitamin A concentrations are reduced as well because this vitamin also moves through the body via transport proteins and vitamin A is needed for healthy development and resistance to disease. In addition, the endocrine disruptors activate enzymes that eliminate thyroxin from the body and reduce the liver’s ability to store vitamin A.”

As if this is not complicated enough, Murk and her PhD students recently discovered that endocrine disruptors’ working is even more complex than they realised. “Cells have molecular pumps that pump the dangerous substances out and substances they need in,” explains Murk. “These are the ABC transporters. We have discovered that many

endocrine disruptors also affect these. For example, they can sabotage the pump that the cell uses to get rid of a disruptor. The concentration of these substances then rises and so their effect increases as well.” This effect on the molecular pumps helps to explain why mixtures of substances in low concentrations nevertheless can have an unexpectedly strong effect.

Outside the laboratory, animals and humans are continuously exposed to mixtures of softeners, PCBs, flame retardants, pesticides and perfluoro compounds in such low concentrations that they are often hardly measurable. Murk suspects that her tests can explain why for example things are going so badly with eels, despite the fact that their habitat has become cleaner.

### Eels

“When an eel swims to the Sargasso sea to reproduce, it no longer eats,” says Murk. “It burns its own fat, which is where the endocrine disruptors are stored. And thus the compounds are released. At the same time, an eel has to change from a freshwater fish into one that can live in salt water and whose only purpose is to reproduce. These processes require the thyroid gland – we know this from experiments with guppies. Guppies can adapt to living in salt water, unless you disrupt the working of their thyroid gland. Then they die within three weeks.”

This may be what happens with eel that leave Europe each year for the Sargasso sea. Experiments in swimming tunnels seem to confirm the theory. In these experiments the eel do not suffer from higher concentrations of PCBs in their body. But if eel have to swim for long periods in salt water they die after about three weeks. At that point they have only swum a thousand kilometres.

If Murk’s disturbing suspicions – ‘there’s no proof yet,’ stresses the professor – turn out to be true, and there are more risks attached to environmental contaminants than we previously thought, then humans are probably also affected. The statistics point in the same direction. “In the Netherlands, one in nine women is affected by breast cancer during her life,” says Murk. “A few years ago, the figure was only one in twelve. In men there has been a similar rise in testicular cancer, even though the percentage of men with this form of cancer is still low. There is definitely a rise in hormone-related cancers. Undoubtedly lifestyle factors also play a role, but I wonder if the endocrine disruptors don’t also have something to do with this increase. Our current and future research will throw more light on the matter.”

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